13th International Conference on Neuronal Ceroid Lipofusinoses (Batten Disease) & Patient Organisation Meeting

28th - 31st March 2012

Royal Holloway College
London, UK

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Windsor Conference Centre, Royal Holloway College, University of London

Conference Organisers

Jon Cooper, Sara Mole, Ruth Williams

Co-Organiser

David Pearce

Scientific Organising Committee

Jonathan Cooper (Institute of Psychiatry, King's College London, UK)
Sara Mole (University College London, UK)
Ruth Williams (Evelina Children's Hospital, Guy's and St Thomas NHS Foundation Trust, UK)
David Pearce (Sanford Children's Health Research Center, Sioux Falls, SD, USA)
Thomas Braulke (University Medical Center Hamburg-Eppendorf, Hamburg, Germany)
David Palmer (Lincoln University, New Zealand)
Mark Sands (Washington University School of Medicine, St Louis, MO, USA)
Jaana Tyynelä (University of Helsinki, Finland)

Local Organising Committee

Jon Cooper, Sara Mole, Ruth Williams, Hannah Mitchison, Claire Russell, Guy Tear, Brenda Williams, Andrea West (BDFA), Heather Band (BDFA)

Welcome

It is our pleasure to welcome all participants to this 13th International Conference on Neuronal Ceroid Lipofusinoses (Batten Disease) & Patient Organisation Meeting, plus many additional satellite meetings, on behalf of the Conference Organisers and Local Organising Committee.

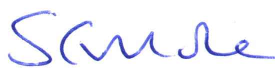
An NCL conference has been held approximately every other year on alternate sides of the Atlantic. This is the only forum that brings together those with interests in basic science and clinical care for this group of devastating diseases. This year we are adding new dimensions to the NCL2012 Scientific Conference. Our co-hosts, the UK **Batten Disease Family Association (BDFA)**, are bringing together Patient Organisations, professionals and affected families for a parallel two day conference, which will include combined sessions with the scientific conference on the final day. We are also hosting many short satellite meetings, including some for participants who will attend just for one event. One of these is the first meeting of the newly formed **Batten Disease International Alliance (BDIA)**. The **BDIA** is a new group of Batten Disease Patient Organisations and Research Foundations who are working together to promote a greater understanding of Batten Disease, by facilitating research, raising awareness and to provide support for affected families. Finally, we will close the conference with a combined and interactive session including a new venture, the 'Market Place', and end our time together with a banquet.

Our primary goal is to provide a conference that will maintain the highest standards of scientific and clinical presentations. However, we also wanted to provide opportunities for other professionals and families to participate in events that were relevant to them. We have chosen this conference venue and organised a program that will bring together the Science Conference, Patient Organisations, the first meeting of the BDIA, and other professionals in different ways, to maximise the opportunities for interactions between attendees.

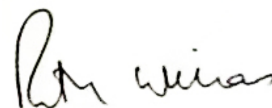
To achieve these goals we have drawn upon the goodwill and expertise of many, such as the Scientific Committee, the Session Chairs, those young scientists who will summarise the scientific sessions each day for attending parents, and experts who will write review articles following this conference to be published in the journal *Biochimica et Biophysica Acta*, as well as conference staff at Royal Holloway College. We thank them for all the work they have already done and will do over the next few days.

We hope that you enjoy this Conference and look forward to meeting all of you over the next few days.

Jon Cooper



Sara Mole



Ruth Williams

On behalf of the Batten Disease Family Association (BDFA), I would like to welcome you all to NCL 2012.

This is the first time that a Patient Organisation has co-hosted an NCL conference and it promises to be a special meeting, not only for its contribution to NCL science but because so many parents, professionals and supporters of those affected by Batten disease are able to attend. As an organisation, it is something that we have always been committed to and on behalf of the BDFA, I would like to express our thanks to Jon Cooper, Sara Mole & Ruth Williams for their vision, and dedication which have helped to make it a reality.

I would also like to thank the BDFA Team of Andrea West (Charity Manager) & Heather Band (Research Trustee) for all their hard work & commitment.

A unique feature of this conference will be the combined sessions, especially on the Saturday, made possible by generous grants from UCL, and Jeans for Genes, which will provide time for scientists and families to meet, exchange information and learn from each other. It is also wonderful to see young scientists offering to spend time during the meeting to help parents better understand the relevance of the scientific sessions. During the parallel 2-day Patient Organisation meeting there will also be seminars, workshops and networking opportunities, which everyone is encouraged to attend.

The recent launch of the Batten Disease International Alliance is a very exciting development and we look forward to hearing about how this will benefit all involved in Batten disease during the Patient Organisation meeting.

We hope that everyone will benefit from attending this meeting and will take full advantage of the opportunities offered to increase and share in the diversity of our knowledge of Batten Disease. Only through forums such as this, with everyone working together, can we achieve our ultimate aim of the advancement of scientific knowledge leading to effective therapies and ultimately a cure to bring light to Batten disease.

Pauline Docherty

Pauline Docherty
BDFA Chair of Board of Trustees

Pauline, Jim &
James Docherty

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Sponsorship

We thank the following organisations for their generous support of **NCL2012**:

University College London - supported by the UCL Public Engagement Unit - funded by HEFCE, the UK Research Councils and the Wellcome Trust as part of the Beacons for Public Engagement initiative

Batten Disease Family Association (UK)

Batten Disease Support and Research Association (USA)

Children's Brain Diseases Foundation (USA)

Bee for Battens (Eire) – including the abstract book

Beyond Batten Disease Foundation (USA)

Beat Batten Foundation (Netherlands)

National Institutes of Health – for travel for USA attendees

International Society for Neurochemistry – including travel support

Company of Biologists (UK) – for the Session 'Disease Mechanisms'

The Genetics Society (UK) – for sponsorship of all Poster Sessions

Ipsen Fund (UK) – for the talk by Prof Andrea Ballabio

BioMarin, Genzyme, Shire HGT

Scientific committee and Local organising committee

Local Organising Committee

Jon Cooper

Sara Mole

Ruth Williams

Hannah Mitchison

Brenda Williams

Claire Russell

Guy Tear

Andrea West

Heather Band

Scientific Organising Committee

Jon Cooper

Sara Mole

Ruth Williams

David Pearce

Mark Sands

David Palmer

Thomas Braulke

Jaana Tyynelä

Session Chairs

Session 1 Genetics & Cell Biology

Sara Mole

Thomas Braulke

Session 2 Disease Mechanisms

David Palmer

David Pearce

Session 3 Links to other diseases

Fran Platt

Steve Walkley

Session 4 Recent Research Findings

Anu Jalanko

Hannah Mitchison

Session 5 New Clinical Perspectives

Angela Schulz

Jonathan Mink

Session 6 Experimental Therapies

Beverly Davidson

Mark Sands

Session 7 Shared with Patient Organisations

Andrea West

Sara Mole

Jon Cooper

Ruth Williams

Venue information - Royal Holloway College and Windsor Building

All events except some meals and social occasions will be held in the Windsor Conference Centre, with our reception and 'Banquet' (informal dress) held in the Founder's Building. Most accommodation is in Tuke Hall (double rooms) and Reid Hall (single rooms). Breakfast will be served in 'The Hub' and lunches will be in the Windsor Building. Evening meals will be in the Founder's Building or 'The Hub'. Our 'Pub Social' will take place in 'The Medicine Bar', which is also open each evening. There is also a quiet area beneath The Hub, called 'Imagine', which is set aside primarily for families and parents. There is a Campus shop, which sells newspapers and a Bank with ATM machines. If you get lost please follow the signs displaying the NCL2012 logo or ask Conference staff for directions.

There is further more detailed information in an appendix on page Ai

conference & banqueting

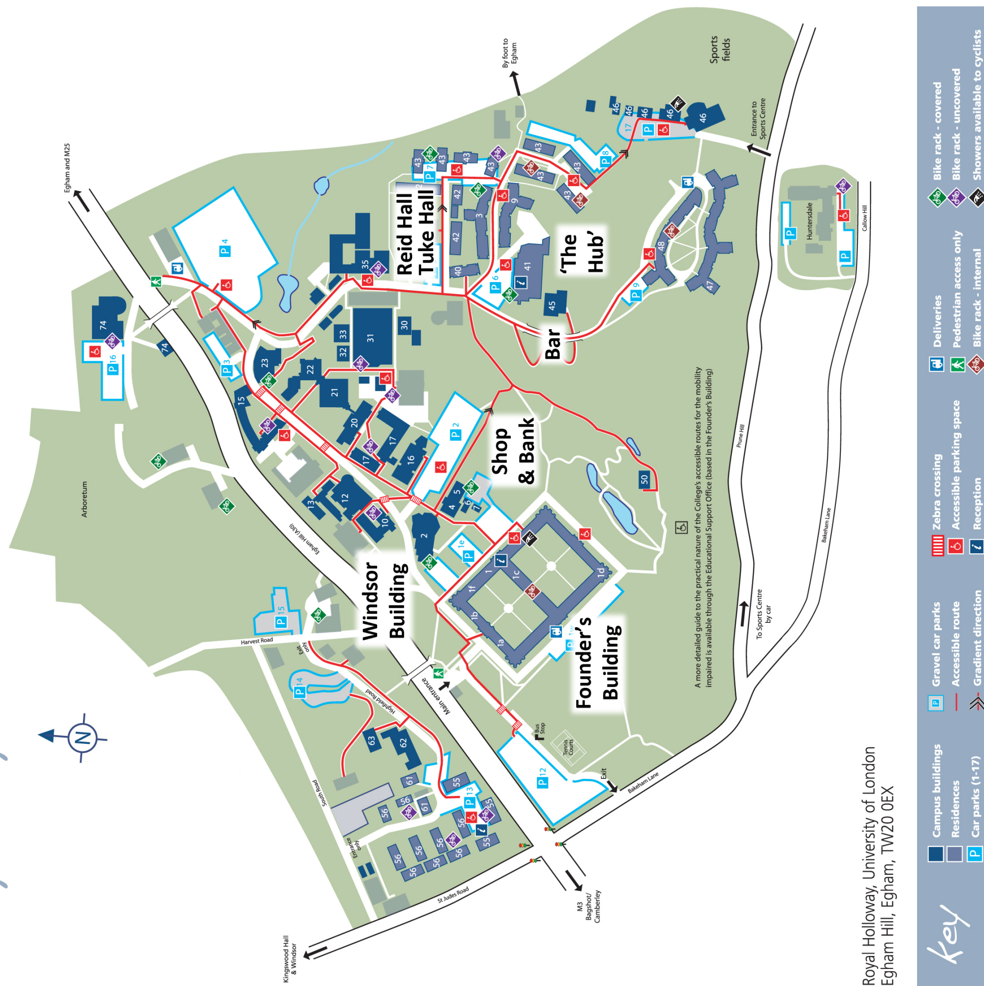
16 The Arts Building
30 Bourne Annexe
31 Bourne Laboratory
1c Founder's Dining Hall & Crosslands
1 Founder's Building
41 The Hub
20 Horton Building
15 International Building
50 Jane Holloway Hall
75 Kingswood Dining Hall, 8Bar9 & Blue Room
1d Main Lecture Theatre
17 McCrea Building
13 Moore Annexe
12 Moore Building & Lecture Theatre
32 Munro Fox Lecture Theatre
33 Munro Fox Seminar Room
1a Picture Gallery
35 Queen's Building
23 Students' Union
74 Sutherland House & Studio Theatre
21 Tolansky Laboratory
63 Wetton's Annexe
62 Wetton's Terrace
22 Wilson Laboratory
2 Windsor Building

accommodation

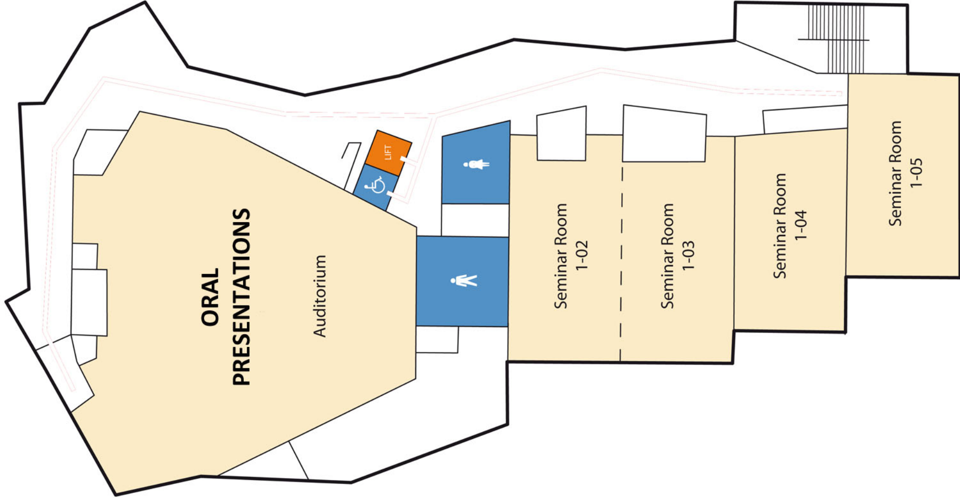
3 Butler Hall
1 Founder's Building
48 Gowar Hall
55 Highfield Court
41 The Hub
75 Kingswood Hall
61 Penrose Court Flats
56 Penrose Court Houses
42 Reid Hall
43 Runnymede Hall
9 Tuke Hall
47 Wedderburn Hall
40 Williamson Hall

other facilities

15 Café Jules
4 The College Bookshop
10 Computer Centre
1b Founder's Chapel
1f Founder's Health Centre
5 Laundry
5 Muslim Prayer Room
45 Medicine Bar & Stumble Inn
7 Cash Machine (ATM)
46 Sports Centre & Gym
6 The Store on Campus

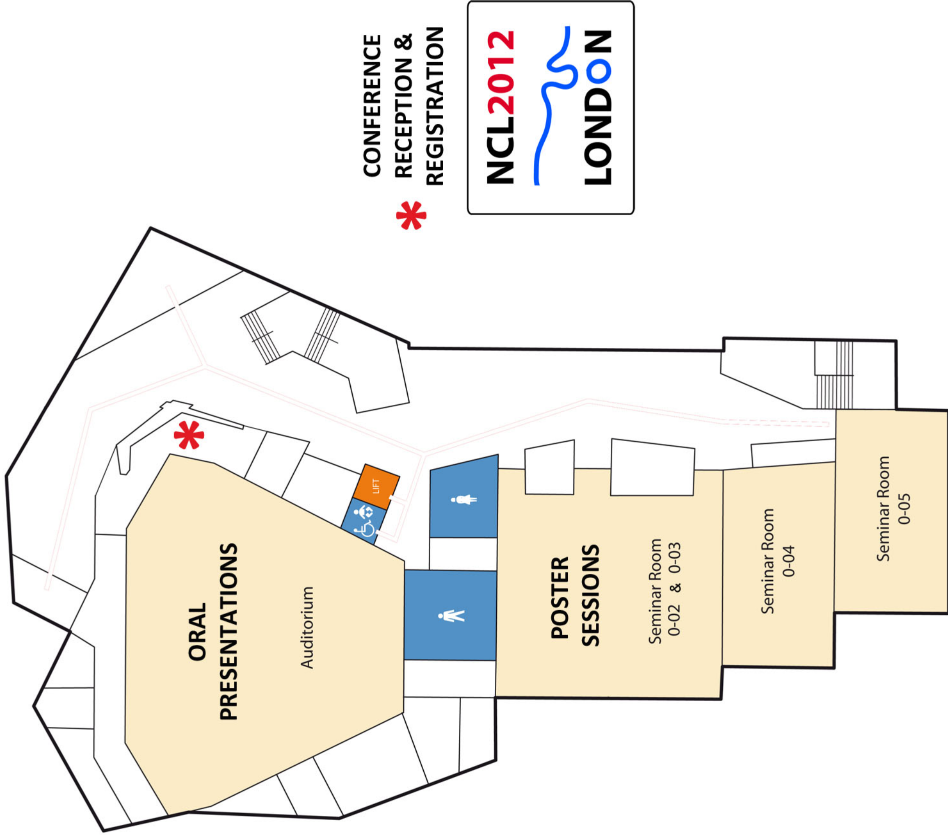


Floorplan of Windsor Building



First Floor

Enter auditorium by first (upper) floor if session has already started



Ground Floor

Conference Wi-Fi and Internet Information

Conference Wi-Fi is provided free of charge in the Windsor Conference Centre and a free wired connection is also available in your room (although you will have to provide your own laptop and only a limited number of Ethernet cables may be available).

To connect to the Wi-Fi

Open Internet Browser

There are 2 options – choose the 'Guest User' option

Terms of Use - confirm acceptance

Enter First Name and Password

First Name: batten

Password: 032012

There is a 60 second count down then you'll be asked to close down and open the browser again. You will only have to complete this registration process the first time, unless you don't use the internet again for 12 hours and then you will have to complete the process again.

To access the network via an Ethernet connection

The same username and password given above will be required if accessing the internet in the bedrooms (although you require an Ethernet cable, available at Reception) and the registration will also be required again.

Social media

We encourage the use of social media to keep those who cannot attend in touch with what is happening over the whole conference. All we ask is that you respect the confidentiality of the details of data presented and discussed before their publication in scientific journals.

Twitter: @ncl2012: #ncl2012, #battendisease

Facebook: NCL 2012

Blogs/diaries:

Perhaps you might consider personal diaries or blogging throughout NCL2012 recording thoughts such as 'What am I hoping for from today? What was good about today? What was difficult? What could have been better? What was unexpected? Who am I glad that I met today? What did I learn?'

Sara Mole and Andrea West (BDFA) would very much appreciate access to these for post-meeting evaluation (in any format), and would be pleased to approve you as one of the authors on **Tumblr:** **http://ncl2012.tumblr.com**, or to follow your own blog.

Post-meeting publications

There will be a series of reviews published in the scientific journal *Biochimica et Biophysica Acta* and many of the Patient Organisations will issue their own 'Perspectives' in their bulletins and newsletters.

NCL2012 Scientific Programme Overview

Wednesday 28th March

14.00-19.00	**Registration**
17.00-18.00	**Welcome and Opening Lectures**
18.00	***Opening Reception followed by Evening Meal***

Thursday 29th March

09.00-12.00	**Session 1 Genetics & Biology of the NCLs** Chairs: Sara Mole (UCL), Thomas Braulke (Hamburg)
12.00-13.00	***Poster Session 1***
13.00-14.00	***Lunch***
14.00-17.00	**Session 2 Disease Mechanisms** Chairs: David Pearce (Sanford Research), David Palmer (Lincoln, NZ)
17.00-18.00	***Poster Session 2***
18.00-18.30	**Science Summary Sessions for Parents**
18.45	***Evening Meal followed by 'Pub Social'***

Friday 30th March

08.30-10.20	**Session 3 Links to Other Diseases** Chairs: Fran Platt (Oxford), Steve Walkley (AECOM, New York)
10.20-10.40	***Break***
10.40-12.00	**Session 4 Recent Research Findings** Chairs: Anu Jalanko (Helsinki), Hannah Mitchison (UCL)
12.00-12.50	***Poster Session 3***
13.00 prompt	***Conference Photograph***
13.10-14.00	***Lunch***
14.00-17.00	**Session 5 New Clinical Perspectives** Chairs: Angela Schulz (Hamburg), Jon Mink (Rochester)
17.00-18.00	***Poster Session 4***
18.00-18.30	**Science Summary Sessions for Parents**
18.45	***Evening Meal together with Patients Organisation Conference***

Saturday 31st March

09.00-12.00	**Session 6 Experimental Therapies** Chairs: Beverley Davidson (Iowa), Mark Sands (Wash U)
12.00-13.00	***Poster Session 5***
13.00-14.00	***Lunch***
14.00-17.00	**Session 7 Joint Session with Patients Organisations Meeting** Session Chairs: Andrea West (BDFA), Sara Mole (UCL), Jon Cooper (KCL), Ruth Williams (Evelina Children's Hospital)
15.15-16.30	**'Market Place'**
16.30-17.30	***Poster Session 6***
18.30	***Conference 'Banquet' for Scientists and Parents***

NCL2012 Scientific Programme

All talks to be held in the main auditorium, Windsor Building.

Wednesday 28th March

14.00-19.00		**Registration**
17.00-18.00		**Welcome and Opening Lectures** Jon Cooper, Sara Mole, Ruth Williams, Andrea West
		Perspectives on NCL disease. *Ruth Willliams (Evelina Children's Hospital)*
		Perspectives on NCL genetics. *Sara Mole (UCL)*
		Perspectives on disease mechanisms and therapies. *Jon Cooper (KCL)*
18.00-19.00		**Opening Reception** (Picture Gallery, Founder's Building)
19.00		**Evening Meal** (Founder's Dining Room, Founder's Building)

Thursday 29th March

09.00-12.00		**Session 1 Genetics & Biology of the NCLs** Chairs: Sara Mole (UCL), Thomas Braulke (Hamburg)
09.00-09.10		Introduction by session chairs
09.10-09.30	O1	Batten disease: A case of retrograde endosome-Golgi protein transport gone bad? *Jeff Gerst (Weizmann Institute)*
09.30-09.50	O2	Identification of potential biomarkers and modifier genes affecting the clinical course of CLN3 Disease. *Georgia Makrypidi (Hamburg)*
09.50-10.00	O3	Brain endothelial cells deficient in CLN3 display abnormal phenotypes associated with alterations in membrane microdomains. *Luis Tecedor (University of Iowa)*
10.00-10.20	O4	CLN3 interacts with motor protein complexes and modifies location of late endosomal/lysosomal compartments. *Kristiina Uusi-Rauva (Helsinki)*
10.20-10.30	O5	Defining the role of mRNA degradation in Batten disease. *Jake Miller (Sanford Research)*
10.30-10.50		***Break***
10.50-11.10	O6	A novel CRMP2/CLN6/KLC4 signaling complex and its role in vLINCL. *Jill Weimer (Sanford Research)*
11.10-11.20	O7	Next generation sequencing identifies the disease causing mutation for NCL in South Hampshire sheep. *Imke Tammen (Sydney)*
11.20-11.35	O8	Proteolytic cleavage of the lysosomal membrane protein CLN7 is associated with CLN7 disease. *Pieter Steenhuis (Hamburg)*

11.35-11.50	O9	Mutations in DNAJC5, encoding cysteine-string protein alpha (CSPα), cause autosomal dominant adult neuronal ceroid lipofuscinosis. *Stan Kmoch (Prague)*
11.50-12.00	O10	A recurrent mutation in DNAJC5 causes autosomal dominant Kufs Disease. *Maxime Cadieux-Dion (Montreal)*
12.00-13.00		***Poster Session 1***
13.00-14.00		**Lunch** (Windsor Building)
14.00-17.00		**Session 2 Disease Mechanisms** Chairs: David Pearce (Sanford Research), David Palmer (Lincoln)
14.00-14.10		Introduction by session chairs
14.10-14.25	O11	Mutant glia impair the health of neurons in Juvenile NCL. *Lotta Parviainen (KCL)*
14.25-14.35	O12	Effects of CLN3 loss on inflammasome activation in microglia. *Tammy Kielian* (*University of Nebraska)*
14.35-14.50	O13	Delayed pathological changes in the thalamocortical system of immunodeficient *Ppt1* null mutant mice. *Thomas Kühl (KCL)*
14.50-15.05	O14	Synaptic dysfunction in motor nerve terminals of knock-out mice lacking Cysteine String Protein-alpha, a protein involved in autosomal-dominant adult-onset neuronal ceroid lipofuscinosis. *Rafael Fernández-Chacón (Sevilla)*
15.05-15.15	O15	Synaptic failure may initiate the neuronal degeneration in cathepsin D deficiency. *Maciej Lalowski (Helsinki)*
15.15-15.25	O16	Early synaptic abnormalities in multiple models of NCL. *Megan O'Hare (KCL)*
15.25-15.45		***Break***
15.45-16.00	O17	Evidence for altered neurogenesis in mouse and sheep models of NCL – an attempt of self-repair? *Sybille Dihanich (KCL/UCL)*
16.00-16.15	O18	*In vivo* intercellular correction in ovine CLN6. *Lucy Barry (Lincoln)*
16.15-16.30	O19	Spatial proteomics identify a novel drug target in JNCL. *Mika Ruonala (Frankfurt)*
16.30-16.40	O20	Early-stage neurologic and non-neurologic abnormalities in $Cln3^{\Delta ex7/8}$ mice precede overt neurodegeneration. *Sue Cotman (MGH Boston)*
16.40-16.50	O21	Autophagy dysfunction in NCL. *Matt Micsenyi (AECOM, New York)*
16.50-17.00	O22	Shared pathological themes between the NCLs and other LSDs. *Sarah Pressey (KCL/UCL)*
17.00-18.00		***Poster Session 2***
18.00-18.30		**Science Summary Sessions for Parents** (Room 1-04)
18.45		**Evening Meal** (HUB Dining Hall) **followed by 'Pub Social'** (Medicine Bar)

Friday 30th March

08.30-10.20		**Session 3 Links to Other Diseases** Chairs: Fran Platt (Oxford), Steve Walkley (AECOM, New York)
08.30-08.40		Introduction by session chairs
08.40-09.05	O23	Modulation of cellular clearance in lysosomal storage diseases. *Andrea Ballabio (TIGEM, Naples & Baylor COM)*
09.05-09.30	O24	Autophagy failure in Alzheimer's Disease and related diseases. *Ralph Nixon (NYU Langone Medical Center/Nathan Kline Institute)*
09.30-09.55	O25	Lysosomal Ca^{2+} homeostasis: role in pathogenesis of lysosomal storage diseases. *Emyr Lloyd Evans (Cardiff)*
9.55-10.20	O26	Astrocytes as neuronal energy providers: putative therapeutic targets in the NCLs? *Luc Pellerin (Lausanne)*
10.20-10.40		***Break***
10.40-12.00		**Session 4 Recent Research Findings** Chairs: Anu Jalanko (Helsinki), Hannah Mitchison (UCL)
10.40-10.45		Introduction by session chairs
10.45-10.55	O27	A novel genetic link between neuronal ceroid lipofuscinosis and the ubiquitin-proteasome system. *John Staropoli (Mass Gen Hospital)*
10.55-11.05	O28	Mutations in the gene encoding Cathepsin F are a cause of type B Kufs disease. *Katherine Smith (Walter and Eliza Hall Institute of Medical Research)*
11.05-11.15	O29	Homozygous mutations in progranulin can cause adult onset recessive NCL. *Katherine Smith (Walter and Eliza Hall Institute of Medical Research)*
11.15-11.25	O30	Exome sequencing reveals ATP13A2 mutations underlying juvenile NCL. *Jose Bras (UCL)*
11.25-11.35	O31	Clinico-pathological features of Kufs disease due to *CLN6* mutation. *Samuel Berkovic (Melbourne)*
11.35-11.45	O32	Age-dependent therapeutic effect of memantine in a mouse model of juvenile Batten disease. *David Pearce (Sanford Research)*
11.45-12.00		Discussion of the implications of recent genetic findings
12.00-12.50		***Poster Session 3***
13.00 prompt		***Conference Photograph***
13.10-14.00		***Lunch*** (Windsor Building)
14.00-17.00		**Session 5 New Clinical Perspectives** Chairs: Angela Schulz (Hamburg), Jon Mink (Rochester)
14.00-14.10		Introduction by session chairs

14.10-14.30	O33	Sex Differences in clinical progression and quality of life in juvenile neuronal ceroid lipofuscinosis. *Heather Adams* (*U. of Rochester)*
14.30-14.50	O34	Multidimensional Clinical Assessment Tool for late infantile CLN2 Disease. *Ruth Williams (Evelina Children's Hospital, London)*
14.50-15.10	O35	Quantitative Brain Volumetric Analysis in Neuronal Ceroid Lipofuscinoses: A tool to precisely monitor disease progression. *Angela Schulz (Hamburg)*
15.10-15.20	O36	Psychopathology in CLN3 disease: Correlation with disease progression and quality of life. *Angela Schulz (Hamburg)*
15.20-15.50		***Break***
15.50-16.10	O37	Sinus node dysfunction in juvenile neuronal ceroid lipofuscinosis. *John Østergaard (Aarhus University Hospital)*
16.10-16.30	O38	Lifelong learning for individuals with Batten disease. *Bengt Elmerskog (Tambartun National Resource Centre)*
16.30-16.40	O39	Mycophenolate mofetil for the treatment of Juvenile Ceroid Lipofuscinosis. *Erika Augustine (University of Rochester)*
16.40-16.50	O40	Biomarker discovery in Batten disease. *Chun-hung Chan (Sanford Research)*
16.50-17.00	O41	Magnetic resonance volumetrics, diffusion tensor imaging and spectroscopy as biomarkers to assess efficacy of gene therapy in a canine model for LINCL. *Fred Wininger (University of Missouri)*
17.00-18.00		***Poster Session 4***
18.00-18.30		**Science Summary Sessions for Parents** (Room 1-04)
18.45		**Evening Meal together with Patients Organisation Conference** (Founder's Dining Room, Founder's Building)

Saturday 31st March

09.00-12.00		**Session 6 Experimental Therapies** Chairs: Beverly Davidson (Iowa), Mark Sands (Wash U)
09.00-09.10		Introduction by session chairs
09.10-09.30	O42	The synergistic effects of CNS-directed gene therapy and bone marrow transplantation for infantile neuronal ceroid lipofuscinosis. *Mark Sands (Wash U)*
09.30-09.50	O43	A small molecule anti-inflammatory enhances the therapeutic effects of AAV-mediated CNS-directed gene therapy for infantile neuronal ceroid lipofuscinosis. *Charles Shyng (Wash U)*
09.50-10.00	O44	Intravenous high-dose enzyme replacement therapy with recombinant palmitoyl-protein thioesterase reduces brain and visceral lysosomal storage in a mouse model of infantile neuronal ceroid lipofuscinosis. *Sandy Hofmann (UT SouthWestern)*

10.00-10.10	O45	Treatment with recombinant human tripeptidyl peptidase-1 (rhTPP1) delays onset of neurologic signs in a canine model of late infantile neuronal ceroid lipofuscinosis (LINCL). *Christine Sibigtroth (University of Missouri)*
10.10-10.30	O46	Nonclinical development of recombinant human Tripeptidyl peptidase 1 (rhTPP1) enzyme replacement therapy (ERT) for Late Infantile Neuronal Ceroid Lipofuscinosis (LINCL). *Brian Vuillemenot (BioMarin)*
10.30-10.50		***Break***
10.50-11.10	O47	AAV-TPP1 transduction of brain ependyma in TPP1-null dogs results in widespread CNS distribution of TPP1 enzyme and improves NCL disease phenotypes. *Beverly Davidson (Iowa)*
11.10-11.30	O48	Gene delivery to the perinatal brain. *Andrew Wong (KCL)*
11.30-11.50	O49	Global CNS gene delivery platform in non-human primates utilizing self-complementary AAV9 vectors. *Steven Gray (UNC)*
11.50-12.00	O50	Post transplantation fate of human neural stem cells in a mouse model of late infantile NCL. *Helen Brooks (KCL)*
12.00-13.00		***Poster Session 5***
13.00-14.00		***Lunch*** (Windsor Building)

14.00-17.00		**Session 7 Joint Session with Patients Organisations Meeting** Session Chairs: Andrea West (BDFA), Sara Mole (UCL), Jon Cooper (KCL), Ruth Williams (Evelina Children's Hospital)
14.00-14.10		Introduction by session chairs
14.10-14.40		Summary of Scientific Sessions by respective Chairs
14.40-14.45		Patient Group Presentation. *Lance Johnston & Irena Newcombe*
14.45-15.00		Prizes and thanks. Announcement of next NCL congress.
15.00-15.15		***Break, with refreshments available during the Market Place***
15.15-16.30		**'Market Place'**
16.30-17.30		***Poster Session 6***
18.30		**Conference 'Banquet' for Scientists and Families** (Founder's Dining Room, Founder's Building)

Microphone carriers
Welcome Session - Sophia kleine Holthaus, Davide Marotta
Session 1 Genetics and Biology - Sophia kleine Holthaus, Francesco Pezzini
Session 2 Disease Mechanisms - Davide Marotta, Katja Kanninen
Session 3 Links to Other Diseases - Christine Sibigtroth, Janos Groh
Session 4 Recent Research Findings - Heidi Larkin, Pieter Steenhuis
Session 5 New Clinical Perspectives - Mervi Kuronen, Matthew Micsenyi
Session 6 Experimental Therapies - Niv Dobzinski, Uma Chandrachud
Session 7 Joint Session - Helen Brooks, Yewande Pearse

NCL2012 Satellite Programme Overview

At various times around or during NCL2012 there will be additional sessions focused on specific topics of interest for particular groups of conference attendees. Some are designed for clinicians or scientists, or particular patient organisations or groups of parents. Many relate specifically to the different work packages of DEM-CHILD, an EU funded collaborative grant, and are thus relevant only to those who are part of this effort. Others are open to those with expertise or interest in the respective area. Some occur in parallel to the main programme. The person responsible is indicated in italics, and additional details for some can be found in the following pages.

Breakfast packs will be provided for those starting at 07.30.

All meetings will be held in the main conference building. If the room for each event is not indicated in the programme below, it will be indicated at the conference information desk prior to its start.

Wednesday 28th March

10.00-12.30	First NCL Mini-Master class: 'Motor disorders in CLN3 disease (Room 0-04) *Ruth Williams (further details provided on page 19)*
10.00-12.00	JNCL PhD Student Meeting (Room 1-02) *Frank Stehr/Danielle Kerkovich*
12.00-16.30	First Meeting of the Batten Disease International Alliance (BDIA) (Room 1-05) *Tony Heffernan/Andrea West*
14.00-16.30	DEM-CHILD WP03 meeting (Epidemiology / Natural history study) + Outcomes and Registry (Room 0-04) *Angela Schulz*

Thursday 29th March

07.30-08.30	NCL Clinical Outcomes Conference (Sept 2012) Planning Session (Room 1-02) *Heather Adams*
10.30-16.00	BDFA Training day for Professionals (Room 1-05) *Andrea West/Ruth Williams (further details provided on page 19)*
17.00-18.00	DEM-CHILD WP01 meeting (Diagnostic gene chip) (Room 0-04) *Angela Schulz*

Friday 30th March

07.30-08.30	DEM-CHILD WP08 (Teaching and dissemination) (Room 0-04) *Prathiba Singhi/Angela Schulz*
17.00-18.00	DEM-CHILD WP07 meeting (Identification of new NCL genes) (Room 0-04) *Sara Mole*
17.30-18.00	International Rare NCL Gene Consortium joins DEM-CHILD WP07 at 17.45 (Room 0-04) *Sara Mole*

Saturday 31st March

07.30-08.30	DEM-CHILD WP06 (Innovative therapies for NCLs caused by mutations in transmembrane proteins) (Room 0-04) *Sander Smith*
16.00-17.00	BDFA UK Professional Networking Event (Room 0-04) *Andrea West*
17.00-18.00	DEM-CHILD WP02 meeting (Automated enzyme testing) (Room 1-04) *Herbert Korall and Susanne Kerlin*
17.00-18.00	Combined DEM-CHILD WP04 and WP05 meetings (CLN1 and CLN3 biomarkers) (Auditorium) *Thomas Braulke and Anu Jalanko*

Sunday 1st April

09.30-15.30	DEM-CHILD Steering group meeting (Moore building) *Angela Schulz*

NCL2012 Satellite Meetings Details

1st International NCL Mini-Master Class: Movement disorders in CLN3 disease

Wednesday 28th March, Room 0.04, 10.00-12.30
Introduction and welcome

Overview of treatment strategies for Parkinsonian Movement disorders in Childhood
Dr Jean-Pierre Lin and Dr Jonathan Mink

Cases and videos – participants

Questions for discussion

- How should movement disorder in CLN3 disease be described?
- What neuronal pathways are likely to be involved?
- Disease mechanisms?
- Optimum treatment strategies?
- Which drugs? Timing of treatment? How should we monitor treatment?

Conclusions and summing up

BDFA Training Day for Professionals caring for children and teenagers with Juvenile Batten disease

Thursday 29th March Room 1.05, 10.30-16.00
This course is suitable for teachers, nurses, therapists, social workers and all professionals involved in the care of children and teenagers with Juvenile Batten disease.

10.30-10.40	Introductions and welcome
10.40-11.00	Introduction to Juvenile onset Batten disease (CLN3 disease) *Ruth Williams*
11.00-11.45	Educational issues – Overview then Q&A session *Barbara Cole*
11.45-12.00	***Break***
12.00-12.50	What are seizures and how are they treated? – Overview, demonstration of emergency seizure management, Q&A session *Ruth Williams*
12.50-13.00	How the BDFA can help *BDFA Family Support Officer*
13.00-14.00	***Lunch***
14.00-15.00	Talking to the children and other family members *Melinda Edwards*
15.00-15.15	***Break***
15.15-16.00	Preparation for the future – maintaining skills and well-being *Sarah Kenrick and team*
16.00	Thanks and close of meeting

Patient Organisations Programme

Families have a dedicated room in the Windsor Conference Centre throughout the conference in **Room 0-05.**

Friday 30th March

10.00		Welcome (Room 1-04) *Pauline Docherty and Heather Band*
10.10-10.30		Update on first official meeting of BDIA (Room 1-04) *Tony Heffernan*
10.30		***Break for coffee***
10.45-12.00		Discussion groups (Room 1-02/1-03)
12.00-12.50		***Poster Session 3***
13.00 prompt		***Conference Photograph***
13.10-14.00		***Lunch*** (Windsor Building)
14.00-17.00		Networking (Room 1-02/1-03) **OR**
		Session 5 New Clinical Perspectives (Auditorium)
17.00-18.00		***Poster Session 4***
18.00-18.30		**Science Summary Sessions for Parents** (Room 1-04)
18.45		**Evening Meal together with Scientific Conference** (Founder's Dining Room, Founder's Building)

Saturday 31st March

09.00-12.00		**Session 6 Experimental Therapies (Auditorium)** Chairs: Beverly Davidson (Iowa), Mark Sands (Wash U)
09.00-09.10		Introduction by session chairs
09.10-09.30	O42	The synergistic effects of CNS-directed gene therapy and bone marrow transplantation for infantile neuronal ceroid lipofuscinosis. *Mark Sands (Wash U)*
09.30-09.50	O43	A small molecule anti-inflammatory enhances the therapeutic effects of AAV-mediated CNS-directed gene therapy for infantile neuronal ceroid lipofuscinosis. *Charles Shyng (Wash U)*
09.50-10.00	O44	Intravenous high-dose enzyme replacement therapy with recombinant palmitoyl-protein thioesterase reduces brain and visceral lysosomal storage in a mouse model of infantile neuronal ceroid lipofuscinosis. *Sandy Hofmann (UT SouthWestern)*
10.00-10.10	O45	Treatment with recombinant human tripeptidyl peptidase-1 (rhTPP1) delays onset of neurologic signs in a canine model of late infantile neuronal ceroid liposfuscinosis (LINCL). *Christine Sibigtroth (University of Missouri)*

10.10-10.30	O46	Nonclinical development of recombinant human Tripeptidyl peptidase 1 (rhTPP1) enzyme replacement therapy (ERT) for Late Infantile Neuronal Ceroid Lipofuscinosis (LINCL). *Brian Vuillemenot (BioMarin)*
10.30-10.50		***Break***
10.50-11.10	O47	AAV-TPP1 Transduction of Brain Ependyma in TPP1-null dogs results in widespread CNS distribution of TPP1 enzyme and improves NCL disease phenotypes. *Beverly Davidson (Iowa)*
11.10-11.30	O48	Gene delivery to the perinatal brain. *Andrew Wong (KCL)*
11.30-11.50	O49	Global CNS gene delivery platform in non-human primates utilizing self-complementary AAV9 vectors. *Steven Gray (UNC)*
11.50-12.00	O50	Post transplantation fate of human neural stem cells in a mouse model of late infantile NCL. *Helen Brooks (KCL)*
12.00-13.00		***Poster Session 5***
13.00-14.00		***Lunch*** (Windsor Building)

14.00-17.00	**Session 7 Joint Session with Patient Organisations Meeting (Auditorium)** Session Chairs: Andrea West (BDFA), Sara Mole (UCL), Jon Cooper (KCL), Ruth Williams (Evelina Children's Hospital)
14.00-14.10	Introduction by session chairs
14.10-14.40	Summary of Scientific Sessions by respective Chairs
14.40-14.45	Patient Organisation Group Presentation *Irena Newcombe and Lance Johnston*
14.45-15.00	Prizes and thanks. Announcement of next NCL congress.
15.00-15.15	***Break, with refreshments available throughout the Market Place***
15.15-16.30	**'Market Place' (Rooms 1-02 to 1-05)**
16.30-17.30	***Poster Session 6***
18.30	**Conference 'Banquet' for Scientists and Families** (Founder's Dining Room, Founder's Building)

Science Summary Sessions 1 & 2: Genetics and Biology; Disease Mechanisms

Chair Lucy Barry, Mariana Vieira, Kim Wager, Megan O'Hare, Andrew Wong, Thomas Kuhle, Lotta Parviainen, Matthew Micsenyi, Mervi Kuronen, Heather Band, Irena Newcombe

Science Summary Sessions 3, 4 & 5 Links to other diseases; Recent Research Findings; New Clinical perpsectives:

Chair Richard Tuxworth, Sarah Pressey, Sybille Dihanich, Katherine R Smith, Katja Kanninen, Nicole Neverman, Charles Shyng, Nadia Mitchell, John Staropoli, Heather Adams, Heather Band, Irena Newcombe

Market Place

Session Facilitators: Hannah Mitchison, Claire Russell

This informal event will allow participants to move between the various 'stalls' and chat to as many groups as they can for about 10 mins each (a bell will ring to mark the time), and there will be refreshments available throughout the hour. The aim of the Market Place is to provide a new way for all at the meeting to find out more about different topics, network, and will lead nicely into the final Poster Session.

Room 1-02

1. **How clinical trials work** – Paul Gissen, Sander Smith, Erika Augustine
2. **Therapy suitable for NCL** – Beverley Davidson, Mark Sands, Andrew Wong
3. **NCL Experts** - Session chairs – Thomas Braulke, Steve Walkley, Anu Jalanko, David Pearce

Room 1-03

4. **How science works**, **How scientists get funding, the Scientific career** –David Palmer, Jill Weimer, Mika Ruonala, Richard Tuxworth
5. **How industry R&D works** – BioMarin - Andy Tincu, Mike Vellard, Brian Vuillemenot, Terence Eagleton

Room 1-04

6. **Clinical discussions** – Ruth Williams, Alessandro Simonati, Alfried Kohlschutter, Heather Adams, John Østergaard, Jonathan Mink, Sarah Kenrick, Amy Vierhile
7. **How is NCL diagnosed**? –Angela Schulz, Nick Lench, Simon Heales, Glenn Anderson, John Staropoli

Room 1-05

8. **Togetherforshortlives** - Disclosure, Hospice - Jane Houghton
9. **Palliative care** – Esther Corker, Toni Wolff, Jane Williams, Katherine Martin
10. **Helen and Douglas House** – Respite care, symptom control assessment and management, transition support, step discharge from hospital and end of life care. From birth to 35 – Rachel Griffith
11. **How to best help children in terms of school and education?** – Barbara Cole, Bengt Elmerskog, Per Fosse, Carrie Mannion

Atrium

12. **Oxford University Press** – Batten disease book – available to browse and purchase

Presentations

Every abstract submitted to NCL2012 is being presented either orally or as a poster. The subjects covered are wide-ranging and include scientific, clinical, educational and family interests. All abstracts were scored by the scientific organising committee solely on the basis of scientific merit, and those scoring the highest were selected as talks. The order and length of talks, in each session was decided by the session chairs. No talks are long, to maximise the number of oral presentations. **All presenters should load their talks onto the computer before the start of the session**, with both PC and Mac available.

Please note that you can enter the main auditorium at both ground floor, and upper (first floor) levels, but **if the session has already started, please try to enter the back of the auditorium via the upper level**. This is because the ground floor entrance is very close to the podium and having people file in during the session is likely to be disruptive.

At the beginning of each session the session chairs will provide an overview so that subsequent speakers can jump straight into their data, referring only briefly to any background necessary to understand the significance of the results presented.

Each presenter will provide a summary slide to be used by session chairs in the final session of NCL2012 (Session 7) where we review the meeting, and each talk will have a slide that summarises the significance of the data in lay terms for those who are not expert in that field.

The appendices at the end of the abstract book summarise useful data on the NCLs to act as a reference during and after the meeting.

Prizes

Prizes will be awarded in the final session for the following categories:

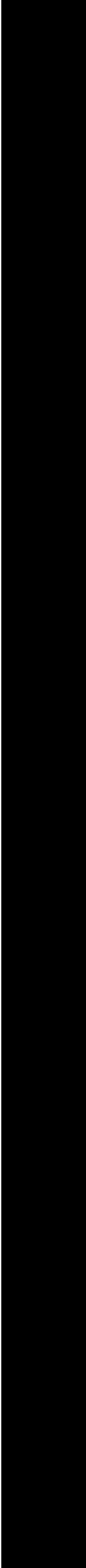
Oral: Best student presentation, Best non-student 'young investigator' presentation, Best clinical presentation

Poster: Best student poster, Best non-student 'young investigator' poster, Best clinical poster and Best Patient Organisation poster.

The **NCL2012 Oral Presentation Review Committee** consists of all Session Chairs: Hannah Mitchison (UCL, London, Chair), Sara Mole (UCL, London), Thomas Braulke (Hamburg), David Palmer (Lincoln, NZ), David Pearce (Sanford Research, USA), Fran Platt (Oxford), Steve Walkley (AECOM, USA), Anu Jalanko (Helsinki), Angela Schulz (Hamburg), Jonathan Mink (Rochester, USA), Beverly Davidson (Iowa, USA), Mark Sands (St Louis, USA), Andrea West (BDFA), Ruth Williams (Evelina Hospital, London), Jon Cooper (KCL, London).

The **NCL2012 Poster Review Committee** consists of Guy Tear (KCL London, Chair), Inés Noher de Halac (Cordoba, Argentina), Imke Tammen (Sydney, Australia), Alfried Kohlschütter (Hamburg, Germany), Brenda Williams (KCL, London), Stephanie Hughes (Dunedin, NZ), Susan Cotman (Boston, USA), Jill Weimer (Sanford Research, USA), Heather Adams (Rochester, USA), Claire Russell (RVC, London), Stephan Storch (Hamburg), Heather Band (BDFA, UK).

Welcome Presentations



Perspectives on NCL disease

Ruth E Williams

Evelina Children's Hospital, Guy's and St Thomas' NHS Foundation Trust, London

I attended my first NCL Congress almost exactly 20 years ago in Hamburg. The first linkage studies were just producing results; the late infantile and early juvenile NCLs had only recently been described; prenatal diagnosis had been attempted in a very small number of families but at a relatively late stage of pregnancy. Then, diagnosis was usually made following rectal or conjunctival biopsy, invasive procedures performed under general anaesthetic, with a long wait for the results. There were discussions regarding the nature and tissue distribution of the storage material, diagnostic histopathology and urine biochemical testing. Supportive clinical management was limited to the use of basic anti-epileptic drugs and nutritional support using gastrostomy tubes was controversial. Anti-oxidants were being investigated as potential disease modifying agents. There was little if any understanding of disease mechanisms. We had a few naturally occurring large animal models, but no prospect of curative treatment in the near future. The atmosphere of the meeting was positive and hopeful.

During this week, twenty years on, we will hear about scientific progress, and increase our understanding of the basic cellular mechanisms that are disrupted in Batten disease. On Friday and Saturday we will learn how some of these scientific advances will be translated into treatments that may alter the relentless neurological decline of the NCL diseases, and the hope that some will be able to reverse the pathology leading to optimism for a cure ' in our time'.

In parallel with huge gains in the scientific NCL world in the last 20 years, there have been profound changes in attitudes to and in the medical care of children with complex neuro-disability and life-limiting neurological conditions. Since 1990, 11 new anti-epileptic drugs have been licensed for use in the UK and gastrostomy placement has become routine in this group of children and young people. Aggressive management of chest and gut complications, improved family support, genetic counselling, family planning choices, symptom control and discussions around choices of treatment and end of life care have become standard. Medical care is practised in partnership with families and parents. Services are increasingly being planned and developed in close collaboration with service users and stakeholders.

We are all here with the ultimate goal of improving Life with Batten Disease. We are powerfully reminded of this goal by the faces and stories of the children and young people directly affected by Batten disease. We are helped in our task by our colleagues past, present and future.

We have several parallel clinical sessions and satellite meetings this week. Our hope is that through opportunities for interaction between scientists, clinicians, families, charities and other agencies, we will become even more aware of the roles and contributions of others, so that all possible routes towards our goal are optimised.

Perspectives on NCL genetics: genes, mutations and genotype-phenotype correlations

Sara E. Mole[1, 2, 3]

[1]MRC Laboratory for Molecular Cell Biology, [2]Molecular Medicine Unit, UCL Institute of Child Health and [3]Department of Genetics, Evolution and Environment, University College of London, London WC1E 6BT, UK

Since the first genes causing NCL were identified in 1995, nearly 400 mutations in thirteen genes have been described that cause NCL disease in families, some of which will be reported for the first time at NCL2012. These genes are *CLN1/PPT1, CLN2/TPP1, CLN3, CLN4/DNAJC5, CLN5, CLN6, CLN7/MFSD8, CLN8, CLN10/CTSD, CLN13/CTSF*, with mutations in *CLN11/GRN*, *CLN12/ATP13A2* and *CLN14/KCTD7* recently described in single families. A further eight genes are reported to cause NCL-like disease in animals (*PPT2, CLCN3, CLCN6, CLCN7, OSTM1, CTSB+L, ARSG*). These genes and their mutations are listed in the NCL Mutation Database (http://www.ucl.ac.uk/ncl). All identified NCL genes lie on autosomes. There is a characteristic disease phenotype known for most NCL genes that is associated with complete loss of gene function, and in most cases so-called 'milder' mutations, where some gene function remains, cause disease that is more protracted or of later onset. Similar disease, notably causing the late infantile NCLs, can arise from loss-of-function mutations in several different genes, (*CLN2, CLN5, CLN6, CLN7, CLN8*), as well as milder mutations in others (*CLN1* and *CLN10*). The genetic basis of cases diagnosed with adult or teenage onset NCL is gradually being revealed, with some cases carrying mild mutations in genes that usually cause NCL in childhood (*CLN1, CLN5, CLN6, CLN10*), and others in genes so far known to cause onset only in adulthood (*CLN13, CLN4*). There are several mutations in NCL genes that cause distinct disease phenotypes, such as progressive epilepsy with mental retardation (EPMR) (missense mutation p.Arg24Gly in *CLN8*), Kufs type A disease (several mutations in *CLN6*), autosomal dominant Parry disease (two mutations affecting adjacent Lys residues in *CLN4*), and even juvenile NCL (common 1 kb deletion in *CLN3*). Single mutations in one gene, *CLCN6*, have been found in two patients, one of which had adult onset and was later found to be compound heterozygous for mutations in *CLN5*, raising the possibility that this specific *CLCN6* allele may be modifying the disease phenotype. *CLN8* has been reported as a candidate modifier gene for type 1 Gaucher disease. Mutations in some recently identified NCL genes also cause other diseases (*CLN11*, *CLN12, CLN14*). Thus, our current understanding of the genetics of NCL now points towards biological consequences that may be shared with other diseases. There remain families diagnosed with NCL for whom the genetic basis remains elusive. It is hoped that recent and future advances in new DNA technologies will provide the means to identify these as yet unknown NCL genes. Finally, these advances in understanding the complexity of the genetic basis of the NCLs leads to a new nomenclature that is gene-based.

Perspectives on disease mechanisms and therapies

Jonathan D Cooper[1,2]*

[1]Pediatric Storage Disorders Laboratory and [2]Department of Neuroscience, Centre for the Cellular Basis of Behaviour, Institute of Psychiatry, King's College London, UK.

Despite the identification of many of the disease-causing genes, very little is known about the underlying disease mechanisms. This situation is compounded by not knowing the normal function of these gene products, or how this is compromised upon mutation. To address questions about disease mechanisms a series of models of different forms of NCL have been generated. These range from simple yeast, through Drosophila and zebrafish, a wide range of genetically modified mice, up to sheep, dog and other large animal models. There is also an increasing use of cellular models of disease, including primary cultures of neurons, glia and other cell types derived from animal models of NCL, patient derived fibroblasts, and induced pleuripotent stem cells, which can theoretically be differentiated unto a variety of human cell types.

Taken together, these different approaches have produced a wealth of new information about NCL pathogenesis. It is now apparent that although widespread at disease end stage, neuron loss is much more selective in its earlier stages. However, the distribution and timing of this neuron loss does not correlate directly with the build-up of storage material. Instead, the localised activation of different types of glia has proven to be the most accurate predictor of subsequent neuron vulnerability. Indeed, emerging evidence suggests the normal biology of glia is also compromised and may impact neuron health. New information about how neurons are affected is also available, with evidence for trafficking defects and pathological targeting of the pre-synaptic compartment.

These studies have not only provided new clues about disease mechanisms, but have also generated series of behavioural and pathological landmarks that can be used to judge the efficacy of a range of experimental therapeutic approaches. With no precise information about the key events that lie downstream of CLN3 mutation, it has not yet been possible to devise a mechanistically based therapy for CLN3 disease. Nevertheless, the effects of glutamate receptor antagonists and both genetic and pharmacological blockade of the autoimmune response have shown some benefit in *Cln3* deficient mice. On this basis a phase I trial of the immunosuppressant *CellSept* has recently been initiated at the University of Rochester, and its results will be awaited with interest.

For classic CLN1 and CLN2 diseases, where the cross correction of deficient neurons is possible, a range of different methods of delivering the missing enzyme have been attempted. Enzyme replacement therapy shows some promise, if the enzyme can access the CNS and neural stem cell (NSCs) transplants appear to provide a source of both PPT1 and TPP-I and produce modest therapeutic benefit. Although a phase I trial of NSCs was undertaken, a subsequent phase Ib trial was cancelled, and the future of this approach for the NCLs is currently unclear. Progress in gene transfer has been more promising, especially in LINCL where newer generations of vectors delivered early in disease progression have proved relatively successful in mouse models. Two phase I trials of intracranial gene therapy with different adenoassociated vectors have been initiated, and novel immunopanning method offers hope that systemically delivered vectors can access the brain to exert a therapeutic effect. INCL has proved a more difficult therapeutic target, but recent studies that combine treating the CNS, together with treating the somatic disease have proved much more effective (as will be presented at this meeting). The challenge with all of these experimental therapies will lie in scaling them up and successfully translating them into a clinical setting.

Oral presentations

Batten disease: A case of retrograde endosome-Golgi protein transport gone bad?

Rachel Kama, Niv Dobzinski, Osnat Zontag, Jeffrey E Gerst

Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 76100, Israel

Despite a myriad of published works, the intracellular localization and functions of CLN3 and its orthologs in other organisms remain unclear. Unfortunately, this conundrum has proven a hindrance for investigators attempting to understand the mechanism of Batten disease onset and to devise new therapeutic approaches/tools for use in its treatment. A key insight towards understanding this disease comes from the plethora of cellular defects that can be observed and quantitated in cells bearing debilitating mutations in CLN3 or its orthologs. This instance of pleiotropy is indicative of CLN3 acting as a central controlling element in a wide number of seemingly disconnected cellular processes.

Results from our laboratory using yeast as a Batten disease model suggest that yeast CLN3 (Btn1) controls the localization of multiple resident *trans* Golgi proteins that confer a variety of intracellular processes. We show Btn1 itself to be a *trans* Golgi protein that regulates an endosomal kinase, which controls SNARE assembly and protein transport into and out of the Golgi [Kama *et al*, 2011 *J. Cell Biol.*]. Defects in Btn1 function lead to a loss in t-SNARE phosphorylation, resulting in defects in SNARE disassembly, and the mislocalization of *trans* Golgi proteins to the late endosomal pathway, without recourse for retrieval. Thus, it may be that the leaching of *trans* Golgi proteins to later compartments disrupts their ability to carry out their specific functions, thus resulting in pleiotropic cellular effects.

In addition to Btn1, we have used yeast to define the functions of two other potential Batten disease-related proteins, Btn2 and Btn3. Like Btn1, Btn2 also acts upon late endosome (LE)-Golgi transport, but works in conjunction with SNAREs and retromer to directly mediate cargo protein retrieval [Kama *et al*, 2007 *Mol. Cell. Biol.*]. In contrast, we identified Btn3 as a Btn2-interacting protein and negative regulator of LE-Golgi transport [Kanneganti *et al*, 2011 *Mol. Biol. Cell*]. Btn3 competes for SNARE binding to Btn2 and, as a consequence relocalizes Btn2 to the cytosol. In addition, Btn3 also inhibits the ability of Btn2 to facilitate the purging of misfolded protein aggregates (*e.g.* prions) from yeast via the endosomal pathway.

By using yeast as a model for Batten disease we predict that the disease state in humans involves defects in endosomal processes, *e.g.* endosome-Golgi protein sorting, the elimination of misfolded protein aggregates, and in TOR pathway signaling to endosomes. These are all likely to occur by the loss of function of CLN3 and ongoing work seeks to verify these findings using mammalian cells.

Identification of potential biomarkers and modifier genes affecting the clinical course of CLN3 Disease

Georgia Makrypidi[1], Anne-Helene Lebrun[1], Sandra Pohl[1], Carolin Schmidtke[1], Sara Mole[2], Susan Cotman[3], Alfried Kohlschütter[1], Thomas Braulke[1], Angela Schulz[1]

[1]Department of Biochemistry, Children's Hospital, University Medical Center Hamburg-Eppendorf, Germany; [2]University College London, MRC Laboratory for Molecular Cell Biology, Molecular Medicine Unit, UCL Institute of Child Health and Department of Genetics, Evolution and Environment; [3]Massachusetts General Hospital, Center for Human Genetic Research

Juvenile neuronal ceroid lipofuscinosis (JNCL) is an incurable pediatric neurodegenerative disorder mostly caused by mutations in *CLN3* gene and characterized by visual loss, epilepsy and psychomotor deterioration. Although, the most common mutation in patients is a 1kb deletion, the disease phenotype appears to be variable.

In this study, we performed a detailed history analysis of 25 CLN3 patients homozygous for 1kb deletion using an established clinical scoring system and an index of relative disease severity classifying them into groups with slow, average and rapid disease progression.

Results: Using a comparative genome-wide expression analysis performed in eight CLN3 patients with different disease progression and matched controls, the clinical variability of the patients could be correlated with changes in gene expression profiles The data showed that five genes (*DUSP2, RGS1, PARP15, POLR2J2, CDC4SE2*) were dysregulated in all CLN3 patients independently of the disease progression which classifies them as potential biomarker genes. Of those, *DUSP2,* was also validated in acutely *CLN3* siRNA-depleted cells and in Cb*Cln3*$^{\Delta ex7/8}$ cerebellar precursor cells. Additionally, thirteen genes were found to be dysregulated in opposite directions in CLN3 patients with rapid versus slow progression of the disease. Among these potential modifier genes the changes in the *RAPGEF1* and *SPIB* mRNA expression were confirmed in *CLN3* siRNA-depleted cells.

Conclusions: These findings indicate that distinct signalling pathways involving e.g. *DUSP2, RAPGEF1* might affect the disease progression in CLN3 disease. Further studies are required to evaluate the role and specificity of the identified biomarker and modifier genes during the pathogenesis of the CLN3 disease.

Brain endothelial cells deficient in CLN3 display abnormal phenotypes associated with alterations in membrane microdomains

Luis Tecedor[1], Colleen S Stein[1], Mark L Schultz[4], Beverly L Davidson[1-4]

Departments of [1]Internal Medicine, [2]Molecular Physiology & Biophysics and [3]Neurology and the [4]Molecular and Cell Biology Program, University of Iowa, Iowa City, IA 52242

Juvenile neuronal ceroid lipofuscinosis (JNCL) is a childhood-onset neuro-degenerative disorder caused by mutations in CLN3, a protein of unresolved function. Using a *Cln3* reporter mouse, we previously showed that *Cln3* is expressed in endothelial cells throughout the brain vasculature. In the current investigation we generated mouse brain endothelial cell lines (MBEC) as an *in vitro* model to study endothelial cell functions.

Results: We found that CLN3 null MBEC display impaired clathrin-independent endocytosis and altered distribution of proteins that participate in endocytosis or vesicular trafficking. In addition, since clathrin-independent endocytosis is dependent on membrane microdomains, we assessed microdomain properties in live MBEC. We discovered that microdomains are abnormal with respect to size and fluidity.

Conclusions: We propose microdomain instability as an underlying cause of endothelial cell defects.

CLN3 interacts with motor protein complexes and modifies location of late endosomal/lysosomal compartments

Kristiina Uusi-Rauva[1], Aija Kyttälä[1], Rik van der Kant[2], Jouni Vesa[3], Kimmo Tanhuanpää[4] , Jacques Neefjes[2], Vesa M. Olkkonen[5], Anu Jalanko[1]

[1]*National Institute for Health and Welfare and FIMM, Institute for Molecular Medicine Finland, Biomedicum Helsinki, PO 104, 00251 Helsinki, Finland*
[2]*Division of Cell Biology, The Netherlands Cancer Institute, 1066CX Amsterdam, Netherlands*
[3]*Department of Human Genetics, David Geffen School of Medicine at UCLA, Gonda Neuroscience and Genetics Research Center, Los Angeles, California 90095-7088, USA*
[4]*Light Microscopy Unit, Institute of Biotechnology, University of Helsinki, PO 56, 00014 Helsinki, Finland*
[5]*Minerva Foundation Institute for Medical Research, Biomedicum Helsinki 2U, Tukholmankatu 8, 00290 Helsinki, Finland*

Juvenile onset neuronal ceroid lipofuscinosis (NCL) usually results from defects in CLN3, an endosomal/lysosomal transmembrane protein. CLN3 has been suggested to affect multiple cellular processes, including membrane trafficking in several intracellular compartments. Previously, the most common disease-associated mutation in *CLN3*, $CLN3^{\Delta ex7\text{-}8}$ (c.462-677del) has been associated with defects in endocytic pathway and abnormal localisation of endosomal/lysosomal compartments [Luiro et al., (2004) Hum Mol Genet 13 (23); Fossale et al., (2004) BMC Neurosci 5 (1)]. This study shows that also a protracted disease-causing mutant, $CLN3^{E295K}$, affects the properties of late endosomes. Expression of $CLN3^{E295K}$ mutant protein in HeLa cells led to perinuclear clustering of late endosomes/lysosomes as well as relocalisation of associated small GTPase Rab7. Lysosomal clustering was also apparent in patient fibroblasts harbouring homozygous $CLN3^{\Delta ex7\text{-}8}$ mutation or compound heterozygous mutation ($CLN3^{\Delta ex7\text{-}8}$/$CLN3^{E295K}$). Together with previously published data this study suggests that CLN3 may have a role in movement of late endosomal/lysosomal compartments via interactions with associated motor protein complexes. Subsequent interaction analyses revealed that CLN3 interacts with both dynein and late endosomal/lysosomal kinesin complexes. Most importantly, CLN3 was found to interact directly with GTP-bound Rab7 and with its effector, Rab7-interacting lysosomal protein RILP. Furthermore, Rab7 and RILP interactions were affected due to two above-mentioned mutations in CLN3. Further analysis with patient cells indicated that the functionality of Rab7 may be severely disturbed by CLN3 disease. Together, our data suggest that CLN3 is a novel Rab7 effector protein directly acting in antero and retrograde trafficking of late endosomal/lysosomal compartments and that defects in these functions have a role in pathogenesis of CLN3 disease.

Defining the role of mRNA degradation in Batten disease

Jake N Miller, Chun H Chan, David A Pearce

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The neuronal ceroid lipofuscinosis (NCLs) are a group of autosomal recessive neurodegenerative disorders of childhood. These disorders are characterized by the accumulation of autofluorescent storage material within lysosomes, and are considered lysosomal storage disorders. Currently, there are ten subtypes of NCL due to mutations in at least ten genes, eight of which have been characterized. Understanding the inherent mRNA degradation mechanisms affecting transcript expression in NCL subtypes is necessary for defining the extent of mutated protein expression within diseased cells. Here we present our research showing decreased expression of *CLN1* and *CLN3* mRNA in human INCL and JNCL lymphoblast cells, respectively. We hypothesize that the decreased mRNA expression levels are due to RNA degradation mechanisms such as nonsense-mediated degradation. The process of nonsense-mediated degradation involves recognition of a variety of mutations within the coding sequence of mRNA, subsequently leading to transcript degradation and the prevention of abnormal protein production.

Several different INCL and JNCL patient-derived lymphoblast cell lines were studied using relative quantification real-time PCR analysis of the *CLN1* and *CLN3* mRNA transcripts. We have found a significant decrease in the expression of several different mutant genotypes in both INCL and JNCL cells when compared to healthy controls.

This marked decrease in *CLN1* and *CLN3* mRNA expression in INCL and JNCL lymphoblast cells leads us to believe that RNA surveillance mechanisms are recognizing these mutant transcripts and causing mRNA degradation, thus leading to decreased mutant protein production within diseased cells.

A novel CRMP2/CLN6/KLC4 signaling complex and its role in vLINCL

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Mechanisms that orchestrate neurite specification, outgrowth, guidance, and maintenance remain at the forefront of our study of neuropediatric disease. CRMP2 is crucial for axon-dendritic specification and axonal extension in the developing brain and contributes to regeneration/degeneration in the mature brain. CRMP2's ability to specify axon/dendrite fate and regulate cargo transport during axonal growth has been shown to be facilitated and/or antagonized through a complex network of alternative protein-protein interactions, including kinesin-1 light chain, dynein, chimaerin, phospholipase D, calmodulin, L1/numb, neurofibromin 1 (NF1), CaV2.2, and, more recently, the neuronal ceroid lipofuscinosis protein, CLN6. Disruption in CRMP2 impedes axonal formation and promotes degeneration and has been associated with many neurological disorders. In this study, we focus on a novel CRMP2 complex with the neuronal ceroid lipofuscinosis (NCL) protein CLN6 and kinesin light chain 4 (KLC4), exploring if this complex provides a unique mechanism for localized CRMP2-dependent signaling and how its disruption facilitates a loss in many CRMP2-dependent processes. Specifically, we highlight how this novel CRMP2/KLC4/CLN6 complex might utilizes CLN6 as a "molecular tag" on a pool of ER-vesicles, allowing the complex to segregate cargo to specific locations in dendrites and axons. Aberrant modulation of this signaling complex could contribute to the pathogenesis of neuropsychiatric disorders, including the variant late infantile NCL associated with mutations in CLN6, through altered axonal/dendritic specification, outgrowth and maintenance. Moreover, we will explore potential therapeutic targets aimed at stabilizing the CRMP2 as a means of preventing and/or restoring neural circuits in vLINCL.

Next generation sequencing identifies the disease causing mutation for NCL in South Hampshire sheep

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The New Zealand South Hampshire sheep have been well characterised as an animal model for variant late-infantile neuronal ceroid lipofuscinosis (vLINCL) in humans. The disease-causing gene had been identified as *CLN6* by linkage analysis and gene expression studies, but the disease causing mutation remained unknown.

A sheep BAC containing *CLN6* and flanking genomic region was sequenced using the 454 sequencing method from Roche to generate a consensus ovine sequence for the region of interest, which served as a reference sequence for mutation screening. Fourteen long range PCRs covering 49kb of the region of interest were amplified in 3 affected and 5 control sheep, the amplicons were sequenced using the ABI SOLID sequencing platform and sequence variations analysed for compliance with the disease phenotype and predicted effect on protein function.

Results
A deletion covering the whole of exon 1 as well as parts of the 5'UTR and intron 1 was identified as the likely disease causing mutation. The deletion was confirmed by Sanger sequencing of an additional 7 animals. A DNA test is currently optimised for the screening of the South Hampshire research flock.

Conclusions
Identification of the disease causing mutation for NCL in South Hamsphire sheep strengthens the usefulness as a model and is a crucial step towards using this model in gene therapy trials. Further work needs to be done to understand gene function.

Proteolytic cleavage of the lysosomal membrane protein CLN7 is associated with CLN7 disease

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Introduction: CLN7 is the polytopic lysosomal membrane glycoprotein that is deficient in CLN7 disease, neuronal ceroid lipofuscinosis late infantile variant phenotype. Based on its subcellular localization and sequence similarities with members of the major facilitator superfamily of transporters, CLN7 is believed to be a lysosomal transporter, but its function is not known. We previously showed that lysosomal targeting of CLN7 is mediated by sorting signals in its cytosolic N- and C-terminal domains.

Results: By analyses of COS-7 cells expressing 3xFLAG-CLN7 we show that full-length CLN7 is proteolytically cleaved twice. The major cleavage event generates an N-terminal *N*-glycosylated 38 kDa fragment comprising 47.3% (± 7.1%, n=6) of cellular CLN7, while the minor cleavage event produces an N-terminal non-*N*-glycosylated 36 kDa fragment comprising 9.5% (± 4.3%, n=6) of cellular CLN7. Therefore, at steady state 56.8% of cellular CLN7 polypeptide is proteolytically cleaved. After removal of both used *N*-glycosylation sites the 38 kDa fragment is no longer present and the 36 kDa fragment comprises 33.9% (± 2.2%, n=6) of cellular CLN7 suggesting increased cleavage due to the absence of *N*-linked oligosaccharides. Elevation of lysosomal pH and retention of CLN7 in the endoplasmic reticulum or at the plasma membrane resulted in inhibition of proteolytic cleavage. In addition, cleavage could be blocked by cathepsin L inhibitors and was not observed in embryonic fibroblasts from cathepsin L-deficient mice. The apparent molecular masses of the cleaved fragments and expression analyses of C-terminally truncated CLN7 polypeptides suggested that both cleavage sites are located within the large lumenal loop L9. Cycloheximide pulse-chase experiments revealed that CLN7 is gradually cleaved and that full-length CLN7 is still present after chase periods of 24 and 48 hours. The known disease-causing mutations p.T294K and p.P412L, localized in lumenal loops L7 and L9 respectively, did not interfere with correct lysosomal targeting of CLN7 but enhanced its cathepsin L-mediated proteolytic cleavage in lysosomes. In contrast, mutations affecting transmembrane domains of CLN7 did not increase proteolytic cleavage of CLN7.

Conclusions: The similar clinical phenotype of paediatric CLN7 patients suggests that all *CLN7/MFSD8* mutations associated with the late infantile variant phenotype result in a complete loss of CLN7 function. Our data suggest that enhanced proteolysis of mutant CLN7 p.T294K and p.P412L contributes to CLN7 disease. Thus, our findings argue that CLN7 is inactivated by proteolytic cleavage and that enhanced CLN7 proteolysis caused by missense mutations in selected luminal loops is associated with disease. The finding that mutations affecting the sequence of transmembrane domains in CLN7 did not alter proteolytic cleavage indicates functional impairment of these mutant proteins by other mechanisms. The presence of distinct 36 kDa and 38 kDa CLN7 fragments, which have considerable stability in lysosomes, suggests that they do not represent degradation intermediates. Functional characterization and identification of the CLN7 substrates are required to determine the importance of proteolytic cleavage for the putative transporter activity of CLN7.

Mutations in *DNAJC5*, encoding cysteine-string protein alpha (CSPα), cause autosomal dominant adult neuronal ceroid lipofuscinosis.

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Autosomal dominant adult neuronal ceroid lipofuscinosis (ANCL) is characterized by accumulation of autofluorescent storage material in neural tissues and neurodegeneration with an age of onset in the third decade of life or later. The genetic and molecular basis of the disease has remained unknown for many years. We carried out linkage mapping, gene expression analysis, exome sequencing and candidate gene sequencing in affected individuals from 20 families and/or simplex cases, and identified in five of them disease-causing mutations in DNAJC5, encoding cysteine-string protein alpha (CSPα). These mutations - p.Leu116del and p.Leu115Arg - are located within the cysteine-string domain of the protein, and affect both palmitoylation-dependent sorting and the amount of CSPα in neuronal cells. The resulting depletion of functional CSPα may cause presynaptic dysfunction and the progressive neurodegeneration observed in affected individuals. Our work represents a major step in the genetic dissection of a genetically heterogeneous group of ANCLs, identifying the long-awaited CLN4. It also confirms a neuroprotective role for CSPα in humans, and advocates detailed investigation of CSPα in the NCLs and other neurodegenerative diseases presenting with neuronal protein aggregation.

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A recurrent mutation in *DNAJC5* causes autosomal dominant Kufs Disease

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The neuronal ceroid lipofuscinoses (NCLs) are a group of inherited neurodegenerative disorders characterized by abnormal lysosomal accumulation of autofluorescent lipopigment in the neurons and in the eye, that are associated with seizures, motor and intellectual deterioration, vision impairments and early death. In the majority of the cases, the pattern of inheritance is autosomal recessive with onset during childhood. Mutations in a total of 8 genes (*PPT1, TPP1, CLN3, CLN5, CLN6, MSFD8, CLN8, CTSD*) have been associated with infantile (INCL, Santavuori-Haltia), late-infantile (LINCL, Jansky-Bielschowsky), and juvenile (JNCL, Batten disease, Spielmeyer-Vogt) forms of the disease. Kufs disease is a rare form of NCL with onset during adulthood. In contrast to the childhood NCLs the vision is normal and the syndrome exhibits either autosomal dominant or recessive inheritance. Mutation in *CLN6* gene, initially described in late-infantile NCL, has been recently reported in cases of recessive Kufs disease.

Kufs disease with autosomal dominant inheritance was first described in 1971 in a large kindred from New Jersey, USA (Parry family; MIM 162350) with more than 18 affected individuals over 4 generations. Additional families have been later described, although with less affected individuals. The molecular basis of the dominant form of adult NCL was obscure but, very recently, Noskova *et al.* reported variants in the *DNAJC5* in 5 families. In parallel, reported here, we mapped the gene on chromosome 20p13.33 causing Kufs disease in the Parry family. We also took advantage of this unique family to identify the causative p.L116del mutation in *DNAJC5* gene by using Next Generation Sequencing (NGS). Sequencing of additional Kufs individuals allowed us to identify the same p.L116del mutation that segregate in another well-characterized autosomal dominant family from our cohort.

The biological studies on the CSPα protein function indicates that it contributes to the rescue of unfolded synaptic proteins, a mechanism that is also underlying various neurodegenerative disorders. Therefore, a better understanding of the genetic mechanisms underlying neurodegeneration in Kufs disease may provide additional insights on the mechanisms causing more common neurodegenerative disorders.

Mutant glia impair the health of neurons in Juvenile NCL

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In all forms of NCLs, localised glial activation occurs early in the disease and accurately predicts the distribution of subsequent neuron loss. However, in *Cln3* deficient mice (*Cln3*$^{-/-}$), this characteristic glial response appears to be attenuated with many astrocytes failing to become hypertrophied and microglia that remain in a ramified state rather than transforming into brain macrophages. Similar morphological findings are also apparent in human JNCL, highlighting the regional specificity of these events. We hypothesised that the normal biology of astrocytes and microglia may also be affected by Cln3 mutation, and given the significant roles glia play impaired biology of astrocytes or microglia (or both) may have a significant impact upon neuron health and function. To begin investigating these issues we established pure primary cultures of either astrocytes or microglia derived from *Cln3*$^{-/-}$ and wild type (C57BL/6J) mice and studied their response to a standardised activation stimulus (Astrocytes: 1μg/ml lipopolysaccharide, 100U/ml Interferon-γ LPS/IFN-γ; Microglia: 50ng/ml lipopolysaccharide) in terms of their morphology, proliferation, and ability to synthesise and secrete a range of proteins. *Cln3*$^{-/-}$ microglia displayed an impaired and delayed morphological response to LPS stimulation, despite normal levels of LPS receptors and no obvious cytoskeletal abnormalities, and normal phagocytic properties. Unexpectedly, their secretion profile revealed significantly reduced secretion of 9 out of 60 cytokines and chemokines. In comparison, *Cln3*$^{-/-}$ astrocytes displayed more pronounced defects in response to LPS/IFN-γ stimulation, with a profoundly impaired morphological response and disrupted actin cytoskeletion, markedly reduced proliferation and much broader defects in the secretion (but not synthesis) of the same panel of 60 cytokines and chemokines. Finally, the *Cln3*$^{-/-}$ astrocytes show an impaired ability to respond to oxidative stress conditions by secretion of one of the most important antioxidants in the brain, glutathione. To begin investigating the potential impact of impaired glial biology in JNCL we grew primary cultures of embryonic neurons from wild type and *Cln3*$^{-/-}$ mice and added mixed glial preparations from either mutant or wild type mice. These co-cultures revealed a negative impact of mutant glia upon the phenotype and survival of wild type neurons, which was much more pronounced upon *Cln3*$^{-/-}$ neurons. In contrast, wild type glia exerted a protective influence upon these mutant neurons, improving their survival and partially reversing their JNCL phenotype. The next challenge will be in determining how these effects are mediated and which types of glia are responsible (astrocytes, or microglia, or both), but these data raise important questions about their role in JNCL pathogenesis. Indeed, revealing that a positive impact upon *Cln3*$^{-/-}$ cortical neurons can be produced solely by correcting glial *Cln3* deficiency may have important therapeutic implications.

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Effects of CLN3 loss on inflammasome activation in microglia

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Juvenile neuronal ceroid lipofuscinosis (JNCL) is a fatal, neurodegenerative lysosomal storage disease that typically presents in children between the ages of 5-10 years, initiating as blindness and progressing to seizures, motor loss, and subsequent cognitive decline. Recent studies by others using CLN3 knockout mice have revealed a correlation between activated microglia and areas of neuronal damage. In addition, microglial activation has been shown to precede evidence of neuronal degeneration, suggesting that microglia may impact JNCL progression. However, the secretory phenotype of activated microglia in the context of CLN3 inactivation remains to be defined. This is a critical issue, since activated microglia may either positively or negatively impact neuronal survival via the production of growth factors versus proinflammatory mediators, respectively. Previous work in our laboratory has revealed a critical role for microglia in initiating inflammatory events immediately following central nervous system bacterial infection. In particular, we have defined the inflammasome as a key molecular pathway in microglia that is responsible for processing the proinflammatory cytokine interleukin-1 beta (IL-1b) into its active form. Depending on the initiating stimulus, activated microglia also produce reactive oxygen species (ROS), which have recently been shown to trigger inflammasome activation, linking the two processes. Prior studies have revealed oxidative imbalance in the brains of CLN3 knockout mice and IL-1b has long been recognized for its neurotoxic properties, suggesting that these pathways may be influenced by CLN3 loss. Our studies will utilize primary microglia isolated from $CLN3^{\Delta 7/8}$ (CLN3 knock-in) and wild type C57BL/6 mice to address the effects of CLN3 loss on ROS production and inflammasome action and potential impact on neuronal survival.

Delayed pathology in the thalamocortical system of immune-deficient Ppt1 null mutant mice

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In all forms of NCL neuron loss is preceded by activation of the innate immune system, but it is unclear whether this contributes directly to disease progression. We have now investigated the role of inflammatory cellular components (lymphocytes and microglia) in mouse models of Infantile NCL ($Ppt1^{-/-}$ mice) and Juvenile NCL ($Cln3^{-/-}$ mice) by identifying, localising and genetically removing immune cells. First, we characterised the types of immune cells present within the CNS and found evidence for progressive infiltration, which is pronounced within the thalamocortical system, by different classes of immune cells in both forms of NCL. In $Ppt1^{-/-}$ mice there is a significant infiltration of CD8 cytotoxic T-cells (and to a lesser extent CD4 Helper T-cells) that occurs early in disease progression when neuron loss first starts within the thalamus, but this only occurs towards the disease end stage in $Cln3^{-/-}$ mice. Both models also display microglial activation, with up-regulation of the macrophage cell recognition molecule sialoadhesin (Sn), which interacts with T-lymphocytes. To analyse the pathogenic impact of lymphocytes, we crossbred $Ppt1^{-/-}$ mice with mice deficient in Rag-1 (which lack T- and B-lymphocytes) and investigated the impact upon well-defined landmarks of disease progression, including regional atrophy, glial activation and neuron loss within the thalamocortical system. As previously documented, in $Ppt1^{-/-}$ mice increased glial activation, neuron and cortical volume loss were evident from 3 months onwards, and worsened with increased age. In $Ppt1^{-/-}$/$Rag\text{-}1^{-/-}$ mice the onset and progression of these pathological measures was delayed, but reached the same disease end point at 7 months of age. Next, we analysed the impact of microglial cells in INCL by crossbreeding $Ppt1^{-/-}$ and $Sn^{-/-}$ mice and found that glial activation and cortical volume loss were also delayed in the absence of this microglial recognition molecule. These observations suggest that both lymphocytes and microglia contribute partially to pathogenesis in $Ppt1^{-/-}$ mice and we are performing similar crosses with $Cln3^{-/-}$ mice. These studies may provide information crucial for developing treatment strategies to block lymphocyte/microglial activation and encourage neuronal survival.

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Synaptic dysfunction in motor nerve terminals of knock -out mice lacking Cysteine String Protein-alpha, a protein involved in autosomal-dominant adult-onset neuronal ceroid lipofuscinosis

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Cysteine String Protein-alpha (CSP-alpha) is a synaptic vesicle protein that prevents neurodegeneration of presynaptic terminals. CSP-alpha knock-out mice suffer from a lethal neurological phenotype that is evident after the second postnatal week (Fernández-Chacón et al., Neuron 42:237-51 (2004); García-Junco-Clemente et al., J Neurosci. 30:7377-91 (2010)). Recently, it has been shown that mutations in DNAJC5, the gene encoding CSP-alpha, cause autosomal-dominant adult-onset neuronal ceroid lipofuscinosis in humans (Nosková et al., Am J Hum Genet. 89:241-52 (2011); Benitez et al., PLoS One 6:e26741 (2011)).

Results

We have found striking changes in the synaptic vesicle cycle in CSP-alpha KO mice using electrophysiology and imaging at the neuromuscular junction (NMJ). Exocytosis at the synaptic terminals is decreased in CSP-alpha KO mice at P16-20. Nerve terminals fail to sustain prolonged release. At these synapses, the SNARE protein SNAP25, is dramatically decreased. That observation could explain a lower number of transmitter release sites observed in mutant synapses. To monitor synaptic exo- and endocytosis in vivo we have generated transgenic mice that express synaptopHluorin (SpH) in neurons but lack CSP-alpha. Combining imaging and electrophysiology, we have uncovered a reduction in the size of the recycling synaptic vesicle pool in mutant synapses. Dynamin-dependent recycling taking place during the stimulus is particularly reduced in the absence of CSP-alpha.

Conclusions

Our results reveal that CSP-alpha might prevent synaptic degeneration in motor nerve terminals by maintaining SNARE complex stability and synaptic vesicle recycling.

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Synaptic failure may initiate the neuronal degeneration in cathepsin D deficiency

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Mutations in the cathepsin D gene cause an aggressive neurodegenerative disease (congenital neuronal ceroid lipofuscinosis) that leads to early death of affected human babies and lambs. In mice, the disruption of cathepsin D gene (*Ctsd*) leads to more prolonged but fatal disease. We have previously shown that mislocalization and aggregation of presynaptic proteins is a prominent feature of man, sheep and mice with cathepsin D deficiency.

In order to identify the early events that lead to synaptic alterations, we investigated synaptic ultrastructure and function in presymptomatic *Ctsd* knockout (*Ctsd* $^{-/-}$) mice. Electron microscopy revealed that there were significantly greater numbers of readily releasable synaptic vesicles present in *Ctsd* $^{-/-}$ mice than in wild-type control mice as early as postnatal day 16. The electrophysiology measurements demonstrated a markedly decreased frequency of miniature excitatory postsynaptic currents at the same age, before the appearance of epilepsy or any morphologic sign of synaptic degeneration.

In a further set of experiments, we systematically compared the synaptic proteome between *Ctsd*$^{-/-}$ and control mice by quantitative iTRAQ labelling method followed by liquid chromatography. We applied in-depth bioinformatic mining of the iTRAQ data to reveal the functional modules associated with the observed proteomic changes. The results of those studies were verified by immunological methods. The data indicate disease specific changes in the cytoskeletal organization, including alterations in acetylation of microtubules and focal adhesion sites. Based on recent literature, focal adhesion kinase (FAK) has a role in neuronal growth cone formation, controlling synapse formation and development of axo-dendritic contact differentiation via SynCAM 1 (Stagi et al. PNAS 2010). Thus, the observed proteomic differences may have serious implications for morphology and polarity of neurons as well as for development and maintenance of synapses, where the electrophysiological measurements indicate early functional abnormalities.

Early synaptic abnormalities in multiple models of NCL

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Synaptic pathology is thought to be an early event in the pathogenesis of multiple forms of NCL. Recently, pre-synaptic abnormalities have been observed in pre-symptomatic CTSD and Ppt1 deficient mice and potentially may occur in other forms of NCL. We are taking advantage of the stereotypical neuronal development of *Drosophila* to identify roles for Cln7 and Cln3, mutated in variant late infantile and juvenile NCL respectively, in synapse development and function. We focus on the neuromuscular junction (NMJ) of the late *Drosophila* larva, a well-characterised model excitatory synapse. We have generated loss-of-function mutations for both Cln7 and Cln3. In both cases synapses develop ostensibly normally. However, the stereotyped development of the *Drosophila* NMJ is quantifiable which has allowed us to identify novel roles for these proteins in synapse biology. In *cln7* deficient animals, synapses providing phasic stimulation are reduced in size yet tonic junctions are unaffected. In contrast, *cln3* deficient animals show an increased number of release sites in junctions providing tonic stimulation yet there is no effect on phasic junctions. This is likely due to oxidative load on neurons in the absence of Cln3 function. Oxidative stress has recently been demonstrated to affect NMJ development in *Drosophila*. We are now expanding these studies to examine synapse pathology in *Cln3* deficient mice. Our initial survey of pre and post-synaptic markers indicates that progressive synaptic pathology is also present in this model. Collectively these data reinforce the suggestion that the synapse is a common and early pathological target in the NCLs.

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Evidence for altered neurogenesis in mouse models of NCL – an attempt of self-repair?

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Emerging evidence suggests that the brain can respond to damage or disease by increasing endogenous neurogenesis (the production of neurons from neural progenitor cells, NPCs). To investigate whether such events occur in the NCLs we used injections of BrdU (5-bromo-2-deoxyuridine) to label the endogenous pool of NPCs and determine their proliferation, survival and subsequent fate. These studies were performed in mouse models of both infantile (*Ppt1*$^{-/-}$ mice) and juvenile (*Cln3*$^{-/-}$ mice) NCL, with BrdU injections made at different stages of disease progression. In both mouse models, BrdU+ve cells were not only found in the neurogenic regions (subventricular zone, SVZ; and hippocampal dentate gyrus, DG), but were also present more widely in the CNS and were much more abundant in *Ppt1*$^{-/-}$ mice. There was a significant increase in the number of BrdU +ve cells in the neurogenic areas of both mouse models during disease progression, but this was both NCL subtype- and regionally-specific, occurring to different extents in the SVZ and DG. For example in *Ppt1*$^{-/-}$ mice the greatest increase in BrdU +ve cells occurred in the SVZ, but in *Cln3*$^{-/-}$ mice the greatest increase was apparent in the DG. Phenotypic analysis of these newly generated cells revealed significantly increased numbers of NeuN +ve neurons in the SVZ or the DG of severely affected *Ppt1*$^{-/-}$ or *Cln3*$^{-/-}$ mice respectively. In contrast, in the regions where no significant increase in BrdU +ve cells was seen, fewer neurons were generated. These findings show that altered neurogenesis occurs in a region specific manner, which differs between forms of NCL. Gaining a better understanding of the mechanisms that lead to these changes in endogenous neurogenesis will be a crucial next step since they may provide targets for future therapeutic approaches.

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In vivo intercellular correction in ovine CLN6

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Intercellular correction has been assumed to be an important aspect of therapies for the NCLs arising from defects in soluble lysosomal proteins. On the other hand the phenomenon of cross-correction, whereby affected cells take up soluble lysosomal proteins from the surrounding environment via endocytosis, is considered unlikely to be effective for NCL forms in which the deficit is in a membrane bound protein, as there is no obvious route to cross-correction. However recent studies suggest that there is cross-correction in the ovine CLN6 NCL which results from a membrane bound protein defect.

In order to test this chimeric sheep were created by mixing blastomeres from homozygous CLN6 affected and normal 16-32 cell embryos and re-implanting the hybrid embryos for development. Brain development and clinical signs were monitored for up to 40 months.

Results: Genotypic and histological examination of the resulting chimeric animals indicated a good degree of colonisation by both cell types in the brain and evidence of cross-cell communication. Computed tomography (CT) scanning of two chimeras revealed brain volumes which were within the normal range and three other animals had progressively recovering brain volumes, with one increasing from far below the affected range to approach a normal volume by two years of age. All normal and recovering-like chimeras presented with reduced or absent disease associated glial activation, no evidence of neurodegeneration, normal cortical thickness and laminar organisation of cells, and no loss of vision long after these symptoms had progressed to terminal disease in affected animals. In addition PSA-NCAM staining for newly generated cells, revealed intense staining along the SVZ and radial migration of cells along white matter tracts with subsequent detection of newly generated cells within the cortical grey matter, not seen in normal control animals, indicating extended neurogenesis in the chimeric animals. Genotyping brain regions of these animals indicated up to 75% of cells were genotypically affected. Despite this storage bodies were rarely observed indicating that storage had been cleared from most cells.

Conclusions: These results suggest that this membrane protein defect may involve the processing of soluble factor(s) and that given the correct environmental milieu disease-affected cells are amenable to correction by normal cells in CLN6 NCL, resulting in an amelioration of disease pathology. Thus therapies based on cross-correction such as gene therapy may be possible even in the membrane bound protein forms of NCL.

Spatial Proteomics Identify a Novel Drug Target in JNCL

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The fact that the pathogenesis of Juvenile Neuronal Ceroid Lipofuscinosis remains unresolved largely accounts for the enigmatic CLN3 protein. Here, we approached the JNCL disease mechanisms from the top-down perspective of spatial proteomics using Multi-Epitope-Ligand-Cartography (MELC). The direct visualization of dozens of JNCL associated cellular components *in situ* in the hippocampus of an adult genetically precise JNCL mouse model (CD1-CLN3$_{\delta}^{ex7/8}$) led to the identification of a novel, drugable metabolic phenotype in JNCL.

Results

The architecture of the protein network formed by over 30 cellular components showed remarkable rearrangement *in situ* in the hippocampus of adult male JNCL mice in comparison to control animals. Careful inspection of the MELC data suggested that the deviations could originate from **a defect in cholesterol homeostasis**, and was verified by biochemical and histological analyses. Investigations for the integrity of the **mevalonate (MVA) pathway** leading to cholesterol synthesis revealed a previously non-reported **accumulation of isoprenoid lipids** as a novel biomarker of JNCL brain tissues. Acknowledging for the slow progression of JNCL we hypothesized that a temporal reduction of the MVA pathway could have an influence on the disease progression in the CNS. Indeed, a short-term **inhibition of HMG-CoA Reductase**, the key enzyme on the MVA pathway, efficiently reverted altered JNCL lysosomal phenotype and **removed proteolipid accumulates** *in vitro* in CLN3 CLN3$^{dex7/8}$ cerebellar granular cells, and reduced the isoprenoid levels and **introduced a halt in the progression** of the disease *in vivo* in adult male CD1-CLN3$^{dex7/8}$ mice. Subsequent analyses showed that the rescue mechanism triggered by HMG-CoA Reductase inhibitor involved at least **enhanced autophagy, reduced mitophagy, increased membrane protein isoprenylation and enhanced endo-lysosomal function**.

Conclusions

Our work here demonstrates the power of the genetically precise JNCL mouse model and our top-down proteomics strategy leading to a potential therapy form. **We propose that the pathogenesis of JCNL and possibly other NCL forms can be therapeutically influenced with MVA pathway modulators.**

Early-stage neurologic and non-neurologic abnormalities in *Cln3*$^{\Delta ex7/8}$ mice precede overt neurodegeneration

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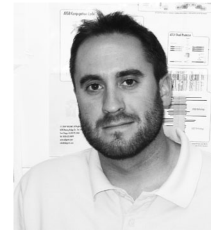
Cln3$^{\Delta ex7/8}$ mice harbor the most common genetic defect causing juvenile neuronal ceroid lipofuscinosis (JNCL). In order to more thoroughly investigate the manifestations of the common JNCL mutation, a ~1-kb deletion excising exons 7 and 8, we carried out a broad mouse phenotyping study following an established screening paradigm, in which we probed morphology and function of both central nervous system (CNS) and non-CNS organ systems of *Cln3*$^{\Delta ex7/8}$ mice, congenic on a C57BL/6N background.

Results: Homozygous *Cln3*$^{\Delta ex7/8}$ mice displayed deficits in sensory and motor tasks including pre-pulse inhibition to acoustic startle and pole-climbing tests at 12-13 weeks of age. Homozygous *Cln3*$^{\Delta ex7/8}$ mice also displayed electroretinography changes reflecting early cone function deficits and a late-onset but progressive decline of retinal post-receptor function. Further, metabolic analysis revealed significant increases in rectal body temperature and minimum oxygen consumption in 13-14-week old mutant *Cln3*$^{\Delta ex7/8}$ mice. Though no significant differences were observed in cardiac function, heart weight was significantly increased in 20-week old *Cln3*$^{\Delta ex7/8}$ mice. In a comprehensive blood analysis, most chemical analytes were unaltered, except for serum ferritin levels, MCV, and reticulocyte count, which were reproducibly elevated in homozygous *Cln3*$^{\Delta ex7/8}$ mice by 12 weeks of age, suggesting CLN3 deficiency leads to abnormalities in hematopoiesis. Finally, an early onset of vacuolation was observed in selected cell types in homozygous *Cln3*$^{\Delta ex7/8}$ mice, including in ~5-15% of peripheral blood lymphocytes, consistent with findings in JNCL patients, and in clear cells of the epididymis of male homozygous *Cln3*$^{\Delta ex7/8}$ mice.

Conclusions: These data establish early phenotypes in homozygous *Cln3*$^{\Delta ex7/8}$ mice that further support a growing body of evidence that CLN3 dysfunction impacts both CNS and non-CNS organ systems.

Autophagy dysfunction in NCL disease



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As an integral part of the lysosomal system, macroautophagy is predictably altered as a secondary pathogenic event in lysosomal disease states. Due to its essential role in maintaining neuronal homeostasis and survival, we have been characterizing how alterations in this pathway contribute to NCL disease pathogenesis. Our studies using mouse models of classic late-infantile NCL (*Cln2*$^{-/-}$) and juvenile NCL (*Cln3*$^{\Delta ex7/8}$) have established that macroautophagy becomes increasingly inefficient with disease progression. Notably, we have identified the accumulation of ubiquitinated protein aggregates within neurons containing the selective autophagosome adaptor proteins p62 and NBR1. p62 and NBR1 function as cargo recognition proteins incorporating ubiquitinated substrates within autophagosomes to be degraded, and the accumulation of these proteins is often used as an indirect marker of macroautophagy function. Unexpectedly, we have found colocalization between p62 and the major lysosomal storage component, subunit c of mitochondrial ATP synthase throughout the CNS. This colocalization is extra-lysosomal and in stark contrast to accumulation in classic membrane-bound storage bodies. We are currently evaluating this feature in the context of macroautophagy impairment through NCL disease course.

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Shared pathological themes between the NCLs and other LSDs

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Investigation of the CNS pathology in mouse models of the neuronal ceroid lipofuscinoses (NCLs) has provided detailed information about the onset and progression of neurodegeneration, glial responses and pathological synaptic changes. This has provided robust landmarks of disease progression. While each NCL model has distinct neuropathological phenotypes, these models share common themes of which brain regions and cell types are affected. To investigate the hypothesis that the precise nature of lysosomal defects influences the resultant neuropathology, we have investigated neuropathological events in mouse models of two other lysosomal storage disorders (LSDs); Sandhoff disease (*Hexb*$^{-/-}$ mice) and Niemann Pick disease type C (*Npc1*$^{-/-}$ mice) to compare these to the landmarks already identified in the NCLs.

We have shown in *Npc1*$^{-/-}$ and *Hexb*$^{-/-}$ mice and the NCL mouse models that the thalamus is an important pathological target of disease. Examination of markers of astrocytosis and microglial activation revealed a particularly pronounced reactive gliosis in a common set of primary thalamic relay nuclei; the ventral posterior nucleus (VPM/VPL), dorsal lateral geniculate nucleus (LGNd) and medial geniculate nucleus (MGN). This was associated with neuron loss early in disease progression in these thalamic nuclei in *Npc1*$^{-/-}$, *Hexb*$^{-/-}$ mice and NCL models. In addition, localised gliosis and neuron loss was also found in interconnecting cortical laminae, demonstrating the vulnerability of interconnected thalamocortical pathways in these models.

In addition, our data indicate that there are common themes in the events leading to neuron loss. For example, immunohistochemical staining for the presynaptic markers VAMP2, synaptophysin and SNAP25 has revealed a reorganisation of these presynaptic proteins in *Npc1*$^{-/-}$ and *Hexb*$^{-/-}$ mice and their accumulation in axonal spheroids, similar to the NCLs. Similarly immunohistochemistry revealed a redistribution of glutamate into astrocytes in the thalamus of both *Npc1*$^{-/-}$ and *Hexb*$^{-/-}$ mice, a phenotype also observed in NCL mouse models.

The systematic and reproducible approach taken in our study has provided a robust basis to make comparisons between models of disease. Taken together, we have shown that while there are distinct pathological events apparent in each mouse model, a series of common events occur in different LSDs. This not only highlights the importance of systematic methodology, but also the value of making comparisons between the NCLs and other LSDs.

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Modulation of Cellular Clearance in Lysosomal Storage Diseases

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We postulated that lysosomal function was subject to transcriptional regulation. Using a systems-biology approach, we discovered a gene regulatory network (CLEAR: Coordinated Lysosomal Enhancement And Regulation) that controls lysosomal biogenesis and function and a master gene, the bHLH-leucine zipper transcription factor TFEB, which binds to CLEAR target sites in the promoter of lysosomal genes and positively regulates their expression. TFEB overexpression induces lysosomal biogenesis and increases the ability of the cell to degrade complex molecules such as mutated huntingtin in a cellular model of Huntington's disease. Subsequent studies showed that TFEB overexpression rescued cell death in a mouse model of Parkinson's disease. We also showed that TFEB directly regulates autophagy, thus providing a link between the regulation of the biogenesis of two cellular organelles, lysosomes and autophagosomes, which cooperate in cellular clearance. Subsequently, we demonstrated the ability of TFEB to promote cellular clearance in several murine models of lysosomal storage diseases (LSDs), both in cell culture and in vivo. Recently, we demonstrated that TFEB colocalizes with the master growth regulator kinase mTOR on the lysosomal membrane. When nutrients are present, phosphorylation of TFEB by mTOR inhibits TFEB activity. Conversely, pharmacological inhibition of mTOR, as well as starvation and lysosomal disruption, can activate TFEB by promoting its nuclear translocation. These data identify an entirely novel lysosome-to-nucleus signaling mechanism that senses and regulates the lysosome via mTOR and TFEB. They also suggest that a pharmacological approach can be used to induce TFEB activity by promoting its nuclear translocation. These findings provide us with novel and innovative tools and strategies to promote cellular clearance. The use of transcriptional activation of lysosomal biogenesis as a tool to promote cellular clearance is a completely new concept and it may impact on the therapy of many LSDs and of common, late onset neurodegenerative diseases.

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Autophagy Failure in Alzheimer's Disease and Related Diseases

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Neurons are particularly vulnerable to dysfunction within the endocytic and autophagic pathways (the "lysosomal network") because of their extreme polar shapes and high levels of vesicular trafficking. In the extensive neuritic dystrophy of Alzheimer's disease (AD), which is a pathological hallmark of the disease, autophagic vacuoles containing incompletely digested proteins selectively accumulate in focal axonal swellings, reflecting defects in both autophagy and axonal transport. Growing evidence indicates that this massive "storage" of waste proteins in neurons, reminiscent of lysosomal storage diseases, mainly reflects a failure of lysosomal proteolytic clearance of autophagic substrates. Compounding the problem is a moderate induction of autophagy, as evidenced by elevated neuronal expression of autophagy genes.

Impaired lysosomal proteolysis is the likely basis for defects in both autophagy and axonal transport leading to the neuritic dystrophy of AD. In living primary cortical neurons expressing fluorescence-tagged markers, LC3-positive autophagosomes in axons rapidly acquired endo-lysosomal markers (Rab7 and LAMP1), underwent retrograde movement, and fused with bi-directionally moving lysosomes that are increasingly numerous at proximal axon levels and in the perikaryon. Disrupting lysosomal proteolysis by either inhibiting cathepsins directly or suppressing lysosomal acidification slowed the axonal transport of autophagy-related organelles but not other organelles and caused their selective accumulation within AD-like dystrophic axonal swellings. Restoration of lysosomal proteolysis cleared accumulated autophagic substrates and reversed the axonal dystrophy.

Defective lysosomal proteolysis is one facet of a continuum of lysosomal system deficits in AD that begin to be evident even prior to amyloid-ß deposition and are driven in part by AD-related genes. The AD-related gene presenilin1 (PS1) is essential for lysosomal proteolysis and autophagy and plays a novel role in lysosome acidification required for protease activation. In cells lacking PS1, including neurons in PS1 mice, a failure to deliver the proton pump vATPase to lysosomes results in autophagy failure. PS1 mutations causing familial AD also confer partial loss of these same functions in fibroblasts from PS-FAD patients and in neurons of AD model mice. Lysosomal and autophagy dysfunction also develops in sporadic AD and in AD mouse models driven in part by other AD-related genes, including amyloid precursor protein and apolipoprotein E. Mutations or polymorphisms of these genes that increase AD risk interfere with cell signaling, markedly upregulate endocytosis, placing stress on autophagy/lysosomal mechanisms by increasing delivery of substrates to this system. Supporting the pathogenic significance of lysosomal system dysfunction in AD, we found that partially restoring deficient autophagy in the CRND8 mouse model of AD by genetically manipulating lysosomal protease activities substantially ameliorates lysosomal pathology, amyloid burden, neuritic dystrophy, and memory deficits.

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Lysosomal Ca^{2+} homeostasis: role in pathogenesis of lysosomal storage diseases

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In addition to their well-known function as a compartment for degradation and recycling there is an emerging body of evidence that indicates an important role for lysosomes in intracellular Ca^{2+} signaling. The lysosome is the second largest Ca^{2+} store within the cell after the endoplasmic reticulum, with an intravesicular Ca^{2+} concentration of ~600mM. The discovery that NAADP, the most potent second messenger for releasing Ca^{2+} from intracellular stores, releases Ca^{2+} from lysosomes via the recently discovered two-pore channels (TPCs) has validated the lysosome as a bona fide intracellular Ca^{2+} store. Several novel lysosomal Ca^{2+} channels are currently being studied including TPCs, TRPML1 and P2X4. We are interested in the mechanisms by which lysosomes utilize Ca^{2+} signaling via these proteins in order to regulate normal lysosomal function and the events that occur following abnormal lysosomal Ca^{2+} signaling. I will summarize our previous findings indicating an important role of abnormal lysosomal Ca^{2+} signaling in the cellular pathogenesis of Niemann-Pick type C disease and will share our recent findings on the role of TRPML1 and P2X4 in altering lysosomal Ca^{2+} levels leading to pathogenesis in other lysosomal storage diseases. The potential for lysosomal Ca^{2+} signaling to be affected in the neuronal ceroid lipofuscinoses will be discussed.

Astrocytes as neuronal energy providers: putative therapeutic targets in the NCLs?

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Despite the fact that the brain represents only 2% of the body weight, energy expenditure associated with brain function is proportionally much more important than for other organs. Neuronal activity is considered to be responsible for the largest part of brain energy costs. In order to face such high energy demands, it has been assumed that direct glucose uptake and oxidation represents the predominant source of ATP for active neurons. However, based on cytoarchitectural and functional characteristics, it was suggested that astrocytes could play a central role in distributing energy substrates from the circulation to active neurons. Indeed, recent studies performed both *in vitro* and *in vivo* have suggested a mechanism by which astrocytes could play a specific role in coupling synaptic activity with glucose utilization. A concept known as the astrocyte-neuron lactate shuttle has been proposed whereby in response to glutamatergic activity, astrocytes promote the glycolytic conversion of glucose into lactate. In parallel, neurons would take up and use preferentially lactate as oxidative substrate for energy production purposes. To ensure this lactate transfer between the two cell types, a group of specific carriers know as monocarboxylate transporters has been identified. Each of them is subject to specific regulations to adapt supply to energy needs in register with activity. Although we begin to have a better understanding of the physiological importance of this process, the possible consequences of impaired metabolic supply from astrocytes to neurons have not been evaluated. There is increasing evidence that astrocytes might be affected in a number of neurodegenerative diseases and could be in fact the initial cause in some instances. In the case of NCLs, recent evidence suggests that astrocyte dysfunction might participate to the etiology of the disease. Such observations raise the possibility that astrocytes (and their metabolic supply role) could represent a putative therapeutic target for such diseases. Indeed, evidence has been provided that boosting lactate transfer can be neuroprotective notably against excitotoxicity. Moreover, different strategies could be envisaged to re-establish adequate astrocytic lactate supply to neurons. Whether such approaches could represent useful therapeutic interventions for NCLs remains to be determined.

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A novel genetic link between neuronal ceroid lipofuscinosis and the ubiquitin-proteasome system

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Introduction

Thirty-five probands in the Massachusetts General Hospital NCL Registry (~8% of total enrollees diagnosed with NCL by conservative clinical and histopathologic criteria) have been ruled out for mutations in the 9 known NCL genes. To further understand the genetic underpinnings of the NCLs, we performed whole-exome sequencing of a Mexican family with a molecularly undefined form of NCL characterized by infantile-onset progressive myoclonic epilepsy, visual loss, cognitive and motor regression, death by the teenage years, and prominent NCL-type storage material in multiple cell types.

Results

Whole-exome sequencing data (NimbleGen SeqCap EZ Human Exome Library v2.0) from two affected siblings and an unaffected sibling were filtered using a recessive model, which identified a single variant predicted to be deleterious: c.550C>T/p.Arg184Cys in exon 4 of KCTD7 (potassium channel tetramerization domain-containing protein 7, MIM 611725). A different mutation in the same gene had been previously reported in a family with progressive myoclonic epilepsy and developmental regression but without other defining hallmarks of NCL. The variant in our index family was found to alter membrane localization of KCTD7 and to abrogate interaction, in a dimeric state, with Cullin-3, a ubiquitin ligase component and previously defined interactor of KCTD7. Intriguingly, cerebellar cells derived from a murine model of juvenile NCL (CLN3) show marked accumulation of endogenous KCTD7 and prominent aggregates of transiently expressed GFP-tagged KCTD7.

Conclusion

Our data suggest that KCTD7 is a candidate gene for a subset of molecularly undefined forms of NCL, in addition to "pure" forms of progressive myoclonic epilepsy, and raise the possibility of complex genotype-phenotype correlations, much as has been described for CLN6. Our findings are consistent with the previously defined role of defective protein processing in NCL and specifically suggest a contribution from the ubiquitin-proteasome system. It will be of interest to determine the substrate(s) of the predicted KCTD7/Cullin-3 ubiquitin ligase complex and their potential role in NCL pathogenesis.

Mutations in the gene encoding Cathepsin F are a cause of type B Kufs disease

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Background: Adult-onset recessive NCL without visual failure is known as Kufs disease. Type A Kufs disease, which presents with progressive myoclonus epilepsy, is caused by mutations in *CLN6*. The molecular basis for Type B Kufs disease, which presents with dementia and motor features, remains to be discovered.

Methods: We used linkage analysis and exome sequencing to investigate two families with Type B Kufs cases in which *CLN6* mutations were not detected. An Italian family with unrelated parents had two affected daughters in whom disease onset occurred at 20 and 32 years, while a consanguineous French Canadian family had one daughter in whom disease onset occurred at 25 years.

Results: The French Canadian family produced a unique linkage peak achieving a maximum LOD score of 2.3 on chromosome 11, while the Italian family had 18 lower linkage peaks, including one that overlapped the other family's single peak. Examination of rare variants detected by exome sequencing revealed that all three cases carried missense mutations in both copies of the *CTSF* gene, which encodes a lysosomal cysteine protease. A previously published mouse model deficient in Cathepsin F develops late-onset neurological disease with characteristic NCL pathology. We sequenced the exons of *CTSF* in 21 cases with suspected Kufs disease and identified one further patient carrying compound heterozygous mutations in *CTSF*.

Conclusions: We have detected five *CTSF* mutations in three families with known or suspected type B Kufs disease. Unlike recessive Kufs A, which seems to be uniformly due to *CLN6* mutations, Kufs B is more genetically heterogenous with mutations in *CTSF* now being identified as one cause.

Talks: Session 4

Homozygous mutations in progranulin can cause adult onset recessive NCL

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Background: We used linkage analysis and exome sequencing to investigate an Italian family with two children affected with adult onset NCL. Onset was at 22 and 23 years, with both patients experiencing convulsions, retinal dystrophy, and cerebellar atrophy.

Results: The parents of the siblings were not known to be related, but we estimated from genomic data that they were approximately second cousins once removed. Linkage analysis yielded two peaks on chromosomes 7 and 17 achieving maximum LOD scores of 3.07 and 2.97. Examination of rare variants detected by exome sequencing revealed that both siblings were homozygous for a 4 bp deletion in the progranulin gene (*GRN)* located within the linkage peak on chromosome 17. Remarkably, this variant is known to be a common cause of frontotemporal lobar dementia in the heterozygous state; it has not previously been observed in the homozygous state.

Re-examination of mice deficient in progranulin revealed fingerprint profiles typical of NCL. We sequenced *GRN* in 20 unsolved cases of known or suspected adult-onset NCL, but did not detect any further homozygous or compound heterozygous mutations in this gene. We were also unable to find any reports of individuals homozygous for the 4 bp deletion in frontotemporal lobar dementia cohorts.

Conclusions: Progranulin mutations are an uncommon cause of adult onset NCL. This finding reveals an unexpected link between NCL and a relatively common neurological disease.

Exome sequencing reveals *ATP13A2* mutations underlying juvenile NCL

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The neuronal ceroid lipofuscinoses (NCL) comprises a heterogeneous group of metabolic storage diseases with ages at onset ranging from around birth to adulthood. Despite the identification of more than nine genes to date there are many families in which the genetic cause remains to be identified. Exome sequencing is the ideal methodology to study this type of families, particularly if kindreds are not large enough for more classical linkage approaches. Here we describe the application of exome sequencing in a family with onset in the teenage years that was well studied clinically and phenotypically, with typical NCL pathology and not of known consanguinity.

A novel single homozygous mutation in *ATP13A2* was found to fully segregate with the disease, which can now also be referred to as CLN12. ATP13A2 belongs to the P-type superfamily of ATPases that transport inorganic cations and other substrates across cell membranes, with ATP13A2 acting in the lysosomal membrane.

Mutations in *ATP13A2* are a known cause of Kufor-Rakeb syndrome, a rare parkinsonian phenotype with juvenile onset. In addition, recently two reports in the NCL dog model demonstrated that mutations in *ATP13A2* were the cause of the disease in that animal. Thus, mutations in this gene cause two clinically distinct diseases. Taken together, these data show that NCL and KRS may share etiological features and further implicate the lysosomal pathway in Parkinson's disease.

Clinico-pathological features of Kufs disease due to *CLN6* mutation

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The clinical and pathological literature on Kufs disease, the paradigmatic form of adult NCL, is confusing. It is challenging to diagnose because the specific ultrastructural inclusions are sparse, and may be confused with normal age lipopigment. We recently described mutations in *CLN6* in recessive Kufs disease.

Here we analyzed clinical data from 8 families (two previously unreported) with Kufs disease and *CLN6* mutations. We also carefully re-examined the ultrastructural features in available material (brain 3 cases, skin 5 cases, rectal mucosa 2 cases, muscle 2 cases).

There were 12 affected subjects; the mean age of onset was 31 years (range 12-51). The typical presentation was of progressive myoclonus epilepsy, although in 3 cases there were definitive cognitive changes or ataxia years before the development of myoclonic or tonic-clonic seizures. In 3 cases, convulsive seizures were not observed and the pattern was of a progressive myoclonic ataxia. The course was slowly progressive, involving ataxia with loss of mobility and dementia. Death occurred approximately 15 years after onset. Fingerprint profiles were the only diagnostic inclusions observed; curvilinear profiles and granular osmiophilic deposits were not found. In the brain, the diagnostic inclusions had restricted distribution in the two whole brains examined, whereas in a limited brain biopsy, diagnostic inclusions were not identified after reevaluation. Definitive fingerprint profiles were found in 2/5 skin biopsies, 1/2 rectal biopsies and in vascular smooth muscle of 1/2 muscle biopsies. All had homozygous or compound heterozygous mutations in *CLN6*; the majority of mutations (8/11) predicted amino-acid substitutions; there were two heterozygous mutations predicting protein truncation and one heterozygous deletion. No cases had mutations predicting total absence of protein.

We conclude that Kufs disease due to mutations in *CLN6* is invariably associated with fingerprint profiles, although they may be difficult to demonstrate. The clinical picture is dominated by progressive myoclonic epilepsy. Analysis of *CLN6* may obviate the need for invasive brain biopsy in cases with the appropriate presentation.

Age-dependent therapeutic effect of memantine in a mouse model of juvenile Batten disease

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Currently there is no treatment for juvenile Batten disease, a fatal childhood neurodegenerative disorder caused by mutations in the *CLN3* gene. The *Cln3*-knockout ($Cln3^{\Delta ex1\text{-}6}$) mouse model of the disease recapitulates several features of the human disorder. $Cln3^{\Delta ex1\text{-}6}$ mice, similarly to juvenile Batten disease patients, have a motor coordination deficit that can be detected as early as postnatal day 14 (Weimer, et al., 2009). Our previous studies demonstrated that acute attenuation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-type glutamate receptor activity by the non-competitive AMPA antagonist, EGIS-8332, in both 1- and 6-7-month-old $Cln3^{\Delta ex1\text{-}6}$ mice results in a significant improvement in motor coordination. Here we show that acute inhibition of N-methyl-D-aspartate (NMDA)-type glutamate receptors by memantine (1 and 5 mg/kg i.p.) had no effect on the impaired motor coordination of one-month-old $Cln3^{\Delta ex1\text{-}6}$ mice. At a later stage of the disease, in 6-7-month-old $Cln3^{\Delta ex1\text{-}6}$ mice, however, memantine induced a delayed but extended (8 days) improvement of motor skills similarly to that observed previously with EGIS-8332 treatment. The age-dependent therapeutic effect of memantine implies that the pathomechanism in juvenile Batten disease changes during disease progression. In contrast to the acute treatment, repeated administration of memantine or EGIS-8332 (1 mg/kg, once a week for 4 weeks) to 6-month-old $Cln3^{\Delta ex1\text{-}6}$ mice did not have a beneficial effect on motor coordination. Histological analysis revealed that the repeated treatments did not have any impact upon microglial activation or the survival of vulnerable neuron populations. Memantine did not affect astrocytosis in the cortex. EGIS-8332, however, decreased astrocytic activation in the somatosensory barrelfield cortex.

Our results show that acute inhibition of NMDA receptors can induce a prolonged (8days) therapeutic effect, identifying NMDA receptors as a new therapeutic target for juvenile Batten disease.

Sex Differences in Clinical Progression and Quality of Life in Juvenile Neuronal Ceroid Lipofuscinosis

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Juvenile neuronal ceroid lipofuscinosis (JNCL) is a childhood-onset neurodegenerative, lysosomal storage disease caused by mutations in the *CLN3* gene. JNCL causes vision loss, seizures, motor and mental decline, and premature death. We examined several datasets to evaluate sex differences in age at symptom onset, symptom progression, and age at death, capability, and quality of life. **Results:** Males may experience first symptom onset on average about one year prior to females (Males' first symptom: 4.88 years old (SD = 1.65); Females' first symptom: 5.65 years old (SD = 1.81)) with significantly earlier onset for behavioral problems: 6.9 vs. 9.33 years; t (58) = 2.46, p. < .05 and for vision loss: Mean = 4.88 (SD = 1.65) vs. 6.38 (SD = 3.31) years; t (69) = 4.0, p < .001. There was no significant difference between males and females in age at onset of seizures, cognitive impairment, or motor symptoms. Despite a later age of symptom onset, females may experience a more precipitous disease course, with average age at death over 1 year prior to males: mean = 20.95 (SD = 4.46) vs. 22.21 (SD = 4.2) years: t (224) = -2.17, p < .05. As well, females were deemed to have lower parent-rated physical quality of life, in relation to their disease. **Conclusions:** If such sex differences exist, this may inform our understanding of biological mechanisms influencing disease course or strategies for targeted therapies.

Multidimensional Clinical Assessment Tool for late Infantile CLN2 Disease

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Aim: To develop and pilot a standardised quantitative scheme for the clinical assessment of children and young people diagnosed with late infantile CLN2 disease.

Method: Ethical approval was obtained. Participating children were recruited through the Batten Disease Family Association (BDFA) and the clinical service at Evelina Children's Hospital (ECH). A schedule for history and examination was devised incorporating existing Batten disease clinical scoring systems and using items drawn from scoring systems used in other conditions. Children underwent a number of assessments over 24 months (not less than 6 monthly). The schedule was revised and simplified part way through the project as some items were found to be repeated or time-consuming and not therefore useful.

Results: Eight individuals were tested on 17 occasions at ages 52-137 months. Five were homozygous for IVS5-1G>C. The results are presented graphically. Due to small numbers in this single centre - single investigator study, statistical analysis was not possible. As expected Hamburg score falls with age, and the UBDRS physical subscale score increases with age. For all CLN2 genotypes both expressive language and postural/locomotor developmental subscales decrease with age. The maximum rate of decline for both is likely to be seen between 48 months and 84 months. For homozygotes the same pattern is seen, but the maximum rate of decline is likely to be seen between 60 months and 84 months.

Conclusion: Standardised assessments of clinical symptoms and developmental skills can be quantified and be meaningful in a clinical setting. The results of Hamburg scores and UBDRS physical subscale are broadly in line with published data. A number of additional subscales including those for developmental motor skills and expressive language skills may be useful additions to clinical outcome measures currently used and deserve further investigation.

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Quantitative Brain Volumetric Analysis in Neuronal Ceroid Lipofuscinoses: A tool to precisely monitor disease progression

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Background: Neuronal ceroid lipofuscinoses (NCLs) are still beyond remedy. The clinical course and its variability in the different NCL forms is widely unknown. The efficacy of experimental treatments presently being developed (gene therapy, enzyme replacement, stem cell transplantation) will have to be evaluated on the basis of sufficient knowledge of the clinical course.
Therefore, a precise and quantitative description of disease progression is urgently needed in order to establish an evaluation tool for these therapies. This can be achieved by collecting volumetric MRI data and linking these to clinical scoring data in different NCL disease forms.
Goals: To develop outcome measures which will be sensitive to treatment effects as well as identification of related changes in cognitive performance.
Methods: Cross-sectional quantitative MRI data were collected for 31 children with documented NCL (CLN1 = 3, age range from 1.4 to 22.5 years, CLN2 = 5, age range from 3.9 to 5.9 years, CLN3 = 23, age range from 7.3 to 29.5 years of age). Volumetric analysis was performed by the automated segmentation. All the volumes used in data analysis were adjusted to the intracranial volume. Volumetric MRI data were correlated with clinical scoring data obtained using scoring methods for late infantile and juvenile NCL respectively.
Results: Volumetric analysis of cerebral MRI data showed that cortical volume declined robustly with age ($p < .01$) and disease progression represented by clinical scoring data. Total grey matter volume decline was similarly significant ($p < .01$) whereas subcortical grey matter volume did not yield significant correlation with age. Corpus callosal, white matter, and cerebellar volumes did not show any significant relationship to the age of subject.
Discussion: Cortical volume atrophy is the main hallmark of NCLs in contrast to the relative spare of white matter, corpus callosum, cerebellum, and deep nuclei volumes. A quantitative volumetric analysis shows that cortical volume atrophy in combination with clinical scoring data might be a potential sensitive outcome measure for evaluation of future therapies.

Psychopathology in CLN3 disease: Correlation with disease progression and quality of life

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CLN3 disease is one of the most common inherited degenerative brain disorders affecting children. Clinical hallmarks are visual loss, dementia, epilepsy, and psychomotor deterioration. Although psychopathologic symptoms represent a great burden on patients and families, few studies have looked at them in detail and at their effect on quality of life (QOL).

The aims of this study were i) to systematically analyse psychopathologic symptoms in CLN3 disease in relation to general disease progression and ii) to determine the effect of psychopathologic symptoms on QOL.

32 patients with genetically proven CLN3 disease were studied (14 male, 18 female, mean age 16.6 ± 4.2 years, range 7.1 to 32.7). Psychopathologic symptoms and psychiatric diagnoses were assessed by a pediatric psychiatrist using psychiatric exploration interviews and other psychiatric measurements. Disease progression was assessed using an established disease-specific clinical scoring system developed by Kohlschütter et al. (1988). QOL was studied using a questionnaire for parents based on elements of established instruments for measuring QOL in children and patients with chronic diseases (EQ-5D, Kidscreen-10, FABEL, SNQ).

Results showed that psychopathologic findings in CLN3 disease are frequent, of significant clinical importance, and sometimes disease-specific. All patients were diagnosed with dementia, combined in most cases (n=20) with additional psychiatric symptoms, mostly hallucinations, depression or mixed symptoms. Eight patients had an additional psychiatric diagnosis such as obsessive compulsive disorder. The symptom severity did not always correlate with general disease progression but had a negative effect on QOL of patients and families. Recognition and successful treatment of psychopathologic symptoms in patients with CLN3 disease will improve their QOL.

Sinus node dysfunction in juvenile neuronal ceroid lipofuscinosis

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Cardiac involvement in neuronal ceroid lipofuscinoses has attracted little attention, previously restricted to a few case and post-mortem studies in juvenile neuronal ceroid lipofuscinosis (JNCL). A progressive cardiac involvement with repolarization disturbances, ventricular hypertrophy and sinus node dysfunction has recently been demonstrated in a Danish population based study comprising 29 individuals with genetically verified JNCL (*Neurology 2011;76:1245-1251*).

The focus of the present paper is to describe the onset and development of sinus node dysfunction in JNCL in this population, seasoned with case reports. Heart rates and heart rate variability were evaluated on consecutive 24-hour ECG recordings. The study group comprised 29 Danish individuals with JNCL aged between 7 and 33 years (16 male and 13 female).

Results: The heart rate significantly decreased with patient age (-1.14 beats per minute per year; p=0.001). Periods with sinus arrest were seen in 9 patients. In one child detected as early as 14 years of age, but otherwise present beyond the age of 18 years. The sinus arrests lasted from 2.1 seconds to 26 seconds. In those individuals complaining sinus arrests lasting more than 10-12 seconds, the patients lost their muscle tone and exhibited either short attacks of head dropping while sitting or more regular syncope, mimicking new types of seizures, and/or complained of increasing fatigue. In one case, a boy 21 years of age, a pacemaker was implanted resulting in a general improvement.

Conclusions: Sinus node dysfunction with long-lasting sinus arrests do occur in JNCL during the adolescence period. From a clinical point of view it is important to study the full extent of these possible life threatening events and frankly discuss the implications. Moreover, knowledge of sinus node dysfunction is important due to a negative impact on the sinus node of some anti-epileptic and anti-psychotic drugs.

Lifelong learning for individuals with Batten disease

A presentation of a textbook for educational practitioners responsible for individuals with Juvenile Neuronal Ceroid Lipofuscinosis (Batten disease)

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Tambartun National Resource Centre for the Visually Impaired in Norway has the national responsibility to follow up education and school attendance for children, youths and adults with Batten disease (hereinafter referred to as the target group). The centre provides services in a lifelong perspective. The services rendered by Tambartun include competence building of educationalists, and local counseling.

Experience shows that individually tailored educational initiatives have a positive impact on the quality of life of the target group. A number of professionals claim, and conferences such as the last ICEVI in Dublin (2009) conclude that goal-oriented educational practices might be the best therapy that can be offered to the target group.

The quality of the Tambartun services for the target group has over time been debated. The target group's education is vulnerable, and often dependent on the quality of the services provided by Tambartun. The quality of services varies from time to time, and is to a large extent a function of the competence and knowledge of the individuals working at Tambartun at any given time. The situation indicated a need for a change.

In 2009 NSVF suggested an initiative to document knowledge and expertise regarding education for the target group. Education for the target group was considered to be a special field of competence. It was decided to write a JNCL educational textbook, and Tambartun was given the editorship. The following goals were addressed: The book should have a lifelong learning perspective and should include an educational concept in order to address the target group's learning needs. The book should be used to improve, standardise and stabilise Tambartun's educational services, and improve local school's educational practices. The textbook shall be used for both formal and informal education of educational staff. The project was concluded in late 2011.

The lecture will include a presentation of the textbook. The contents of the book are built upon the philosophy behind ICF (the International Classification of Functioning). The core messages and themes of the book are learning and maintenance of skills through participation in an inclusive setting, every-day cognitive and physical stimulation, learning "windows" and learning phases, and the need for recurrent educational assessments. The textbook considers mainstream school subjects such as reading and writing Braille/black print, mathematics, English, home economics and use of ICT to facilitate learning and communication. The textbook discusses Individual Educational Plans (IEP) and Individual Plans (IP) for persons with Batten disease and the need to plan for transitions. The textbook is also illustrating different organizational aspects regarding learning and maintaining skills according to differences in age and modes of living. The presentation will put a special focus on the importance of intensifying the development of reading and writing skills in early age, from visually to tactile and/or auditory modalities.

Mycophenolate mofetil for the treatment of Juvenile Ceroid Lipofuscinosis

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Juvenile Ceroid Lipofuscinosis is the most common lysomosal storage disease with symptom onset around age 5 to 6 years, beginning with vision loss, followed by seizures, cognitive decline and death in the late second or early third decade of life. There are no current disease modifying treatments and only particular symptoms, such as seizures, can be treated. However, studies in knock-out JNCL affected mice have shown that treatment with mycophenolate mofetil, an immunosuppressant, slows the motor symptom progression and also leads to an overall slower progression of the disease.

The University of Rochester Batten Center team is currently funded through the FDA and the Batten Disease Support and Research Association to determine if mycophenolate mofetil is a reasonable treatment option for children with JNCL. Since the use of mycophenolate mofetil in this instance is different than its' initial approval indication, our team must first establish that the medication is both safe and well tolerated in children with JNCL.

We plan to enroll 30 children with JNCL into the study, using the Unified Batten Disease Rating Scale to determine any affect on seizures, motor skills and cognition. The study will consist of 2 8-week arms: one arm will consist of treatment with mycophenolate mofetil and one with placebo. The study team will be blinded and safety and tolerability will be closely monitored using local physicians as co-investigators to aid in monitoring the children's health. Visits to the University of Rochester will occur at the beginning and the end of each 8-week arm with the local doctor visits occurring in between. The main aim of the study is to assess safety and tolerability, so safety assessments such as blood work, ECGs and assessment of adverse events will be closely monitored. Some preliminary efficacy assessments will be conducted as well but it is our thought that 8 weeks of treatment with mycophenolate mofetil is not likely to alter the outcome of the disease.

If the results of the study determine that mycophenolate mofetil is well tolerated and safe for use in children with JNCL, then an open-label trial to test for efficacy will be likely be considered.

Biomarker Discovery in Batten Disease

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The Neuronal Ceroid Lipofuscinoses are a family of recessively inherited neurodegenerative diseases having a worldwide incidence of up to 1 in 12, 500 live births. Although these disorders result from mutations in one of at least eight distinct genes, termed CLN1-8, they share common clinical manifestations including blindness, loss of cognitive function, seizures, decline in motor skill and premature death. Similarities are also observed at the cellular level where the intracellular accumulation of autofluorescent storage materials and selective loss of neuronal subpopulations are two of the most prominent features. The close resemblance of clinical symptoms among these genetically unrelated disorders often leads to misdiagnosis since it is almost impossible to distinguish between the various members of this disease family based on clinical observations alone. Currently, genetic testing is the only accurate means by which to differentiate between the various NCL disorders. However, since NCLs can result from multiple mutations in one of up to 8 different genes (182 mutations have been reported thus far), this can be a very complex and time-consuming process that can only be performed in a handful of facilities equipped to do such work. Therefore the development of new, simple diagnostic tools will greatly aid in the diagnosis and treatments of these disorders. To this end, we have used multiple proteomic approaches, including two dimensional difference in-gel electrophoresis (DIGE) and multiplex suspension arrays (Luminex assays) to profile protein levels in blood plasma from Batten disease patients. In addition, we have begun using UHPLC-MS techniques to measure levels of metabolites in blood plasma in the hopes of identifying biological pathways that may be affected by this disease.

Results: Using multiple proteomic approaches, we show small but significant changes in the levels of proteins that are associated with the immune response, including complement factors and serine protease inhibitors (SERPINS), and in the levels of apolipoprotein A-IV. Metabolomic profiling of blood plasma in Batten patients indicated changes in levels of metabolites associated with cholesterol metabolism, triglycerides, and fatty acid metabolism. In addition, levels of ganglioside G2, normally expressed in the cerebellum and peripheral nerves, were elevated in Batten disease patients.

Conclusions: Our current work has identified a number of putative proteomic and metabolomic biomarkers for Batten disease, and we are currently validating these results using additional techniques and patient samples. It is hoped that this work will eventually be expanded to encompass the full spectrum of NCL disorders and provide a panel of proteomic and metabolomic biomarkers that can be used to distinguish between the various forms of this family of diseases.

Magnetic resonance volumetrics, diffusion tensor imaging and spectroscopy as biomarkers to assess efficacy of gene therapy in a canine model for LINCL

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Dogs with a null mutation in *TPP1* develop a neurodegenerative disorder that is comparable to late infantile neuronal ceroid lipofuscinosis (LINCL). Accurate ante-mortem biomarkers are essential for evaluating efficacy of therapies in this dog model. Magnetic resonance (MR) cerebral findings in these animals include generalized atrophy with sulcal margin widening, decreased interthalamic adhesion size and generalized ventriculomegaly. A quantitative volume measurement of atrophy is necessary for more sensitive disease assessment, and for assessing therapeutic interventions. In addition to structural measurements, functional MR is being assessed in the dog model. Diffusion tensor imaging (DTI) is a quantitative means of evaluating white matter integrity. Magnetic resonance spectroscopy (MRS) can identify chemical byproducts of neuronal degradation. We hypothesized that 3-dimensional volumetric assessment of vetriculomegaly would correlate with overall disease progression and be a useful means to assess treatment efficacy. As a secondary aim, we evaluated pilot DTI and MRS sequences in LINCL dogs. Normal and LINCL dogs with and without AAV.TPP1 therapy were imaged with a 3T Siemens trio with a high resolution MPRage sequence at 3, 6, 9 and 12 months. Concurrent DTI and MRS sequences were performed on corticospinal tracts and motor cortex respectively. 3D modeling of the ventricular system along with grey and white matter was performed using the Brainsight program^{tm}. At 9 months LINCL dog ventricular volume was 7.5 times greater than normal dogs. Preliminary data in affected animals also suggest that intracerebral grey and white matter atrophy occurs similarly, and for MRS, we note a decrease in N-acetylaspartate consistent with human reports. In two affected dogs treated with low dose vectors, atrophy was improved to 3.8 and 4.9 times that of normal, with concomitant improvements in clinical signs. While MRS and DTI are under further investigation as useful biomarkers, data to date suggest that an increase in ventricular volume is a viable biomarker of disease progression and therapeutic efficacy in the LINCL dog.

The synergistic effects of CNS-directed gene therapy and bone marrow transplantation for infantile neuronal ceroid lipofuscinosis

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Infantile neuronal ceroid lipofusciniosis (INCL) is an inherited childhood neurodegenerative disorder caused by the loss of palmitoyl protein thioesterase-1 (PPT1) activity. Affected children suffer from blindness, epilepsy, motor dysfunction, cognitive decline, and premature death. The *Ppt1*$^{-/-}$ mouse shares most of the histological and clinical features of INCL. Previous single-therapy approaches using small molecule drugs, gene therapy, or neuronal stem cells resulted in partial histological correction, with minimal improvements in motor function or lifespan. Here we combined CNS-directed AAV2/5-mediated gene therapy with bone marrow transplantation (BMT) in the INCL mouse.

Results: AAV2/5-mediated gene therapy alone resulted in histological correction with significant decreases in both autofluorescent accumulation and inflammatory markers. This single approach also improved motor function and increased the median life span from ~8mo to ~12mo. In contrast, BMT alone provided no improvement in any measure. However, the addition of BMT to CNS-directed gene therapy further and dramatically increased the lifespan to ~18mo and led to normal motor function out to 13mo of age. Interestingly, autofluorescent accumulation and inflammatory markers were not significantly different from normal mice in 18mo old INCL mice treated with the combination of CNS-gene therapy and BMT.

Conclusions: These data are truly striking given the fact that BMT alone is deleterious yet it synergizes with CNS-directed gene therapy to dramatically increase efficacy. These findings could form the basis of an effective therapeutic strategy for INCL. In addition, these data provide insights into the underlying mechanisms of disease and identify potential new therapeutic targets.

A small molecule anti-inflammatory enhances the therapeutic effects of AAV-mediated CNS-directed gene therapy for infantile neuronal ceroid lipofuscinosis

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Infantile neuronal ceroid lipofusciniosis (INCL) is the most rapidly progressing form of Batten disease. INCL is a profoundly neurodegenerative disorder caused by the loss of the lysosomal enzyme palmitoyl protein thioesterase-1 (PPT1). Affected children first present with blindness that progresses to intractable seizures, motor dysfunction, cognitive decline, and premature death. The murine model of Ppt1-deficiency shares most of the histological and clinical features of INCL. Neuroinflammation characterized histologically by increased GFAP (astrocytosis) and CD68 (activated microglia) expression is a prominent feature of INCL. Therefore, we administered a potent anti-inflammatory drug (Minozac) alone and in combination with CNS-directed gene therapy to INCL mice in an attempt to treat this invariably fatal disease. Minozac is a new class of small molecule anti-inflammatory that readily crosses the blood brain barrier and inhibits the production of pro-inflammatory cytokines. This drug has been shown previously to provide significant clinical benefit in other models of neuroinflammation.

Results: As demonstrated previously, AAV2/5-mediated gene therapy alone resulted in reduced brain atrophy, improved motor function and increased the median life span from ~35wk to ~45wk. Animals treated with Minozac alone had a small but statistically significant reduction in brain atrophy but no improvement in motor function or life span. INCL animals treated with both AAV and Minozac had significantly improved motor function and increased life span (~47wk) compared to animals treated with AAV alone. Detailed biochemical (PPT1 activity, cytokine levels, etc.) and histological (autofluorescence, GFAP, CD68, etc.) analyses of the treated animals are currently underway.

Conclusions: These data confirm the therapeutic benefits associated with CNS-directed, AAV-mediated gene therapy as a potential treatment for INCL. Unfortunately, treatment with this potent anti-inflammatory alone provides little or no benefit. This suggests that the inflammatory process is so severe or is sufficiently downstream in the disease process that simply targeting this aspect of disease will not be therapeutic. However, targeting the inflammation while simultaneously providing a persistent source of the deficient enzyme can provide additional therapeutic benefit. These findings could form the basis of an effective therapeutic strategy that incorporates several approaches that target different aspects of the disease process.

Intravenous high-dose enzyme replacement therapy with recombinant palmitoyl-protein thioesterase reduces brain and visceral lysosomal storage in a mouse model of infantile neuronal ceroid lipofuscinosis

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These authors contributed equally to the work.

Talks: Session 6

Introduction. PPT1-related neuronal ceroid lipofuscinosis (NCL) is a lysosomal storage disorder caused by deficiency in a soluble lysosomal enzyme, palmitoyl-protein thioesterase-1 (PPT1). While the disease is characterized by accumulation of autofluorescent storage material throughout the brain and other tissues, neurodegeneration dominates the clinical picture. Homozygous PPT1 knockout mice reproduce the known features of the disease, developing signs of motor dysfunction at 5 months of age and death by 8.5 months. In the current study PPT1 knockout mice were treated with purified recombinant PPT1 (0.3 mg) administered intravenously weekly either 1) from birth; or 2) beginning at 8 weeks of age.

Results. The treatment was well tolerated and neither anaphylaxis nor neutralizing antibody formation was observed. In mice treated from birth, survival increased from 236±1.3 to 271±16.6 days (median ± S.E.), and the onset of motor deterioration was similarly delayed. In mice treated beginning at 8 weeks, no increases in survival or motor performance were seen. Neuropathology was improved in these mice, but only in the group treated beginning at birth. In visceral tissues, substantial clearance of autofluorescent storage material was observed, especially from macrophages in spleen, liver and intestine, and pancreatic acinar and renal tubular cells.

Conclusions. These findings suggest that enzyme replacement therapy may be an option for addressing visceral storage as part of a comprehensive approach to PPT1-related NCL. More efficacious delivery methods to target the brain are needed.

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Treatment with recombinant human tripeptidyl peptidase-1 (rhTPP1) delays onset of neurologic signs in a canine model of late infantile neuronal ceroid liposfuscinosis (LINCL).

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Late infantile neuronal ceroid lipofuscinosis (LINCL) is a neurodegenerative lysosomal storage disease characterized by loss of TPP1 activity with progressive neurodegeneration and death during childhood. TPP1-null Dachshunds recapitulate the human disease. In this study, TPP1-null dogs were treated with approximately 20 doses of rh-TPP1 (4 mg/dose) or artificial cerebrospinal fluid (vehicle) via intracerebroventricular or intrathecal administration. Doses were given every other week from approximately 9 weeks of age until euthanasia.

Results. A vehicle-treated dog initially exhibited cerebellar ataxia at 32 weeks of age, followed by loss of menace response, and manifestation of intention tremor and pelvic limb proprioceptive deficits that progressed to involve all limbs [**Figure 1**].

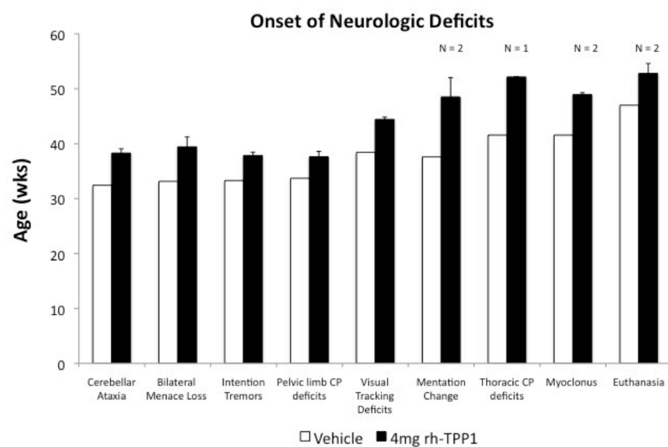

Figure 1. Dogs treated with 4mg rh-TPP1 exhibit a later onset of neurologic deficits. A single vehicle-treated dog consistently exhibited earlier onset of neurologic deficits than dogs treated with rh-TPP1 (n = 3 except when otherwise denoted). Data for rh-TPP1 treated dogs are represented as the mean age at onset ± S.E.M of deficits.

End-stage disease signs, consisting of loss of cognition, severe mentation abnormalities, loss of visual tracking and persistent myoclonic jerks, necessitated euthanasia at 47 weeks of age in the vehicle-treated dog. Similar signs necessitating euthanasia in two untreated TPP1-null dogs were reached at 46 and 49 weeks. In rh-TPP1 treated TPP1-null dogs (n=3), cerebellar ataxia was not initially observed until 38.3 ± 0.8 weeks of age, followed by loss of menace response, and appearance of intention tremor and proprioceptive deficits [**Figure 1**]. Two of the rh-TPP1 treated dogs did not reach the disease stage necessitating euthanasia until 51 and 55 weeks of age. One of these dogs developed a mass in the same ventricle as the ICV catheter. Histopathologic studies are underway to provide a diagnosis of the mass. A third rh-TPP1 treated dog has exhibited no loss of cognition or myoclonic jerks at its present age of 52 weeks. This dog is currently exhibiting only mild neurologic deficits. No long term systemic side effects of the rh-TPP1 treatment have been observed. ***Conclusions.*** These preliminary results indicate that CNS administration of rhTPP1 delays the onset of neurologic deficits and prolongs lifespan in TPP1-null Dachshunds. Additional dogs are being assessed to extend these findings.

Nonclinical development of recombinant human Tripeptidyl-peptidase 1 (rhTPP1) enzyme replacement therapy (ERT) for Late Infantile Neuronal Ceroid Lipofuscinosis (LINCL)

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BioMarin Pharmaceutical Inc.

LINCL is caused by lack of the enzyme TPP1. LINCL patients exhibit accumulation of lysosomal storage in the CNS accompanied by neurodegeneration, loss of function, and death. rhTPP1 ERT is in development for treatment of LINCL. To bypass the blood-brain barrier, the enzyme will be administered directly to the CNS into the cerebrospinal fluid (CSF). The pharmacology, pharmacokinetics, and toxicology of rhTPP1 were evaluated in TPP1-knockout (KO) mice, TPP1-null and normal dogs, and normal cynomolgus monkeys. The pharmacodynamic profile was assessed after repeat intrathecal (IT) and intracerebroventricular (ICV) administration to TPP1-KO mice and TPP1-null dachshunds, two relevant animal models of LINCL. rhTPP1 administration resulted in reduction of lysosomal storage accumulation, improvement in neurological function, and survival extension compared with vehicle treated controls. Pharmacokinetics (PK) in CSF and plasma, as well as CNS tissue distribution, were assessed in single and repeat IT and ICV administration studies in monkeys, beagles, and TPP1-null dachshunds. Peak CSF exposure was 200- to 1000-fold higher than in plasma and remained above the lysosomal K_{uptake} for 2-3 days post-dose. rhTPP1 was present in many brain structures at concentrations 2- to 6-fold the endogenous level. PK and CNS distribution were generally similar in monkeys after a single IT (lumbar) or ICV administration, although with greater deep brain delivery after ICV administration. The toxicity profile was evaluated after a single ICV administration to monkeys and repeat ICV and IT administration to TPP1-null dachshunds. ICV/IT dose administration has been well tolerated, with a few clinically manageable anaphylactoid reactions after repeat administration to dachshunds, attributable to the canine immune response to a human protein. Inflammatory infiltrates along the ICV/IT catheter tracks have been observed in all animals, including vehicle controls, which were attributable to the implantation and presence of a CNS delivery device. No other effects indicative of toxicity to rhTPP1 have been observed. This nonclinical program supports the continued development of rhTPP1 via clinical evaluation.

AAV-TPP1 Transduction of Brain Ependyma in TPP1-null Dogs Results in Widespread CNS Distribution of TPP1 Enzyme and Improves NCL Disease Phenotypes

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Late infantile neuronal ceroid lipofuscinosis (LINCL) is a recessively inherited genetic disease due to deficiency in tripeptidyl protease 1 (TPP1), a lysosomal hydrolase. Children with LINCL show developmental delay at 18-24 mo, seizures and blindness, and there is progressive atrophy in the CNS. To date, only palliative therapies are available for this uniformly fatal disorder. We proposed that enzyme replacement to the CNS can be achieved by creating a cellular reservoir for enzyme secretion into, and distribution by, the CSF. To evaluate this approach to therapy for LINCL, we screened various AAV serotypes for transduction of ventricular lining cells (the ependyma) expressing reporter genes following intraventricular delivery of the AAV vectors. In mouse, AAV2/4 effectively transduced ependyma, allowing reversal of CNS deficits in mouse models of lysosomal storage disease mice. To translate this to human therapy, we performed similar studies in a TPP1-null dog model with a disease course similar to LINCL children. In dogs, AAV2/2 was most efficient at transducing ependyma. Following delivery of AAV2/2.TPP1 to ventricles of LINCL dogs, we noted extensive enzyme activity in brain regions from cortical structures to the spinal cord, ranging from 5-10% to more than 20% of heterozygous levels. Volumetric analysis of MRI scans revealed significantly attenuated brain atrophy. The progression of clinical symptoms were also improved. Finally, in NHP, we show that this approach can achieve TPP1 activity throughout various brain regions, supporting the utility of this method for recombinant ERT in LINCL children.

Gene delivery to the Perinatal Brain

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A number of lysosomal storage diseases present irreversible pathology or result in death during the neonatal period e.g. neuronopathic Gaucher disease or the congenital form of neuronal ceroid lipofuscinosis, respectively. Conventional medicine offers no suitable treatment and so gene therapy may be an alternative approach. Given the early onset of such diseases, the gene delivery vector would require to be administered to either the fetal or early neonatal brain.

We have investigated the use of various gene delivery vectors administered to the brain of fetal or neonatal mice by either direct intracranial injection or intravenous administration. We demonstrate that intracranial injection of adeno-associated virus serotypes 8 and 9 results in extensive and widespread delivery when administered to the E15 fetal brain. Intravenous administration of AAV9 to fetal and neonatal mice resulted in transduction of the entire central nervous system (brain, spine, retina) and evidence of gene expression in the peripheral nervous system (myenteric plexus and innervating nerves). Furthermore, the gene expression profile within cells of the CNS was developmental stage dependent i.e. fetal administration resulted in predominantly neuronal transduction, whereas neonatal injection targeted expression mainly to protoplasmic astrocytes. None of the injections produced any measurable microglia-mediated immune response.

These findings support the view that intracranial injection of viral vectors to the perinatal brain represents a powerful research tool for investigating animal models of neurodegenerative diseases. However, the minimally invasive intravenous route has potential as a therapy and realistic translation to the clinic.

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Global CNS gene delivery platform in non-human primates utilizing self-complementary AAV9 vectors

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Several forms of Batten Disease, including those caused by loss-of-function mutations in PPT1 (306 aa) and TTP1 (563 aa), are amenable to gene replacement therapeutic approaches. In these cases, production of functional enzyme from the delivered transgene in a small number of targeted cells can exert a therapeutic effect on a larger area of the CNS, since the enzyme can be secreted from expressing cells and taken up by neighboring cells via the mannose-6-phosphate pathway. Numerous published proof-of-concept experiments have established that AAV-based gene replacement for CLN1 and CLN2 can exert a therapeutic effect. However, the efficacy and translation of Batten gene therapy has been limited by the extent to which a transgene can be widely delivered to both the brain and spinal cord in a large animal and in humans.

We report a gene delivery platform that would facilitate the translation of gene therapy for multiple forms of Batten Disease to large animals and humans. We have tested intrathecal administration of self-complementary (sc) AAV9/GFP vectors in mice, pigs, and non-human primates (NHPs). In pigs (n=3), 50-100% of spinal cord motor neurons were transduced, along with extensive transduction of neurons and glia in the brain at a dose of 1.7x10e11 vg/kg. In NHPs (n=6), approximately 2% of the entire brain and spinal cord area was GFP positive at a dose of 1.9x10e12 vg per 3-6 kg animal. Importantly, the transduction was evenly spread throughout the entire brain and spinal cord after a single injection, with minimal delivery to peripheral organs. Also, we have observed in 3 NHPs that the presence of pre-existing neutralizing antibodies against the AAV9 vector (1:32, 1:32, and 1:128) did not interfere with successful CNS gene delivery.

The results we've obtained in rodents, pigs, and non-human primates rely on the use of sc AAV vectors, which are approximately 20- to 50- fold more efficient for gene delivery compared to traditional single-stranded (ss) AAV vectors. However, the sc AAV vectors can only package up to ~2.1 kb of foreign DNA (versus ~4.5 for ss AAV vectors). Using a modified chicken beta actin promoter with a bovine growth hormone polyA signal, a transgene of up to 1050 bp (350 aa) can be packaged, and we have documented strong, ubiquitous, and stable GFP expression in mice with this AAV construct for up to 60 weeks. By testing different combinations of promoters, polyAs, and introns, we have identified a synthetic promoter and polyA combination that allows ~1890 bp (~630 aa) of space for transgene packaging into a sc AAV vector. Using the synthetic minimal promoter and polyA, we have documented stable and ubitquitous expression of GFP in mice with this AAV construct for 12 weeks.

We predict that the described gene delivery platform would be amenable for multiple Batten Disease gene therapy approaches, and it would enhance the efficacy seen in previous gene therapy studies that relied on direct intracranial injections. The intrathecal injection is a routine non-surgical procedure with minimal complications. This platform would provide widespread enzyme production throughout the entire brain and spinal cord and would be readily scalable from rodents to large animals to humans.

Post transplantation fate of human neural stem cells in a mouse model of late infantile NCL

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[2]StemCells, Inc., Palo Alto, CA, USA

Neural stem cell (HuCNS-SC) grafts are one means to supply the Tri-Peptidyl Peptidase 1 (TPP1) enzyme that is deficient in late infantile NCL (LINCL), and grafted cells may also replace dead or dying neurons. This approach has previously been tested in a mouse model of infantile NCL, in which very few of the transplanted HuCNS-SC adopted a neuronal phenotype. We have now explored the post-transplantation fate of the same HuCNS-SCs transplanted into a NODSCID mouse model of LINCL, either as neonates or in juvenile mice. As a first step we defined the extent of neuropathology in ungrafted *Tpp1*$^{-/-}$/NODSCID mice and have documented regional-specific atrophy, localised glial activation and selective neuron loss, which is pronounced within the thalamocortical system. In grafted *Tpp1*$^{-/-}$/NODSCID mice SC121+ve HuCNS-SC were found in dense cell clusters, but had also migrated widely within the CNS with many HuCNS-SC present in the rostral migratory stream and the subventricular zone. Colocalisation with markers that identify neurons (Fox3), astrocytes (S100B), or oligodendrocyte (O4) revealed that HuCNS-SC adopted a variety of different fates, which varied according to the age of grafting and where these grafts were placed. This included a variable proportion of HuCNS-SC that remained in a relatively undifferentiated state, but many appeared to adopt a neuronal fate. We are currently investigating whether these grafted HuCNS-SC deliver TPP1 *in vivo*, as they are capable of doing *in vitro*. Taken together these data set the scene for assessing the impact of these HuCNS-SC grafts upon pathological markers of disease progression, and testing their therapeutic efficacy.

Supported by a Medical Research Council (UK), DTA studentship.

Poster Presentations

Poster Presentations

Posters are listed by their theme, presenting author and poster session

Poster Session 1: 12.00-13.00 Thursday 29th March
Poster Session 2: 17.00-18.00 Thursday 29th March
Poster Session 3: 12.00-13.00 Friday 30th March
Poster Session 4: 17.00-18.00 Friday 30th March
Poster Session 5: 12.00-13.00 Saturday 31st March
Poster Session 6: 17.00-18.00 Saturday 31st March

Theme 1 Genetics & Biology of the NCLs

P1 Regulation of endosomal protein trafficking by starvation and its link to Batten disease. *Niv Dobzinski (Weizmann Institute, Israel)*, **Poster Session 1**.

P2 CLN9, CLN5, CLN8 proteins and ceramide synthases. *Rose-Mary Boustany (Beirut, Lebanon)*, **Poster Session 2**.

P3 CLN2 is the most frequent NCL in Latin America. Update of the phenotypes and mutational spectrum. *Romina Kohan (Cordoba, Argentina)*, **Poster Session 4**.

P4 Using tags to clarify CLN5 topology, maturation and localization. *Heidi Larkin (Quebec, Canada)*, **Poster Session 1**.

P5 Calnuc, a new protein involved in ceroid lipofuscinosis? *Heidi Larkin (Quebec, Canada)*, **Poster Session 2**.

P6 Study of the Golgi complex in juvenile NCL. *Davide Marotta (UCL, London)*, **Poster Session 4**.

P7 Defective phagocytic maturation in Cln3-deficient retinal pigment epithelium. *Hannah Mitchison (UCL, London)*, **Poster Session 1**.

P8 Identification of novel mutations in variant Neuronal Ceroid Lipofuscinosis. *Fillippo Santorelli (Pisa, Italy)*, **Poster Session 2**.

P9 Analysis of CLN6 mutations in ovine Batten disease. Nicole Neverman *(Dunedin, New Zealand)*, **Poster Session 4**.

P10 Late infantile neuronal ceroid lipofuscinosis: mutations in the CLN2 gene and clinical course in Spanish patients. *María S Pérez-Poyato (Barcelona, Spain)*, **Poster Session 1**.

P11 Evidence of Autophagy in Human CLN6 Fibroblasts. *Francesco Pezzini (Verona, Italy)*, **Poster Session 2**.

P12 Studies on CLN3 – A practical approach to its structure. *Juliana Ramos Moreno (Frankfurt, Germany)*, **Poster Session 4**.

P13 Molecular events underlying the endocytic defects found in JNCL. *Mark Schultz (Iowa City, USA)*, **Poster Session 1**.

P14 The MGH NCL Patient Biorepository: a shared resource to test and generate hypotheses about NCL pathophysiology. *John Staropoli (Boston, USA)*, **Poster Session 2**.

P15 Creating fission yeast strains to identify new therapeutic targets for Batten disease. *Mariana Vieira (UCL, London)*, **Poster Session 4**.

Theme 2 Disease Mechanisms

P16 Altered protein prenylation, mevalonate pathway and peroxisomes in JNCL. *Ilona Ahonen (Frankfurt, Germany)*, **Poster Session 1**.

P17 Increased expression of TNF-α, IL-1β, TGF-β and IL-10 in the brains of sheep with CLN6 NCL. *Lucy Barry (Lincoln, New Zealand)*, **Poster Session 2**.

P18 Behavioural studies and magnetic resonance imaging in the Merino sheep NCL model. *Imke Tammen (Sydney, Australia)*, **Poster Session 3**.

P19 Induced pluripotent stem cells (iPSCs) as a model for NCL diseases. *Tea Blom (Helsinki, Finland)*, **Poster Session 1**.

P20 Clusters of newly generated neurons in the cortex of sheep and human CLN6 deficiency. *Sybille Dihanich (KCL, UK)*, **Poster Session 2**.

P21 Immune cells are pathogenic mediators in the visual system of two mouse models of neuronal ceroid lipofuscinosis**.** *Janos Groh (Würzburg, Germany)*, **Poster Session 3**.

P22 A cell-based screen to identify modifiers of autophagy in JNCL. *Uma Chandrachud (Boston, USA)*, **Poster Session 1**.

P23 Metal accumulation and activation of cellular signaling pathways in disease-affected brain regions of ovine CLN6 neuronal ceroid lipofuscinosis. *Katja Kanninen (Melbourne, Australia)*, **Poster Session 2**.

P24 Investigating behavioral and biochemical correlates of disease in CLN6 mice. *Anthony White (Melbourne, Australia)*, **Poster Session 3.**

P25 Canine neuronal ceroid lipofuscinoses as models for understanding disease mechanisms and developing therapies for the human NCLs. *Martin Katz (Missouri, USA)*, **Poster Session 3**.

P26 Spatio-temporal analysis of glial activation and neuron loss in the *Cln8* mouse model of neuronal ceroid lipofuscinosis. *Mervi Kuronen (Helsinki, Finland)*, **Poster Session 1**.

P27 *In vitro* modeling of juvenile neuronal ceroid lipofuscinosis (JNCL): Patient fibroblasts and their reprogrammed derivatives as human models of JNCL. *Xenia Lojewski (Dresden, Germany)*, **Poster Session 2**.

P28 Retinal degeneration and microglial activation in mouse models of neuronal ceroid lipofuscinoses. *Myriam Mirza (Regensburg, Germany)*, **Poster Session 5**.

P29 Murine model of variant late infantile neuronal ceroid lipofuscinosis recapitulates disease progression. *Jill Weimer (Sioux Falls, USA)*, **Poster Session 1**.

P30 The specificity of storage of subunit c of mitochondrial ATP synthase in the ceroid lipofuscinoses. *David Palmer (Lincoln, New Zealand)*, **Poster Session 2**.

P31 Displaced axon initial segment localisation in *Cln3* deficient neurons. *Lotta Parviainen (KCL, London)*, **Poster Session 5**.

P32 Defective mitochondrial bioenergetics in murine homozygous $Cln3^{\Delta ex7/8}$ cerebellar neuron precursor cells precede subunit c storage. *Anton Petcherski (Frankfurt, Germany)*, **Poster Session 1**.

P33 Disturbed lipid metabolism, defective myelination and early microglial activation in *Cln5*-deficient mice and severe neuropathology of double-knockout mice lacking *Cln1* and *Cln5*. *Mia-Lisa Schmiedt (Helsinki, Finland)*, **Poster Session 2**.

P34 Distribution of GM1 ganglioside is altered in Cb $Cln3^{\Delta ex7/8}$ cells. *Aleksandra Somogyi (Frankfurt, Germany)*, **Poster Session 4**.

P35 Development of a Disease Model for Late-Infantile Neuronal Ceroid Lipofuscinosis Using Patient Specific Induced Pluripotent Stem Cells. *Michael Vellard (BioMarin, USA)*, **Poster Session 1**.

P36 Electroretinography in aging $Cln3^{\Delta ex7/8}$ KI mutant mice. *Cornelia Volz (Regensburg, Germany)*, **Poster Session 2**.

P37 Regional and subtype-specific glial activation and neuron loss in human NCL. *Andrew Wong (KCL, London)*, **Poster Session 5**.

Theme 3 Links to Other Diseases

P38 Cerebellar neurobiology of Niemann-Pick type C1 disease. *Ian Williams (Oxford, UK)*, **Poster Session 1**.

Theme 4 Recent Research Findings

P39 Anti-retinal antibodies in Juvenile Neuronal Ceroid Lipofuscinosis (JNCL). *Arlene Drack (Iowa City, USA)*, **Poster Session 2**.

P40 CLN3 mutation associated with altered dendritic cell phenotype. *Samantha Hersrud (Sioux Falls, USA)*, **Poster Session 5**.

Theme 5 New Clinical Perspectives

P41 Epilepsy in Juvenile Neuronal Ceroid Lipofuscinosis is Usually Characterized by Well-Controlled Generalized Tonic-Clonic Seizures. *Amy Vierhile (Rochester, USA)*, **Poster Session 3**.

P42 Statistical properties of the jNCL scoring system according to Kohlschütter (1988). *Angela Schulz (Hamburg, Germany)*, **Poster Session 4**.

P43 Experiences of providing a genetic diagnostic service for the neuronal ceroid-lipofuscinoses / Batten disease in the U.K. *Clare Beesley (Great Ormond Street Hospital, London)*, **Poster Session 5**.

P44 Neuronal Ceroid Lipofuscinosis in Norway. *Ingrid Helland (Oslo, Norway)*, **Session 3**.

P45 Characteristics of neuronal ceroid lipofuscinosis in patients from the West Balkan region. *Ruzica Kravljanac (Belgrade, Serbia)*, **Poster Session 4**.

P46 Clinical Application of a proposed axial classification system for the Neuronal Ceroid Lipofucinoses. *Ruth Williams, (Evelina Children's Hospital, London)*, **Poster Session 5**.

P47 Spectrum of mutations in French patients with ceroid lipofuscinosis. *Catherine Caillaud (Paris, France)*, **Poster Session 3**.

P48 Neuronal Ceroid Lipofuscinosis in Italy: Epidemiological Assessment of Childhood Forms in the Molecular Age. *Alessandro Simonati (Verona, Italy)*, **Poster Session 4**.

P49 DEM-CHILD - A Treatment-Oriented Research Project of NCL Disorders as a Major Cause of Dementia in Childhood. *Angela Schulz (Hamburg, Germany)*, **Poster Session 5**.

P50 Symptom Care Planning for young children affected by NCL. *Ruth Williams, (Evelina Children's Hospital, London)*, **Poster Session 3**.

P51 Multidimensional Clinical Assessment Tool for juvenile CLN3 Disease. *Ruth Williams, (Evelina Children's Hospital, London)*, **Poster Session 4**.

Theme 6 Experimental Therapies

P52 Novel approaches to chaperone therapy of Batten disease. *Glyn Dawson (Chicago, USA)*, **Poster Session 5**.

P53 Glutamate receptors as a therapeutic target for the treatment of INCL. *Rozzy Finn (Sioux Falls, USA)*, **Poster Session 3**.

P54 Galactosylceramide (GalCer) as a potential treatment for juvenile neuronal ceroid lipofuscinosis (JNCL). *Hayat Harati (Lebanon, Beirut)*, **Poster Session 4**.

P55 Gene therapy in ovine Batten disease – pre-trial vector testing in neuronal cultures. *Stephanie Hughes (Dunedin, New Zealand)*, **Poster Session 5**.

P56 Evaluation of the efficacy of a calpain inhibitor as an anti-neurodegenerative agent in Borderdale sheep with neuronal ceroid lipofuscinosis (CLN5). *Hannah Lee (Lincoln, New Zealand)*, **Poster Session 3**.

P57 Developing viral vector gene therapy for CLN5 and CLN6 Batten disease in ovine models. *Nadia Mitchell (Lincoln, New Zealand)*, **Poster Session 4**.

P58 Nonsense-suppression does not rescue the NCL-like phenotype of a zebrafish model of late infantile neuronal ceroid lipofuscinosis. *Claire Russell (RVC, London)*, **Poster Session 5**.

P59 TFEB-mediated lysosomal enhancement as a therapeutic strategy for Batten disease. *Marco Sardiello (Houston, USA)*, **Poster Session 3**.

P60 Exploring the potential of mouse neural stem cell grafts in Infantile NCL. *Andrew Wong (KCL, London)*, **Poster Session 4**.

Theme 7 Shared Session with Patient Organisations

P61 NCL Foundation – a concept to fight an orphan disease? *Frank Stehr (Hamburg, Germany)*, **Poster Session 5**.

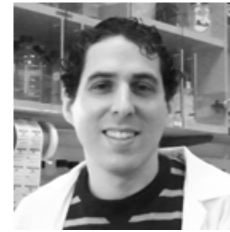
P62 The Batten Disease Family Association (BDFA). *Andrea West, Heather Band (UK)*, **Poster Session 3**.

Additional Posters

P63 'Working Together' - A Child Development Team's Support to Children with a Rare Neurometabolic Condition. *Esther Corker (Nottingham, UK)*, **Poster Session 5**.

P64 Children with juvenile neuronal ceroid lipofuscinosis: how do they present to paediatricans and when? – UK data from the past 16 years. *Anne Marie Winstone, Lesley Stellitano, Chris Verity (Cambridge, UK)*, **Poster Session 1**.

Regulation of endosomal protein trafficking by starvation and its link to Batten disease



Niv Dobzinski, **Jeffrey E Gerst**

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The yeast, *Saccharomyces cerevisiae*, contains orthologs of certain human disease-related genes therefore it can serve as a model organism for the study of inherited genetic disorders. We employ yeast as a model system to dissect the mechanics of the *CLN3* ortholog, Btn1, whose deletion leads to up-regulation of *BTN2*. *BTN2* encodes a protein involved in late endosome (LE)-Golgi retrograde protein transport and its deletion results in the mislocalization of *trans* Golgi proteins like Yif1 (a regulator of Rab family GTPases) and Kex2 (a subtisilin-like protease) to the vacuole. Our lab recently demonstrated that the deletion of *BTN1* mimics that of *BTN2*, and that Btn1 is a regulator of Golgi SNARE assembly. The mislocalization of *trans* Golgi proteins and their accumulation in the vacuole in *btn1Δ* and *btn2Δ* cells parallels the accumulation of material in the lysosomes of neurons in Batten disease patients.

Results

In ongoing studies, I observed that GFP-tagged Yif1 is mislocalized to the vacuole in wild-type cells upon either amino acid starvation, rapamycin (an inhibitor of TOR kinase) treatment, or by inactivation of both TOR genes (*e.g. TOR1* and *TOR2*). This observation in yeast hints at possible involvement of the TOR signaling pathway in the mislocalization of proteins in Batten disease. In addition to the mislocalization of Yif1 under these conditions, we have identified other proteins that are mislocalized from the Golgi to the vacuole, indicating that this is a general phenomenon. Interestingly, despite the connection to TOR signaling, protein mislocalization does not involve the autophagic machinery. However, it does necessitate the vacuolar protein sorting (VPS) pathway, which is known to control key steps in the delivery of ubiquitinated proteins to the vacuole. Importantly, we find that deletion of specific E2 and E3 ligases results in the retention of Yif1 in the Golgi upon starvation.

Conclusions

The mechanism and involvement of TOR signaling and ubiquitination in the relocalization of Golgi proteins is currently under study. We hope to establish a link between Tor signaling, protein ubiquitination, and the onset of Batten disease.

CLN9, CLN5, CLN8 proteins and ceramide synthases

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Objective: Four patients with a new form of juvenile Neuronal Ceroid Lipofuscinoses (NCL), a childhood neurodegenerative disorder, previously known as CLN9 variant are now reclassified as having CLN5 disease. The various clinical, pathological and biochemical data pertaining to these patients are now attributable to this CLN5 variant. Despite efforts at defining the CLN5 protein, its function remains elusive. CLN5-deficient ($CLN5^{-/-}$) fibroblasts obtained from these patients demonstrate adhesion defects, increased growth, apoptosis and decreased levels of ceramide, sphingomyelin, and glycosphingolipids. The CLN8 protein (CLN8p) corrects growth and apoptosis in $CLN5^{-/-}$ cells. This study consolidates the relationship between CLN5 and CLN8 proteins and highlights their role as activators of (dihydro)ceramide synthase (CerS) in a ceramide species–specific manner.

Methods and results: Homozygosity mapping using microarray technology led to identification of CLN5 as the culprit gene. Ceramide synthase activity and ceramide species measured by mass spectrometry are decreased in $CLN8^{-/-}$ cells, similarly to $CLN5^{-/-}$ cells. Comparison of normal vs. $CLN5^{-/-}$ cell CerS1-bound proteins by immunoprecipitation, differential gel electrophoresis and mass spectrometry, revealed absence of γ-actin in $CLN5^{-/-}$ cells. The γ-actin gene sequence is normal in CLN5-derived DNA. The following γ-actin-bound proteins, vimentin and histone proteins H2Afz/H3F3A/Hist1H4, were absent from the γ-actin protein complex in $CLN5^{-/-}$ cells.

Interpretation: The functions of these proteins and their failure to bind to γ-actin could explain the CLN5 cellular phenotype. We explore the role of the CLN5 and CLN8 proteins in sphingolipid *de novo* biosynthesis and suggest that CLN5 and CLN8 proteins are more closely related than previously believed.

CLN2 is the most frequent NCL in Latin America. Update of the phenotypes and mutational spectrum.

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The Neuronal Ceroid Lipofuscinoses (NCLs) are metabolic lysosomal disorders characterized by the intracellular accumulation of autofluorescent ceroid-lipofuscin like bodies in peripheral tissues and in the brain. Ten human disease genes have been identified: *CLN10/CTSD*, *CLN1/PPT1*, *CLN2/TPP1*, *CLN3*, *CLN4/DNAJC5*, *CLN5*, *CLN6*, *CLN7/MFSD8*, *CLN8* and *CLCN6*, with more than 380 mutations and 49 polymorphisms reported (http://www.ucl.ac.uk/ncl). In the Center for the Study of Inborn Errors of Metabolism of the Children´s Hospital, Córdoba-Argentina 82 patients were diagnosed since 2003: CLN1 (2; 2.4%); CLN2 (23; 28.2%); CLN3 (6; 7.3%); CLN5 (2; 2.4%); CLN6 (2; 2.4%); CLN7 (3; 3.7%); CLN8 (2; 2.4%). The remaining 42 (51.2%) individuals showed clinical and electron microscopy data compatible with NCL but do not have molecular diagnosis. Among the 23 CLN2 patients, the majority (16, 69.6%) showed the classic phenotype but the remainder (7, 30.4%) evidenced a more protracted course. This juvenile phenotype was however clinically distinguishable from the JNCL "classical" CLN3, beginning with severe seizures at 5-9 years, residual TPP-I activity, and with visual decline appearing later on in the evolution of the disease; whilst the classical CLN3 generally initiates with visual impairment. The CLN2 mutation spectrum included: 6 new missense mutations, E7 p.Asp276Val, E8 p.Arg339Gln, E8 p.Arg350Trp, E11 p.Ala453Val, E11 p.Ala453Asp, and E13 p.Gly535Arg; 3 nonsense, including the new: E4 p.Leu104X and previously known, E6 p.Arg208X and E3 p.Gln66X; 3 known intronic, I2 c.89+5G>C, I5 c.509-1G>C and I8 c.887-10A>G; and 1 new deletion, E9 c.1107-1108delTG. The most frequent mutation (p.Asp276Val) was found in 15 alleles (35.7%). The next frequent mutations were the p.Arg208X in 4 alleles (9.5%), followed by p.Leu104X, p.Gln66X and c.887-10A>G in 3 alleles (7% each). The new missense changes were validated in 200 chromosomes of controls with same ethnicity by PCR and sequencing, restriction enzymes or ASO. The prediction of mutation pathogenicity was supported by analysis using bioinformatics resources: ClustalW2, PolyPhen-2, SIFT, PoPMusic 2.1, and PDB/PyMol 1.1. Founder effects were assumed for the most frequent mutations, and all others appeared private.

Using tags to clarify CLN5 topology, maturation and localization

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Background: Late-infantile variants of Neuronal Ceroid Lipofuscinosis (vLINCL) are neurodegenerative diseases caused by mutations in *CLN5* gene. This gene encodes CLN5, a 407 amino acid protein with a predicted molecular weight of 46 kDa and with an unknown function. Controversial data have been reported on the localisation, maturation, solubility and topology of this protein. CLN5 has been suggested to contain one or two transmembrane (TM) domains and an unusual signal peptide (SP) cleavage site located after the first TM domain. CLN5 has also been shown to be secreted in the extracellular media and to be distributed in the ER, Golgi or lysosomes depending on the antibody used. In this study, we propose to elucidate CLN5 topology, maturation and localization by introducing epitope tags at different sites in the protein.

Methods: We generated three CLN5 constructs: an N-terminal tagged CLN5 (HA-CLN5), a C-terminal tagged CLN5 (CLN5-HA) and an internally tagged CLN5 in which a FLAG epitope was inserted after the putative signal peptide (SP-Flag-CLN5). These constructs were transiently expressed in HeLa cells and analyzed by western blotting, [^{35}S]methionine pulse-chase, glycosidase and proteinase K treatment assays, membrane fractionation as well as confocal microscopy to better characterize CLN5 processing and localization *in cellulo*.

Results: HA-CLN5 is detect as three bands, a 75 kDa glycosylated form, a 50 kDa unglycosylated form and a short 18 kDa fragment corresponding to the cleaved signal peptide. CLN5-HA and SP-Flag-CLN5 are detected as 35 and 60 kDa bands, corresponding respectively to the unglycosylated and glycosylated forms of CLN5 on which the SP has been cleaved. The proteolytic cleavage of CLN5 resulting in a 60 kDa mature form is observed immediately after a 5 min pulse and most likely represents cotranslational signal sequence cleavage. At steady state, HA-CLN5 is located in the endoplasmic reticulum while CLN5-HA and SP-Flag-CLN5 are mainly distributed in the Golgi where they partially colocalized with mannose-6-phosphate receptor. Treatment of microsomes with proteinase K indicated that CLN5 precursor contains one TM domain with the N-terminus facing the cytoplasm and the C-terminus protected in the lumen. Membrane fractionation assays indicated that all tagged-CLN5 proteins were associated to the membrane fraction. Unexpectedly, we observed that the tagged-CLN5 were present in the extracellular medium after one hour chase at 37°C. We are presently using these tagged-CLN5 constructs to characterize the consequence of introducing pathologic mutations (FIN minor, EUR, SWE) on CLN5 processing and localization.

Conclusion: The preproform of CLN5 is a type 2 transmembrane protein located in the endoplasmic reticulum. The transmembrane region is removed from CLN5 preproform following SP cleavage resulting in a 60 kDa highly glycosylated mature form. Mature CLN5 is associated to the membrane, located in the lumen of the Golgi and is eventually secreted. This study clarifies our knowledge on the basic properties of CLN5 for a better understanding of late-infantile lipofuscinosis.

Calnuc, a new protein involved in ceroid lipofuscinosis?

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Background: The efficient intracellular transport of lysosomal enzymes has important consequences in human health. Improper delivery of lysosomal cargo give rise to a range of neurodegenerative pathologies classified as lysosomal storage disorders such as the neuronal ceroid lipofuscinosis (NCL). The transport of soluble luminal lysosomal enzymes to the lysosome is carried out by the Mannose-6- phosphate receptors (MPR) and Sortilin. For anterograde traffic from Golgi-to-the endosome, newly synthesized lysosomal cargos binds to the receptors in the Golgi and is packaged into clathrin coated vesicles. When the receptor/cargo complex reaches the more acidic environment of the endosomes, the cargo dissociates from the receptor and is transported to the lysosomes while the unoccupied receptor is recycled back to the Golgi for another round of sorting. Ceroid Lipofuscinosis Neuronal protein-5 (CLN5), a protein involved in late-infantile variants of NCL, has been recently implicated in the lysosomal receptors retrograde transport *(Mamo A, Jules F, Costantino S, Lefrancois S. The role of CLN5 in endosomal sorting. Mol Biol Cell 2010; 21 (suppl): abstract#1625*). We recently obtained evidences that Calnuc, an ubiquitous Ca^{2+} binding protein present on the Golgi and endosomes, regulates the endosomal sorting of receptors to the Golgi. The overall objective of this study is to determine whether Calnuc is involved in trafficking of lysosomal receptors and their cargo and to verify its potential implication in NCL.

Results: Biochemical assays showed that Calnuc interacts directly with MPR and Sortilin. Confocal microscopy indicated that Calnuc colocalizes with MPR and Sortilin in the Golgi and endosomes. Depletion of Calnuc by small interfering RNA (siRNA) missorted MPR and Sortilin in the lysosomes, enhanced its lysosomal degradation and leads to the secretion of the lysosomal hydrolase cathepsin D into the culture medium. In concordance, immunofluorescence assay revealed that the LAMP2-labeled lysosomes are greatly enlarged in Calnuc-depleted cells, suggesting an engorgement of the lysosomes. These observations indicate that Calnuc interacts with lysosomal receptors and is important for their efficient recycling to the Golgi. We next investigated the potential link between Calnuc and CLN5. We showed that Calnuc directly interacts with CLN5 and colocalizes with it in the Golgi. Finally, using siRNA knockdown, we observed that the depletion of Calnuc decreased the level of CLN5 while the depletion of CLN5 does not affect Calnuc's level.

Conclusion: Our results support a role for Calnuc in controlling the itinerary of the lysosomal sorting receptors by regulating CLN5 protein levels. Most importantly, our work supports a potential implication of Calnuc in the pathological dysfunction leading to variant late infantile NCL.

Study of the Golgi complex in juvenile NCL

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Defects in *CLN3* lead to the juvenile form of neuronal ceroid lipofuscinosis (juvenile CLN3 disease, JNCL or Batten Disease). CLN3 is a multi-pass trans-membrane protein highly conserved in single-celled eukaryotes such as the yeasts *S. pombe* and *S. cerevisiae*, suggesting a fundamental role for this protein. CLN3 has been linked to many different cellular processes such as autophagy, lipid synthesis and/or modification, lysosomal homeostasis, cytoskeleton organisation and trafficking. Despite these endeavours, the function of CLN3 remains elusive. The yeast orthologous protein, Btn1, was first shown be located at the Golgi complex in fission yeast, and later at the same organelle in budding yeast. In *S. pomb*e loss of Btn1 function affects Golgi homeostasis, including number and morphology, and protein trafficking. In *S. cerevisiae* Golgi retrograde transport is affected, via Sed5 phosphorylation. We have embarked on a study of the role of Btn1 and CLN3 at the Golgi complex, and the effect of mutations, in yeast and mammalian cells, making use first of light and electron microscopy to study morphological effects. This work may be relevant to understanding the molecular basis of NCLs caused by mutations in CLN6 and CLN8 proteins, which are located upstream of the Golgi apparatus, in the endoplasmic reticulum.

Defective phagocytic maturation in Cln3-deficient retinal pigment epithelium

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We are using *Cln3*-deficient mice to investigate the molecular basis of loss of vision in juvenile Batten disease (JNCL; CLN3) since JNCL patients can be blind-only for several years before CNS deterioration is evident. Research into JNCL visual failure will be important in developing retinal therapies for this critical period of life and may shed light on the reasons for subsequent CNS neurodegeneration. Visual loss in JNCL arises from degeneration and loss of photoreceptors, specialised neurons in the retina that translate light into visual signals. Photoreceptor viability relies on a high turnover of their outer segments, which are shed in large numbers daily to be replaced with newly formed outer segment membranes carrying the phototransductive proteins. This renewal process relies on the neighbouring retinal pigment epithelium (RPE) cells to phagocytose and remove shed material. Their phagocytic mechanism requires intact lysosome function since newborn phagosomes undergo a maturation step changing them into digestive structures after degradative hydrolase enzyme delivery via fusion with the endo-lysosome compartments. We previously described defective lysosomal cathepsin activity in *Cln3*-deficient cells (Metcalf *et al* Traffic 9:1905, 2008), and we therefore investigated whether the phagocytic pathway is compromised in *Cln3*-deficient RPE. Electron microscopy of dissected *Cln3* retinae showed significantly increased numbers of mature RPE phagosomes while numbers of early phagosomes were unchanged. Cryo-EM immunostaining was used to assess lysosome-phagosome fusion efficiency using rhodopsin and cathepsin D to label phagosomes and lysosomes respectively. *Cln3*-deficient RPE cells showed a marked reduction in the number of cathepsin D-positive 'phagolysosomes' which result from fusion. Compared to controls the number of immature phagosomes located close to or docked onto lysosomes but still cathepsin D-negative was increased, while the number of mature phagolysosomes positive for both markers was less frequent, suggesting that lysosome-phagosome fusion was deficient. These results demonstrate that RPE phagocytosis is defective in JNCL, apparently due to deficient phagosome maturation via lysosome-phagosome fusion. Consistent with the retinal degeneration that arises from loss of Cln3 function, we conclude that Cln3 is involved in the RPE phagocytic process that is essential for photoreceptor renewal and viability.

Identification of novel mutations in variant Neuronal Ceroid Lipofuscinosis

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The neuronal ceroid lipofuscinoses (NCLs) are a group of genetically inherited neurodegenerative diseases exhibiting both locus and phenotypic heterogeneity. The common characteristics of this group of diseases include progressive cognitive and motor deterioration, seizures, visual loss, dementia, and early death. The disease phenotype usually presents during infancy or early childhood and rarely in adolescence and adulthood.

In Italy, variant-late infantile NCL (vLINCL) forms account for over 70% of cases, and mutations are found in the *CLN1*, *CLN5*, *CLN6*, *CLN7*, and *CLN8* genes. We report on novel vLINCL patients who displayed new mutations. Through CLNet, a nation-wide network of child neurology units, we selected 8 new patients with features suggestive of NCL. Four patients were born in either the Middle East or in the Indian peninsula. Following EEG, MRI, and ultrastructural examinations, 3 cases were suitable for genetic testing. In one child we detected a reported c.335G>A/p.R112H mutation whereas in another Pakistani adopted child we found a new c.526_527insA variant in *CLN5*. In an Egyptian girl we discovered a new c.611G>A (p.R204H) change in *CLN8*. Mutations were homozygous in all patients, heterozygous in their parents, and not detected in 350 control chromosomes. Our findings suggest that: i) the array of gene in associated with NCL in general and vLINCL in particular is higher that imagined; ii) immigration in Europe from Far East or Arabic countries will account for a significant number of new cases to be discovered; iii) more rapid and complete sequencing methods ("exome *a la carte*") are needed in the face of the several genes in NCL and the many more to be found.

Analysis of CLN6 mutations in ovine Batten disease

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Naturally occurring forms of Batten disease have been found in three breeds of sheep, CLN6 in New Zealand South Hampshires and Australian Merinos, and CLN5 in New Zealand Borderdales. Flocks have been established which provide excellent large animal models to study both the CLN5 and CLN6 forms of the disease, including the validation of potential treatment strategies.

The aim of this study was to use primary sheep neural cells obtained from foetal South Hampshire sheep deficient in CLN6 ($CLN6^{-/-}$) and unaffected Coopworth controls ($CLN6^{+/+}$) to study early changes in cellular pathology. Specifically, we examined lysosomal pH and ER pathology between healthy and CLN6-deficient sheep neural cells.

A significant decrease in lysosomal acidity was observed in the CLN6-deficient primary neural cultures as compared to the healthy controls using the dye LysoTracker Red ($P < 0.0001$ n = 3 per group, ANOVA).

Confocal analysis of calnexin immunostained sheep neural cells revealed a visible reduction of endoplasmic reticulum staining in the $CLN6^{-/-}$ cells as compared to control cells. Preliminary analysis showed this difference to be statistically significant ($P = 0.036$ n = 3, unpaired t-test). These results are being extended with more animals and compared with western blots using both anti-calnexin and another endoplasmic reticulum marker, anti-protein disulphide isomerase (PDI).

Future studies will transduce $CLN6^{-/-}$ primary neural cells with wild-type CLN6-containing lentivirus and use the LysoTracker and endoplasmic reticulum assays to test for functional correction after gene therapy treatment. These assays may well provide an important means by which to test the *in vitro* success of potential treatments for the NCLs.

Late infantile neuronal ceroid lipofuscinosis: mutations in the CLN2 gene and clinical course in Spanish patients

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Background: Late infantile neuronal ceroid lipofuscinosis (LINCL, Jansky-Bielchowsky disease) is caused by mutations in the *CLN2/TPP1* gene.

Aim: Describe the clinical course and results of biochemical, neuropathological and genetic studies to assess the chronological evolution of the disease.

Patients and methods: Twelve Spanish patients mean age 7.4 years (range 4–14) were evaluated in this study. The assessment of psychomotor impairment included age at onset; first symptom; age at regression of acquired skills; epilepsy and salient signs and symptoms of the disease.

Results: Age at onset ranged from 18 months to 3.7 years (mean 2.2). Febrile seizures followed by afebrile seizures and delayed speech were observed as presenting symptoms. Patients lost the ability to speak in full sentences at a median age of 3.7 years (95% CI 3.1–4.3). Loss of walking ability and absence of language appeared at a median age of 4.5 years (95% CI 4.3–4.6 *vs.* 95% CI 4.1-4.8). Partial seizures occurred by age 3.4 years (95% CI 2.9–3.8) and myoclonic jerks were present at a median age of 3.7 years (95% CI 3.4–4). Ataxia and cognitive decline were observed at a median age of 4 years (95% CI 3.9-4.1 *vs* 95% CI 3.5-4.4) and blindness at a median age of 5.1 years (95% CI 4.6–5.6). We report four novel mutations in the *CLN2* gene.

Conclusions: The results of this study provide detailed information about the natural history of LINCL. The clinical progression of LINCL seems to be independent of the mutation involved.

Evidence of Autophagy in Human CLN6 Fibroblasts

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In all forms of NCL, but one, cells accumulate and store ceroid lipofuscin, which appears mostly to be comprised of dolichol lipids and the hydrophobic protein, subunit c of mitochondrial ATP-synthase. Neuronal death due to impaired cellular function accounts for the progressive cerebral atrophy and retinal degeneration. Pathogenetic mechanisms leading to cell death are not yet understood, even if some evidences for a primary role of apoptotic cell death have been provided in knock-out mice (and to a lesser in humans). Cell compartments, such as the mitochondrial network and the endoplasmic reticulum, are also affected in these conditions, providing a further contribution to the impairment of cell viability. *CLN6* codes for a polytopic membrane protein, localized in the endoplasmic reticulum (ER), which can dimerize; its sequence is highly conserved, but its function it is still unknown. Mutations in *CLN6* are associated with either variant Late Infantile (vLI) or adult onset NCL, with clinical similarities and striking differences in terms of disease courses. After reviewing peripheral, extra-cerebral biopsies from 5 molecularly identified CLN6 patients (3 vLI and 2 adults), we detected ultrastructural findings consistent with autophagy in some cases. Investigations on the behaviour of the lysosomal compartment in fibroblasts obtained from 5 CLN6 patients showed a remarkable distortion and enlargement of the ER cisternal network ultrastructurally, along with the presence of wide vacuoles lined by a single membrane, either empty or containing cytoplasmic material. No lysosomal aggregates were detected within the enlarged ER cisternae. Ultrastructural presence of the phagophore and the autophagosome was consistent with the autophagic origin of the vacuoles. These findings were supported by the presence of punctate LC3-positive cells (immunofluorescence) and decreased LC3-I/LC3-II ratio (Western blotting) in basal condition. The unchanged LC3-I/LC3-II ratio seen following Rapamycin treatment (an inducer of autophagy) was consistent with the hypothesis of saturated autophagic activity in CLN6 cells. Markedly increased lysosomal compartment was shown ultrastructurally and confirmed by using cellular markers of the acidic organelles (Lysotracker) and lysosomal membranes (LAMP2) only in fibroblasts from variant LINCL cases. Our results agree with previous evidence that defects in ER-resident CLN6 protein lead to lysosomal dysfunction and storage. Likewise the lysosomal (as well as proteasomal) pathways seem to be sufficient to prevent the aggregation of the mutant CLN6 polypeptides in the ER. Autophagy represents a rescue mechanism to facilitate the removal of oligomeric complexes of misfolded proteins; however, it may become a negative event for the cell, leading to self-digestion and death. The significance of the activated autophagy, and the relationship between stressed ER and autophagy in CLN6 disease is still lacking, since these findings have never been reported in neuronal cells. Improved knowledge of the mechanisms correlating ER stress with autophagy and lysosomal accumulation may help to identify biomarkers characterizing the likely different disease mechanisms explaining vLI and adult forms of CLN6.

Studies on CLN3 – A practical approach to its structure

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The full-length CLN3 has been described as a hydrophobic and highly glycosylated integral membrane protein of unknown function. Many efforts have been made in defining its membrane topology; controversial experimental and computational studies suggest up to eleven transmembrane domains. In this respect, it is fundamental to determine the CLN3 structure at an atomic resolution to get a better understanding of its function(s). Aiming for an x-ray structural analysis of CLN3, we are working on the production of the human CLN3 protein using the continuous exchange cell-free expression system based on *Escherichia coli* extracts. In contrast to conventional heterologous protein expression methods, our system (i) avoids cytotoxic effects and metabolic problems caused by protein overexpression in living cells, (ii) lacks membrane barriers, (iii) allows the supply of compounds for stabilizing the protein, and (iv) permits the direct incorporation of the produced CLN3 into membranes of chosen composition.

Results

We established the expression of the full-length human CLN3 protein in analytic scale. Furthermore, we were able to solubilize and isolate the product from the cell-free reaction mixture. We are currently up-scaling the production, aiming to yield the preparative amounts of pure CLN3 required for crystallization screens. Addition of detergents to the cell-free reaction is also underway in order to directly obtain the soluble and folded form of the protein.

Conclusions

The cell-free expression system has shown its benefits towards the production of CLN3. Further characterization of this membrane protein is required for a full comprehension of its biological function(s). Additionally, precise structural information would assist in the development of high-affinity antibodies. As a distant goal, revealing the structure might even provide angles for novel therapy strategies.

Molecular events underlying the endocytic defects found in JNCL.

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Juvenile neuronal ceroid lipofuscinoses (JNCL) is the most common neurodegenerative disorder in children. JNCL is caused by mutations in the *CLN3* gene, which encodes CLN3p, a protein of unknown function. Cell lines derived from patients or mice with CLN3p deficiency have impairments in actin regulated processes such as autophagy, vesicular trafficking, clathrin-independent endocytosis, and wound healing. Consistent with these phenotypes, CLN3p has been shown to associate directly or indirectly with the actin regulatory proteins myosin-IIb, fodrin, and hook1. We hypothesize the link between the impaired cellular processes and these actin regulatory proteins is the small GTPase, Cell division control protein 42 (Cdc42). Activated Cdc42 recruits effectors such as p21-activating kinase-1 (PAK-1), LIM domain kinase (LIMK) and Wiskott-Aldrich syndrome protein (WASP) to membranes promoting actin polymerization. These actin polymerization events cause filopodia formation, neuronal growth cone maintenance, and endocytic vesicular trafficking to occur. Cdc42 is a regulator of myosin-IIb, fodrin, hook1, LIMK and WASP, and is therefore poised to impact these processes if inappropriately activated.

Conclusions: Here we show that Cdc42 is constitutively active in multiple CLN3p deficient cell lines causing misregulation of downstream signaling events and multiple actin dependent processes**.** Investigations into Cdc42 activity in JNCL cells may provide insight into how CLN3p deficiency impairs normal cellular physiology and provide a novel target for therapeutic development.

The MGH NCL Patient Biorepository: a shared resource to test and generate hypotheses about NCL pathophysiology

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Introduction
Significant progress has been made in the animal modeling of NCL, but ultimately, mechanistic hypotheses about and therapeutic approaches to the disease must be validated in patient-derived material. To this end, we have consolidated and begun to characterize a significant biorepository of lymphoblastoid and fibroblast cell lines representing most defined molecular subtypes of NCL, as well as undefined forms. A valuable resource that has arisen from this effort is a collection of induced pluripotent stem (iPS) cell lines derived by reprogramming of patient fibroblasts. We have used both fibroblast and iPS lines to probe the possible interaction between autophagy and mitochondrial homeostasis suggested by our previous phenotypic characterization and gene expression analysis of cerebellar cell lines derived from a genetically precise knock-in murine model of juvenile NCL (JNCL, CLN3).

Results
Electron microscopic analysis of iPS cell lines from 2 unrelated patients with *CLN3* mutations revealed a robust accumulation of large vacuoles that likely correspond to the structures highlighted by the lysosome-specific marker LAMP1, suggesting improper resolution of autophagolysosomal structures. GRP75 staining of CLN3 fibroblast and iPS cells revealed an elongation and reticulation of the mitochondrial network that was more extensive than in control cells, possibly reflecting abnormal mitochondrial turnover or biogenesis, consistent with previous observations in murine CLN3-deficient cells. Analysis of fibroblasts from an atypical and unusually early-onset case of CLN5 also carrying a variably penetrant *POLG1* mutation showed significantly decreased mtDNA copy number (~30% reduction), decreased mitochondrial membrane potential (~50% reduction), and increased accumulation of LC3 (~12-fold increase) compared to control cell lines.

Conclusion
Phenotypes in human JNCL fibroblasts and iPS cells further validate murine JNCL genetic models and provide additional support for early defects in mitochondrial and endosomal-lysosomal physiology in human JNCL and possibly other forms of NCL, such as CLN5. These cell culture models are valuable tools for generating additional hypotheses about NCL pathophysiology as well as assaying potential targets of early pathology in NCL and other neurodegenerative disorders in which the roles of autophagic and mitochondrial pathology are becoming increasingly evident.

Creating fission yeast strains to identify new therapeutic targets for Batten disease

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The gene underlying classic juvenile NCL in humans (*CLN3*) encodes a polytopic membrane spanning protein of unknown function. CLN3 has been implicated in a diverse array of cellular processes, including lysosomal homeostasis, autophagy, trafficking, lipid modification and cytoskeletal organisation. Due to the apparent complexity of CLN3 function, the study of these processes in simple model systems continues to prove highly informative. The fission yeast *Schizosaccharomyces pombe* provides an ideal model organism for the study of CLN3 function, due to its simplicity, genetic tractability and the presence of a single orthologue of CLN3 (Btn1). Btn1 is 30% identical and 48% similar to human CLN3, and exhibits a functional profile comparable to its human counterpart. Further, it has been demonstrated that CLN3 can complement for the loss of Btn1 in *S. pombe*, demonstrating that these two proteins are functional orthologues.

A number of studies have highlighted the importance of the extent of CLN3 and Btn1 expression in the accurate analysis of protein localisation and function. Despite the advances made in determining the function of Btn1 in *S. pombe*, the majority of these studies used ectopic expression of *btn1* from strong promoters. Genomic integration of *btn1* mutants would be highly desirable, providing information more representative of the pathological phenotype. In addition, the integration of *gfp-btn1* fusions should allow more accurate analysis of Btn1 localisation.

A series of strains containing *gfp-btn1* fusions and mutations in *btn1* previously characterised in *CLN3*, including the most common 1-kb intragenic deletion and others with milder effects on the phenotype, are being derived. A comparison between these different strains (wild-type; *btn1*Δ, where *btn1* is deleted; a mutant containing the 1-kb deletion; and a mutant containing the equivalent to the mild p.E295K mutation) for gene expression and the rescue of the appropriate marker phenotypes will further elucidate the function of Btn1 and the pathways it mediates, and also, will disclose novel targets or tools for therapy.

Altered protein prenylation, mevalonate pathway and peroxisomes in JNCL

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The juvenile form of Neuronal Ceroid Lipofuscinosis (JNCL) is caused by mutations in CLN3, a protein of unknown function. Consequently, the exact pathological mechanism of JNCL on a molecular level remains unraveled. In JNCL, accumulation of dolichol is indicative for a defect in its utilization and/or production. Dolichol is generated from isoprenoid lipid farnesyl pyrophosphate (FPP) via the mevalonate pathway. FPP and its derivative geranylgeranyl pyrophosphate (GGPP) are substrates for protein prenylation, a posttranslational modification of membrane-targeted proteins. In our previous study we have determined elevated levels of FPP and GGPP in the brain of an adult $Cln3^{\Delta ex7/8}$ mouse. Acknowledging the close relation of dolichol and isoprenoid lipids, this indicates a general defect in the prenylation machinery and/or mevalonate pathway under JNCL physiology. Current knowledge on the localization of the prenylating enzymes is ambiguous, while the key enzymes of the mevalonate pathway are reported to localize in the peroxisomes. In this study, we investigated the membrane association of normally prenylated proteins, and screened for alterations in the subcellular localization of the enzymes catalyzing the reactions of protein prenylation and the mevalonate pathway.

Results

Fractionation analyses on $Cln3^{\Delta ex7/8}$ mouse brain showed a decrease in the membrane association of normally prenylated proteins. This indicates that these proteins are in an inactive, soluble form and thus not fully capable of performing their function. An immunofluorescence-based screening using Multi-Epitope-Ligand-Cartography (MELC) on cerebellar granular cells from a wild-type mouse showed that the prenylating enzyme farnesyl transferase, the key enzymes of the mevalonate pathway, and the CLN3 protein all localized in the peroxisomes. The level of the localization was altered in cerebellar cells from a $Cln3^{\Delta ex7/8}$ mouse. Additionally, the subcellular distribution of peroxisomes was altered in the $Cln3^{\Delta ex7/8}$ cells, which is indicative for a novel, severe phenotype in JNCL.

Conclusions

We conclude that i) protein prenylation is defective in JNCL, ii) the CLN3 protein could be associated with the events of protein prenylation and the mevalonate pathway, and iii) peroxisomes might be a location of relevant events regarding the mechanisms underlying JNCL.

Increased expression of TNF-α, IL-1β, TGF-β and IL-10 in the brains of sheep with CLN6 NCL

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Traditionally it has been thought that neurodegeneration in NCL is a direct consequence of the accumulation of storage material and lack of the enzyme activity in lysosomes. However time course studies of sheep affected with the CLN6 form revealed glial activation in prenatal, presymptomatic ovine brain which proceeded in a progressive, regionally specific manner. This was followed by neurodegeneration that also developed regionally following the same order, implicating a primary role for glial activation in pathogenesis.

Glial cells are both the primary source and target of many cytokines in the CNS, and the balance between pro- and anti-inflammatory cytokines is crucial in the escalation and resolution of the inflammatory cascade. Failure or dysfunction of anti-inflammatory control mechanisms may play a role in the establishment of a chronic neuroinflammatory response.

Expressions of the pro- and anti-inflammatory cytokines, TNF-α and IL-1β, and TGF- β and IL-10, respectively, were measured via qPCR and Western in different regions of the brain blot and at different stages of disease development to gain an insight into changes in cytokine expression with disease progression.

Results: Western blot analysis revealed that all four cytokine concentrations were increased in the affected cortex, but not in affected spleen, indicating specific neuroinflammation. Cytokine mRNA expressions were elevated in frontal, parietal and occipital affected cortical regions at 6 months, well before clinical disease is evident, indicating that the altered gene expression began before detectable neuronal loss and acute disease symptoms. Expression increased further until 18 months and to similar relative extents, despite big differences in copy numbers, varying from 600 copies/µl cDNA (*IL-10*) to greater than 94,000 copies/µl cDNA (*TGF-β*), after which expressions of both IL-10 and TNF-α declined.

Conclusions: These data highlight a progressive and atypical increase in cytokine expression in affected animals, suggesting that an uncontrolled inflammatory response is underway and indicate an involvement of these cytokines in neuroinflammation.

Behavioural studies and magnetic resonance imaging in the Merino sheep NCL model

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Neuronal ceroid lipofuscinoses (NCL) have been reported in a variety of animal species and some of these species have been used extensively as models for the disease in humans. Animal studies, especially in large animals, are important for improving the understanding of the disease mechanisms as well in therapeutic pre-clinical trials. Merino sheep affected with NCL have a missense mutation (c.184C>T) in the ovine CLN6 gene and are recognised as a model for late-infantile neuronal ceroid lipofuscinosis (vLINCL) in humans. To develop disease progression measures in these sheep for future therapeutic trials, behaviour observation and magnetic resonance imaging (MRI) studies were conducted in the NCL Merino research flock in 2010 and 2011. After a preliminary behavioural trial in 2010, in 2011 nine observation sessions on 4 affected, 2 carrier and 2 control Merino sheep, which occurred between 26 to 54 weeks of age, were conducted to observe posture, behaviour and reaction to visual and auditory stimuli, weight measurements, as well as activity measurements using an IceTag™ activity sensor. For the MRI study, sheep were scanned using T2, FLAIR and 3D T1 isometric volume imaging on a Esaote VetGrande 0.25 Tesla system at about 5 (n=2 affected and 2 controls), 8 (n=3 affected and 3 controls) and 14 (n=3 affected and 3 controls) months of age to identify changes in the grey and white matter and volumetric measurements.

Results

When comparing NCL affected lambs with controls in the behavioural study over time, NCL affected sheep showed changes to their feeding behaviour, had a slower increase in live weight, showed an increase in the average number of steps taken per day and exhibited a gradual decline in the performance of 'appropriate' flocking behaviour response to visual stimuli. Preliminary MR findings indicate that a number of MR imaging changes are already present at 5 months of age, including enlargement of the ventricles, cerebral atrophy.

Conclusions

Preliminary analyses suggest that both behavioural studies and MRI can be useful in measuring changes to disease progression in future pre-clinical therapeutical trials. Both approaches allow repeated observation in sheep over extended periods of time and thus will be useful to reduce the number of animals needed in future trials. Further work is needed to develop appropriate grading systems to attempt to quantify the degree of change over time seen in both MRI and behavioural studies.

Induced pluripotent stem cells (iPSCs) as a model for NCL diseases

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Reprogramming of mouse and human somatic cells to a pluripotent state, described by Takahashi and Yamanaka (Cell 2006), represent a powerful method to generate patient-specific cell lines to be used in disease modeling, for example in neurodegenerative diseases. Neuronal ceroid lipofuscinoses (NCLs) offer a well studied model to test the possibilities of the induced pluripotent stem (iPS) cell technology both in neurological research and in medicine.
The aim of the study is to characterize and compare iPS cells derived from the NCL mouse models and human patients. The iPS cells will be grown from fibroblasts of human CLN1 and CLN5 patients, both diseases enriched in Finnish population, as well as from mouse embryonic fibroblasts (MEFs) of the respective *Cln1* and *Cln5* deficient mouse models previously established by the research group. These iPS cells will be characterized for their embryonic stem (ES) cell like properties. Fully reprogrammed iPS cells are then differentiated into neural precursor cells (NPCs) and further into neurons and glial cells, which represent the most affected cell types in these disorders.

Results We are currently characterizing the iPS cells derived from *Cln1*$^{-/-}$ and *Cln5*$^{-/-}$, as well as from *Cln1*$^{-/-}$/*Cln5*$^{-/-}$ MEFs. The iPS colonies show ES cell like morphology and express ES cell specific surface markers alkaline phosphatase (AP) and SSEA-1. RT-PCR confirmed the expression of endogenous ES cell marker genes, as well as the silencing of the transgene expression. Embryonic bodies (EBs) formed from iPS cells are analyzed for expression of all three embryonic germ layer markers by immunocytochemistry. EBs are further differentiated *in vitro* into specific cell types, particularly to neurons, glial cells (astrocytes, oligodendrocytes, microglia) and macrophages. We will next proceed with the human NCL patient fibroblasts, and finally, compare the feasibility of these different iPS cells as disease models.

Conclusions These data will provide us better knowledge of the validity of mouse and human iPS cells as a disease model for NCL, and may help us finding new disease pathways that hopefully will also benefit future therapy for NCL.

Clusters of newly generated neurons in the cortex of sheep and human CLN6 deficiency

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Mounting evidence points towards altered levels of neurogenesis in disorders that display pronounced neuron loss, and may represent an attempt at brain repair. Compared to mouse models the degree of neuropathology, and it's regionally selective nature, are particularly pronounced in sheep models of NCL. As such, we undertook an immunohistochemical survey of markers for newly generated neurons in the neurogenic niches and cortex of CLN6 deficient South Hampshire sheep, comparing our findings with human CLN6 cases. Staining for doublecortin and PSA-NCAM was largely unaltered in the dentate gyrus, but was progressively increased in the subventricular zone (SVZ), which appeared thicker in CLN6 sheep. Numerous positively stained cells appeared to exit the SVZ, but there was no evidence for any overt migration of these cells over significant distances. Surprisingly, however, immunostaining also revealed dense clusters of doublecortin positive cells and fibres within the cortex of CLN6 sheep, which become more complex with disease progression. Viewed in Nissl stained sections these cell clusters were prominent along the boundary of lamina II of the entorhinal and parieto-occipital cortex, the cortical region where pathology is most pronounced in these affected sheep. More detailed analysis revealed that these cell clusters were composed of cells at different stages of differentiation, with smaller cells in their centre staining positively for immature neuronal markers like Sox2 and doublecortin, but not for GFAP. Staining for the neuronal marker NeuN, a marker of more mature neurons, was observed at the outer margins of the cell clusters, and many of these NeuN expressing cells appeared to receive synaptophysin positive synaptic inputs. Preliminary data reveal evidence for similar cell clusters in the CNS of CLN5 Borderdale sheep and human CLN6 deficiency, but similar structures were absent in NCL mouse models. Taken together these data provide novel evidence for the generation of new neurons in more than one form of NCL, but are only apparent in species where cortical pathology is especially pronounced. It is not clear what mechanism(s) underlie these events or whether these can be manipulated for therapeutic ends.

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Immune cells are pathogenic mediators in the visual system of two mouse models of neuronal ceroid lipofuscinosis

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The NCLs display pronounced neurodegeneration, often with early affliction of the visual system. Neurodegeneration is typically accompanied by a low grade of inflammation, the pathogenic impact of which is not clear. We are investigating the role of inflammatory cellular components (lymphocytes and microglia) in two mouse models of NCL: early onset Infantile NCL (*Ppt1*$^{-/-}$ mice) and the slower progressing Juvenile NCL (*Cln3*$^{-/-}$ mice).

First, we characterised the types of immune cells present within the optic nerve and retina, and found evidence for progressive infiltration by different classes of immune cells in both forms of NCL. In *Ppt1*$^{-/-}$ mice there is a significant infiltration of CD8+ cytotoxic effector T-cells (and to a lower extent CD4+ Helper T-cells) that occurs early in disease progression when neuron loss starts. Consistent with the later onset of juvenile NCL, neuroinflammation occurs much later in *Cln3*$^{-/-}$ mice (from 15 months of age onwards). Both models also display microglial activation, with upregulation of the macrophage cell recognition molecule sialoadhesin (*Sn*), which is supposed to interact with T-lymphocytes.

To analyse the pathogenic impact of lymphocytes, we crossbred *Ppt1*$^{-/-}$ mice with mice deficient in *Rag-1*, (which lack T- and B-lymphocytes) and scored axonal damage and neuronal survival in the optic nerve and retina using immunohistochemistry, electron microscopy and retrograde labelling techniques. In *Ppt1*$^{-/-}$ mice, progressive neuropathic alterations of retinal ganglion cells were detected from 3 months onwards but were significantly reduced in *Ppt1*$^{-/-}$*Rag-1*$^{-/-}$ mice. Moreover, bone marrow transplantation experiments revealed a crucial role of CD8+ cytotoxic T-cells in the formation of these neuropathic alterations. Next, we analysed the impact of microglial cells in INCL by crossbreeding *Ppt1*$^{-/-}$ and *Sn*$^{-/-}$ mice and found that axonopathic changes were also robustly diminished in the absence of *Sn*. In addition to the amelioration of morphologic alterations, immune-compromised *Ppt1*$^{-/-}$ mice presented with improved clinical phenotype and a substantially extended survival time.

Similarly, when we investigated 18 month old *Sn*-deficient *Cln3*$^{-/-}$ mice, we also observed significantly reduced axonal damage and improved neuronal survival in the optic nerve and retina compared to *Cln3*$^{-/-}$ mice with Sn+ microglia, indicating a similar detrimental contribution of the innate immune system in models of different forms of NCL.

These observations suggest that both lymphocytes and microglia contribute to pathogenesis in *Ppt1*$^{-/-}$ and *Cln3*$^{-/-}$ mice. These studies are providing significant insights into the pathogenesis of individual forms of NCL and may provide information crucial for developing treatment strategies to block lymphocyte/microglial activation and foster neuronal survival.

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A cell-based screen to identify modifiers of autophagy in JNCL

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Juvenile NCL (JNCL) is caused by mutations in *CLN3*. The most common *CLN3* mutation is a ~1kb deletion eliminating exons 7 and 8. We have previously generated a mouse model that recapitulates the common JNCL mutation, and we have established cerebellar neuronal precursor cell lines from these mice. Using these accurate genetic models of JNCL, we have previously shown evidence of early onset autophagy pathway disruptions that likely lead to the accumulation of the c subunit of the mitochondrial ATP synthase, though the downstream impact of the autophagy defects on neuronal cell survival requires further study. Nevertheless, an improved understanding of the pathways that regulate the autophagy defects in JNCL is likely to give important new insight into JNCL pathophysiology and potential strategies to delay or ameliorate disease. Therefore, we have established wild-type (Cb*Cln3*$^{+/+}$) and homozygous mutant (Cb*Cln3*$^{\Delta ex7/8/\Delta ex7/8}$) cell lines stably expressing GFP-LC3, a well-known autophagy marker, and a cell-based screening assay using these cells that will aid in the identification of JNCL disease modifiers.

Results: Under normal growth conditions, we first established that Cb*Cln3*$^{\Delta ex7/8/\Delta ex7/8}$ cells had ~2-fold more GFP-LC3-labeled vesicles, compared to wild-type cells. To identify drugs that would significantly increase or decrease the number of GFP-LC3 labelled vesicles in our Cb*Cln3*$^{\Delta ex7/8/\Delta ex7/8}$ cell system, we then screened several focused compound libraries and an unbiased library of known FDA-approved drugs and natural products. Numerous hit compounds were identified in our screen, particularly from the focused libraries, that significantly affected GFP-LC3 vesicle accumulation in both wild-type and homozygous Cb*Cln3*$^{\Delta ex7/8}$ cells. A comparison of our hit list with those reported by other groups who have similarly performed GFP-LC3 small molecule screens in other cell systems revealed strong overlap, giving us confidence in our newly developed assay. Further analysis of our screening data and follow-up dose response studies revealed that only one of our hit compounds exhibited genotype-selectivity, whereby homozygous Cb*Cln3*$^{\Delta ex7/8}$ cells were more sensitive to treatment. The genotype-selective compound was identified as thapsigargin, which inhibits SERCA, a calcium channel of the ER, and is known to thus raise cytosolic calcium levels from intracellular stores. This mechanism of action was validated in our cerebellar cell culture system.

Conclusions: Given the altered sensitivity of the homozygous Cb*Cln3*$^{\Delta ex7/8}$ cells to thapsigargin, we hypothesize that the common JNCL mutation in the *CLN3* gene leads to early defects in calcium homeostasis. A possible role for CLN3 in calcium-mediated events is supported by previous studies demonstrating CLN3 interaction with calsenilin, a neuronal calcium-binding protein, and a potential alteration in intracellular calcium levels in JNCL patient cells.

Metal accumulation and activation of cellular signaling pathways in disease-affected brain regions of ovine CLN6 neuronal ceroid liopfuscinosis

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Mutations in the *CLN6* gene cause a variant late infantile form of neuronal ceroid lipofuscinosis (NCL). *CLN6* mutations result in loss of function of the endoplasmic reticulum resident CLN6 protein and lead to disease clinically characterized by vision impairment, motor and cognitive dysfunction, and seizures. Accumulating evidence suggests that alterations in metal homeostasis and aberrant cellular signaling pathways are implicated in several neurodegenerative and developmental disorders, yet little is known about their role in the NCLs. To explore the disease mechanisms of CLN6 NCL, metal levels and expression of proteins implicated in cellular signaling pathways were assessed in brain tissue from the South Hampshire ovine CLN6 model. Metal level analyses revealed robust zinc and manganese accumulation in disease-affected regions of CLN6 ovine brain at 12-14 months of age, particularly in the occipital and parietal lobes. Altered expression of zinc transporters was also identified in the affected brain regions. These changes occurred at the time of observable symptomatic disease onset and concomitantly with alterations of synaptic proteins and aberrant modulation of the Akt/GSK3 and ERK/MAPK cellular signaling pathways. Taken together, these results demonstrate that altered metal homeostasis, synaptic protein loss, and disturbances of cellular signaling pathways are early characteristic features and part of the pathogenic mechanisms that occur in the CLN6 form of NCL. Therapeutic approaches aimed at restoration of metal homeostasis and inhibition of aberrant kinase activation may be beneficial and are clearly attractive targets for further study.

Investigating behavioral and biochemical correlates of disease in CLN6 mice

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Mutations in the CLN6 gene cause a variant late infantile form of neuronal ceroid lipofuscinosis (vLINCL). The CLN6 gene codes for a highly conserved protein that localizes to the endoplasmic reticulum. The function of the CLN6 protein is currently unknown. Affected children develop epilepsy and neuronal degeneration that lead to loss of vision and progressive mental and motor deterioration. The nclf (CLN6) mouse is homozygous for a 1-bp insertion that causes a frame-shift mutation in the CLN6 protein after P105. This same mutation has been found in vLINCL patients, and the CLN6 mouse recapitulates many of the features seen in vLINCL including progressive retinal atrophy, widespread cortical astrocytosis, accumulation of storage material, Wallerian degeneration of the spinal cord and brain stem, hindlimb paralysis, and terminal seizures. This model provides a powerful tool for investigating the underlying pathological basis of vLINCL and related forms of NCL. However, there is currently no detailed behavioural analysis of this model and only limited investigation of molecular and cellular pathology.

Results: We are performing a detailed longitudinal study of disease-associated behaviour including assessment of motor coordination and balance using rotarod, stride-length analysis, test of grip strength, and visual assessment. In parallel, tissues from mice are being examined at 1, 3 and 6 months and end stage for molecular and cellular markers of early neuronal degeneration. In preliminary studies on the South Hampshire ovine CLN6 model we have observed substantial alterations to regional brain zinc, manganese and cobalt levels and correlative metal transporter expression and cell signal kinase activity. Therefore, we are currently investigating metal homeostasis, metal transporter expression, and cell signaling pathway activity in comparative CLN6 murine tissues together with the correlative behavioural changes.

Conclusions: These studies may offer novel insights into molecular changes associated with CLN6 protein loss early in disease progression.

Canine neuronal ceroid lipofuscinoses as models for understanding disease mechanisms and developing therapies for the human NCLs

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Animal models have played key roles in helping us to develop a better understanding of the mechanisms that underlie the neuronal ceroid lipofuscinoses (NCLs) and are currently being used to assess potential approaches for treating or curing people with these disorders. Mouse models have been developed for most of the NCLs. While these models have been of great value, they are of limited utility in the development of therapeutic interventions. The limitations of the mouse models stem largely from the small size and simplicity of the mouse central nervous system relative to that of humans. The size, structure and complexity of the central nervous systems of dogs are much more similar to those of humans. Therefore dogs are likely to be better models for assessing and optimizing potential therapeutic interventions for the NCLs.

NCL has arisen through spontaneous mutation in many dogs. Various forms of NCL have been documented in at least 15 different dog breeds to date. The first canine cases of NCL were described in English Setters by Nils Koppang in the 1950s. Dr. Koppang developed a research colony of NCL-affected English Setters that were maintained and studied for many years. Subsequently, many cases of canine NCL were reported in the veterinary literature, but none of the other early NCL cases led to the development of enduring research models.

Advances in molecular genetic technology, and the generation of the canine genome reference sequence, and the identification of the mutations responsible for most of the human NCLs have facilitated efforts to identify the genetic bases of the canine NCLs. . In 2005, the mutations responsible for NCL in English Setters (*CLN8*) and Border Collies (*CLN5*) were discovered. Subsequently, NCL-causing mutations were identified in American Bulldogs (*CTSD*), Miniature Dachshunds (*CLN2/TPP1* and *CLN1/PPT1*), Australian Shepherds (*CLN6*), Tibetan Terriers (*ATP13A2*) and American Staffordshire Terriers (*ARSG*). Research is currently under way to identify the NCL mutations in other dogs with recently reported cases of NCL.

Of the canine NCLs, only the Miniature Dachshund with a recessive null mutation in *TPP1* has been developed as a model for research. The *TPP1* mutation was identified in a pet dog that died at approximately 11 months of age. Both parents of the affected dog were located and were acquired to establish a research colony. This colony has been developed over the past several years and is now at the point where 2 to 3 affected puppies can be produced each month. These dogs are currently being employed to assess enzyme replacement and gene therapy approaches for treatment. Early results from these studies suggest that one or both approaches are likely to be efficacious in treating children with late infantile NCL.

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Spatio-temporal analysis of glial activation and neuron loss in the Cln8 mouse model of neuronal ceroid lipofuscinosis

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Neuronal ceroid lipofuscinoses (NCLs; Batten Disease) are inherited lysosomal storage diseases, which are the most common cause of neurodegeneration in children. Mutations in the *CLN8* gene have been described in Finnish and Mediterranean NCL patients. The *Cln8* deficient *motor neuron degeneration (mnd)* mouse recapitulates the major hallmarks of the human disease, and is used as a model system to study the pathology in CLN8 deficiency.

To understand the pathogenesis of CLN8 deficiency we have here characterized the central nervous system of *mnd* mice*,* especially at early stages of disease progression. Through a combination of quantitative histology and mRNA gene expression profiling, we aim to form a comprehensive picture of the regional, cellular and molecular disease progression in the *Cln8*^{mnd} mouse brain. Based on previous studies of other NCL mouse models, we have concentrated primarily on the sensory thalamo-cortical pathways, in which the extent and timing of glial activation and neuron loss were quantified. Neurons were counted stereologically and glial activation monitored by using GFAP and CD68 as markers for reactive astrocytes and activated microglia, respectively. The age points studied covered the disease pathology from early to moderately symptomatic (1, 3 and 5 months) and late symptomatic (8 months) mice.

In *Cln8* deficiency, the somatosensory pathway comprising the thalamic ventral posterior nucleus and the primary somatosensory cortex was found to be the most affected relay system. Astrocytosis and microglial activation were apparent from 5 months of age. Loss of thalamic relay neurons accompanied glial activation at this age, and became more pronounced with disease progression. Similarly, although appearing later in the disease, visual relay neurons in the lateral dorsal geniculate nucleus, were lost before their target neurons in the primary visual cortex.

In order to identify the molecular changes leading to the observed neuron loss and astrocytosis, we have performed mRNA microarrays of cerebral cortical and thalamic tissue of *mnd* mice and this analysis is ongoing.

In conclusion, thalamic sensory relay nuclei are affected before their cortical targets, which is most pronounced in the somatosensory system. The relatively delayed pathology of the visual thalamocortical pathway distinguishes *Cln8* deficiency from previously studied NCL models. Thus, in *Cln8* deficiency, spatio-temporal disease progression shares similarities, but also differs from other forms of NCL disease.

In vitro modeling of juvenile neuronal ceroid lipofuscinosis (JNCL): Patient fibroblasts and their reprogrammed derivatives as human models of JNCL

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JNCL (Batten disease) is caused by mutation of *CLN3*. It has a prevalence of 1 in 100,000 and usually arises between 4 and 10 years of age. First symptoms include considerable vision loss due to retinitis pigmentosa, associated with seizures, psychological degeneration and premature death. New animal models of JNCL provided the ability to study neuropathology during the course of disease. Nevertheless, major questions about molecular pathogenesis remain unanswered. There is thus a pressing need of a human cell model of JNCL.

The development of induced pluripotent stem (iPS) cells by Takahashi and collaborators has revolutionized the work on *in vitro* cell models for common human diseases. Researchers are able to generate any kind of cell type that might be affected in a given disease such as neurons to gain insights into disease pathophysiology.

Thus, we have developed JNCL-specific induced pluripotent stem cells (iPSCs) by reprogramming skin fibroblasts. Patient fibroblasts were infected with retroviruses encoding four transcription factors, *OCT4*, *SOX2*, *KLF4* and *cMYC*. Retroviral silencing, karyotyping, immunocytochemistry, and germ layer differentiation (meso-, endo- and ectoderm) gave insights about the quality of the generated iPSCs.

Through phenotyping of the JNCL iPSC lines we have found that, as in the murine model of JNCL, human JNCL iPSCs display abnormalities in the lysosomal and mitochondrial subcellular compartments. To further investigate the specific consequences of CLN3 deficiency in human JNCL neurons, we have generated neural precursor cell (NPC) lines as well as subtype-specific neurons from the diseased iPSC lines. Experiments on the molecular pathogenesis of those cell types which are mainly affected by JNCL will give further insights into the disease mechanism.

Results: Generation and phenotypic analysis of iPSC and NPC lines from patients suffering from mutations in the *CLN3* gene.

Conclusions: Patient fibroblast can be reprogrammed into iPSCs, which can give rise to NPCs and subtype-specific neurons, thus providing a suitable cell culture model for JNCL.

Retinal degeneration and microglial activation in mouse models of neuronal ceroid lipofuscinoses

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Purpose: Neuronal ceroid lipofuscinoses (NCL) are early onset lysosomal storage disorders characterized by vision loss, mental and motor deficits, and spontaneous seizures. Notably, massive accumulations of autofluorescent material in neurons lead to progressive neuronal degeneration and cell loss. Neuropathological analyses of human autopsy material and brain from NCL animal models revealed neuroimmune processes closely associated with neuronal degeneration. It is currently unclear whether this phenomenon is confined to the brain or also occurs in the retina. The aim of our study was to characterize the relationship between retinal degeneration and microglia activation in different mouse models of NCL.

Methods: Retinal degeneration of the NCL mutant mouse strains $Cln3^{\Delta ex7/8}$ KI and $Cln6^{nclf}$ was characterized by detailed structural analyses at different ages. Microglial morphology and migration was analyzed by immunohistochemistry. Visual acuity and retinal function was determined by measuring the optokinetic response in an Optomotry system and by use of electroretinography, respectively. Retinal and brain neuro-degenerative gene expression markers were compared in effort to determine if degeneration occurs in both tissues co-incidentally.

Results: Our data show that there is a migration of microglia from the plexiform layers to the nuclear layers in $CLN6^{nclf}$ and $Cln3^{\Delta ex7/8}$ KI retinas, which is consistent with an alerted state of microglia. Moreover, the shape of these cells changed from a ramified form to an amoeboid form. Histological analyses revealed that this microglial activity was accompanied by a prominent retinal degeneration. Optomotry tests showed that the $CLN6^{nclf}$ and $Cln3^{\Delta ex7/8}$ KI mice had a progressive decline in visual acuity as they aged. These results were further confirmed by electroretinograms. Finally, neuro-inflammatory markers, notably microglial activation markers, are expressed earlier and at higher levels in $CLN6^{nclf}$ mouse retina compared to brain.

Conclusions: Our results identified a coincidence of microglia activation, retinal degeneration, and vision loss in $CLN6^{nclf}$ and $Cln3^{\Delta ex7/8}$ KI mice. Furthermore, the early expression of neuro-inflammatory markers in the retina suggests therapies aimed at modulating these markers could be helpful in preserving vision and ameliorate neuronal brain degeneration in NCL patients.

Posters: Theme 2

Murine model of variant late infantile neuronal ceroid lipofuscinosis recapitulates disease progression

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Collectively, the neuronal ceroid lipofucinoses (NCLs) are the most common neurodegenerative pediatric disorder. There are currently ten variants of this lysosomal storage disorder, which are classified clinically based upon age of onset and progression of disease symptoms. Further pathological classification is based upon ultrastructrual patterns of accumulated storage material within the lysosomes as well as the sequence analysis of the mutated gene. Mutations in *CLN6* result in variant late infantile onset neuronal ceroid lipofuscinosis (vLINCL). The protein product of the *CLN6* gene, CLN6, is an endoplasmic reticulum membrane protein with an unknown function. In this study we set out to characterize the disease condition of the Cln6^{nclf} mouse and validate its use for the study of vLINCL. Using qRT-PCR we validate the stable expression of a mutant mRNA of a novel truncated Cln6 protein in the Cln6^{nclf} mouse. Using the rotarod we further demonstrate that the mutant mouse exhibits deficits in motor coordination prior to the onset of previously reported paralysis seen in the mouse. In conjunction with the motor deficit decline in the mouse we present evidence that there is a loss of neurons within specific regions and lamina of the cortex correlation to motor coordination. In addition to laminar thinning within the cortex we also report astrocytic activation within the cortex and specific nuclei of the thalamus. Loss of specific GABAergic interneuron populations are also seen within defined regions of the cortex as well as the hippocampus. Finally, we provide evidence for a loss of dendritic spines found on the apical dendrite of glutamatergic pyramidal neurons within the S1BF and motor regions of the cortex. Together this data demonstrates that the Cln6^{nclf} mouse recapitulates many of the disease phenotypes seen within other animal models of vLINCL, other NCLs, as well as human vLINCL cases. These results confirm that the Cln6^{nclf} mouse is a valid model for vLINCL and the use of this mouse will be vital in the screening of possible therapies.

The specificity of storage of subunit c of mitochondrial ATP synthase in the ceroid lipofuscinoses

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Until the late 1980s the dogma was that the stored material in the NCLs was ceroid/ lipofuscin, with a fluorescent matrix of cross-linked peroxidised lipids and proteins. Careful analysis of storage bodies isolated from ovine CLN6 NCL showed this to be wrong, and that they consisted of normal lysosomal lipids and a specific protein, subunit c of mitochondrial ATP synthase. This hydrophobic membrane protein is very difficult to work with and is not amenable to standard proteomic methods. It is extremely insoluble, prone to aggregate and reacts very poorly with Coomassie blue and other protein stains. Standard mass spectrometric and LC-MS methods are uninformative. These factors have led to considerable confusion about subunit c storage, the role of ceroid/lipofuscin and specificity in the NCLs.

However the matrix within storage bodies is easily purified and dissolves in lithium dodecyl sulphate (LDS) or chloroform/methanol/ammonium acetate. Subunit c stains well with a special 2-step silver stain and is the only major protein component of storage bodies, recognised by sequencing and Western blotting. Its amino acid sequence dominated when total storage bodies proteins were sequenced by automatic Edman degradation, to the extent that a sequence of over 40 amino acids was determined. Calculations showed that over 67% of the storage body protein is subunit c, far more than in purified ATP synthase. No ceroid/lipofuscin-like polymers were detected and aggregates reconstituted from non-fluorescent subunit c and phospholipids exhibited lipofuscin-like fluorescence indicating that this as a property of aggregation.

A number of workers have used direct protein sequencing to establish specific subunit c storage in CLNs 2,3,5,6,7 and 8 in humans, sheep, cattle, mice and dogs, also indicated by immunohistochemistry. Immunohistochemistry also indicated subunit c accumulation in MPS IIIb but only in specific brain regions, whereas subunit c storage is generalised in the NCLs.

Direct protein sequencing is now an old technology, largely replaced in protein analysis by mass spectrometry and LC-MS methods. Dominant subunit c storage has been shown in NCLs in sheep, mice, humans, dogs and a cat by this technology using specialised columns and solvent systems. In all cases the molecular mass data shows that the complete and normal subunit c is stored including normal trimethylation of lysine 43. This feature of animal subunit c allows packing of only 8 c subunits into the ATP synthase driving ring and thus more efficient ATP production in animals than is possible in yeasts and other life forms. However the relationships between the genes implicated in the various NCLs, the pathogenesis of the diseases and the accumulation of subunit c are not clear. More needs to be known about the normal methylation, demethylation and turnover of subunit c before we can infer a causal relationship between disruptions of these processes and the NCLs.

Displaced axon initial segment localisation in *Cln3* deficient neurons

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With very little known about the normal role of Cln3 in neurons, it has been difficult to define how their biology has been compromised in JNCL. Primary neuron cultures offer a means to systematically address these issues in a controlled *in vitro* environment, and we have been characterising the phenotypes of neuronal cultures derived from Cln3 deficient mice (*Cln3*$^{-/-}$). Compared to wild type cultures, *Cln3*$^{-/-}$ cortical neuronal cultures initially grow normally, but with increased time in culture a variety of phenotypes emerge. The mutant neurons appear smaller, having much shorter processes, mislocalised or redistributed presynaptic proteins and an abnormal cytoskeleton. The axon initial segment (AIS) is an important structure within healthy neurons that determines those proteins that reach the axon and modulates neuronal excitability and the initiation of axon potentials. However, the position of the AIS is not fixed and moves distally along the axon when wild type cultures are chronically depolarised with high levels of potassium. This is thought to represent a compensatory response to chronic depolarisation, altering the probability of neuron firing. Since such properties may have relevance to the seizure phenotype of JNCL, we investigated the location of the AIS in *Cln3*$^{-/-}$ cortical neurons using immunostaining for Ankryn G, one of the major protein components of the AIS. Upon K^+ induced depolarisation wild type neurons displayed the expected distal displacement of the AIS along the axon. Surprisingly, in *Cln3*$^{-/-}$ cultures the AIS was already significantly displaced away from the cell soma, and did not move further upon depolarisation. These data suggest that these JNCL neurons may have altered firing properties or may be compensating for altered electrical activity. Although these possibilities await experimental verification, our preliminary data suggest that this displacement of the AIS is influenced by glia. In wild type neurons grown in the presence of mutant astrocytes and microglia the AIS is significantly displaced, whereas in mutant neurons grown together with wild type glia the mislocalisation of the AIS is partially reversed. These data not only reveal novel consequences of Cln3 deficiency in neurons, but also suggest that these may be targeted therapeutically. It will be important to determine if similar phenotypes are also evident *in vivo* and in the human disease.

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Defective mitochondrial bioenergetics in murine homozygous *Cln3*$^{\Delta ex7/8}$ cerebellar neuron precursor cells precede subunit c storage

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A hallmark of the pathology of juvenile neuronal ceroid lipofuscinosis (JNCL) is the formation of autofluorescent storage material throughout the body. As the main component of storage material in JNCL is the c subunit of mitochondrial F_1F_0-ATP synthase, the involvement of mitochondria in JNCL pathology has long been suspected. Accordingly, mitochondrial defects, such as morphological abnormalities, decreased β-oxidation of lipids, compromised function of oxidative phosphorylation complexes and increased mitochondrial reactive oxygen species content have been shown in different JNCL models and in patient cells. The genetically precise knock-in mouse model that recapitulates the most common 1.02 kb *CLN3* deletion of exons 7 and 8 was shown to accurately mimic JNCL pathology. Cerebellar neuronal precursor cells (CbCs) derived from these mice show progressive storage of subunit c upon aging at confluent density. Mutant cells also exhibit abnormal mitochondrial morphology, increased sensitivity to oxidative stress and reduced basal ATP levels under sub-confluent growth conditions. However, extensive assessment of mitochondrial bioenergetics in this model has not yet been performed. Using Multi-Epitope-Ligand-Cartography (MELC), an imaging technique to perform 3D-proteomic studies *in situ*, we discovered a novel, mitophagy-associated phenotype in JNCL CbCs aged under confluence. Based on this and previous findings we assed mitochondrial function by respirometry and measurement of mitochondrial membrane potential in CbCs.

Results:
We found that colocalization of the mitochondrial outer membrane channel protein, VDAC, with Lysotracker was increased in aged mutant CbCs, consistent with an involvement of mitophagy in the accumulation of storage material. In sub-confluent mutant CbCs this phenotype was not observed; instead, mitochondrial respiration was overall mildly compromised, and this correlated with a significant decrease in maximum respiration capacity. Additionally, the mitochondrial membrane potential was significantly reduced in sub-confluent mutant CbCs, compared to wild-type CbCs.

Conclusions:
In a murine neuronal cell model of JNCL, the formation of storage material depends on mitophagy and is preceded by defects in mitochondrial bioenergetics. The contribution of these pathways to JNCL pathology remains to be fully elucidated, but improving mitochondrial function may prove to be an effective strategy to influence the course of the disease.

Disturbed lipid metabolism, defective myelination and early microglial activation in *Cln5*-deficient mice and severe neuropathology of double-knockout mice lacking *Cln1* and *Cln5*

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Mutations of *CLN5* lead to CLN5 disease, late infantile variant phenotype neuronal ceroid lipofuscinosis formerly known as Finnish variant late infantile NCL (vLINCL $_{Fin}$). Loss of *Cln5* in mice has been shown to result in loss of thalamocortical neurons, and glial activation, but the underlying mechanisms are still poorly understood and further addressed in this study. Studies of the molecular interactions between NCL proteins have revealed that CLN5 interacts with many NCL proteins including CLN1. In order to study the possible disease-related effects of these interactions, we developed a novel mouse model lacking both *Cln1* and *Cln5* genes and analyzed its pathology and affected pathways.

We present here that Cln5 expression in the mouse brain increases gradually with age and differs between neurons and glia, with the highest gene expression in microglia. In Cln5*ko* mice, we documented early and significant microglial activation that was already evident at 3 months of age. Loss of *Cln5* also leads to defective myelination in vitro and in the developing mouse brain. This was accompanied by early alterations in serum lipid profiles and lipid transport in Cln5*ko* mice. Furthermore, our novel mouse model lacking both *Cln1* and *Cln5* presents an overall more severe phenotype with earlier starting symptoms compared to the single knockout mice. We observed strong accumulation of autofluorescent material and widespread astrocytosis and microglial activation at 3 months of age. Gene expression profiling of cortex derived from *Cln1/5* dko mice pointed out several affected pathways, including neurodevelopment, lipid metabolism and inflammation.

In summary, these data provide significant new information about events associated with Cln5-deficiency, revealing altered myelination and disturbances in lipid metabolism, together with an early neuroimmune response. Also, our very recent data of the *Cln1/5* dko mouse model indicates that CLN1 and CLN5 might share common pathways, which is currently being further investigated.

Distribution of GM_1 ganglioside is altered in Cb $Cln3^{\Delta ex7/8}$ cells

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Gangliosides play a major role in the neuronal development, particularly in neurite generation and cell survival. Besides of being a putative member of cholesterol enriched membrane microdomains, by inhibition assays the GM_1 ganglioside has been shown to modulate the binding properties of neuronal cell adhesion molecules (NCAM) and N-cadherin. Previously, using Multi Epitope Ligand Cartography (MELC), an automated multidimensional fluorescence microscopy technique, we have shown that the membrane distribution of GM_1 is severely affected in the hippocampus of adult JNCL mouse brains. In order to investigate how this phenotype contributes to the pathological course of JNCL at cellular level we used MELC imaging on cerebellar granule neuronal precursor cells carrying a $Cln3^{\Delta ex7/8}$ knock-in mutation to examine the spatial proteome of GM_1 associated proteins.

Results

Like in adult mice MELC data obtained from Cb $Cln3^{\Delta ex7/8}$ cells depicted decreased intensity levels and mislocalization of GM_1. Quantitative analysis confirmed the reduction in total GM_1 and subsequent FACS analysis revealed that the plasma membranes of JNCL cells were depleted from GM_1. Careful inspection of the MELC data connected the alternated distribution of GM_1 to autophagy related processes. In line with this observation are our findings from quantitative microscopy showing increased colocalization of an autophagy marker and GM_1 using 3D confocal laser scan microscopy.

Conclusions

The phenotype detected here indicates that the presence of truncated CLN3 protein induces severe disturbances in the composition of cellular membranes. A mislocalization of GM_1 arouses malfunctions in cell signalling, endocytosis, autophagy, vesicle fusion and fission and other membrane-related events. Several of these functions were previously shown to be affected in JNCL, suggesting an impact of the truncated CLN3 protein on the synthesis of GM_1 or the pathway by which it is transported to the plasma membrane.

Development of a Disease Model for Late-Infantile Neuronal Ceroid Lipofuscinosis Using Patient Specific Induced Pluripotent Stem Cells

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Late-infantile neuronal ceroid lipofuscinosis (LINCL) is a hereditary and fatal neurodegenerative disease of childhood that is caused by mutations in the gene *CLN2* encoding the lysosomal protease tripeptidyl-peptidase 1 (TPPI). Hallmarks of the disease are accumulation of autofluorescent storage material and accumulation of mitochondrial subunit C of ATP synthase in the lysosome. Typically symptoms start at age 2-4. Clinically the disease is characterized by loss of vision, seizures, and psychomotor deterioration, and results in premature death at age 7-15 years. Studies of pathogenesis of LINCL in humans are strongly limited by lack of availability of patient specific central nervous system (CNS) material. Novel cellular reprogramming technologies allow derivation of induced pluripotent stem cells (iPSCs) from patient somatic cells and further terminally differentiate them into desired cell types, which can be used for disease modeling, cell based assays, drug screenings and validation. Here, we describe our effort in generating a valid cellular disease model for studying molecular mechanisms of LINCL pathology in vitro. We have derived LINCL patient specific iPSCs, which express canonical pluripotency markers (Tra 1-60, Tra 1-81, Oct ¾) and are karyotype normal. We have successfully differentiated them into Sox1 and nestin positive neural stem cells (NSCs) and subsequently into disease specific neurons. Microscopic analysis revealed mild accumulation of lipofuscin in disease-specific neurons not observed in normal neurons. Western blot revealed an increased accumulation of ATP synthase subunit C in LINCL neurons. The disease model described here is the only in vitro model utilizing human LINCL specific neurons.

Electroretinography in aging Cln3$^{\Delta ex7/8}$ KI mutant mice

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Purpose: Neuronal ceroid lipofuscinoses (NCL) are characterized by lysosomal accumulation of autofluorescent material and lead to degeneration of the central nervous system. Patients affected by the juvenile form of NCL (JNCL), the most common form of the disease, develop visual failure prior to mental and motor deficits. It is currently unclear if the corresponding mouse model, Cln3$^{\Delta ex7/8}$ KI, develop the same retinal phenotype and electroretinogram as patients. The aim of our study was to investigate the visual disease progression in a mouse model using elctroretinography.

Methods: Retinal function of the NCL mutant mouse strain Cln3$^{\Delta ex7/8}$ KI was characterized by scotopic and photopic electroretinography at different ages. The results were then compared with age-matched wild-type controls

Results: The amplitudes of the a-wave and b-wave decrease significantly in normal mice with increasing age, while the implicit time remains constant. There is only a small decrease in the b/a ratio in these mice, indicating that normal aging affects the outer and inner retina similarly. Young Cln3$^{\Delta ex7/8}$ KI mice have normal amplitudes and b/a-ratios. In these mice amplitudes also decrease with increasing age. In older Cln3$^{\Delta ex7/8}$ KI mice, there is a significantly higher b-wave amplitude loss. The a-wave amplitude reduction is comparable with the one occurring in wild-type mice. This results in a clear drop in the b/a ratio

Conclusions: Electroretinography can be used to characterize disease progression in a mouse model for JNCL. Our results indicate a decline of the inner retinal function in Cln3$^{\Delta ex7/8}$ KI mice, similar to human disease. In the context of a therapeutic trial, electroretinography could provide important information about disease course under treatment and identify successful approaches.

Regional and subtype-specific glial activation and neuron loss in human NCL

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There is a complex relationship between neuron loss, astrocytosis and microglial activation in the hippocampal formation in human NCL. We have now extended these studies to other CNS regions (focusing on the frontal, visual and primary motor cortex) in cases of infantile (n=2), late infantile (n=2) and juvenile (n=2) NCL, staining for markers of astrocytosis (GFAP) and microglial activation (CD68, MHC-II). These cortical regions not only serve different functions, but are also affected to different extents in NCL mouse models. The degree of cortical neuron loss varied dramatically between forms of NCL, being nearly complete in INCL with only very few neurons persisting, mostly in the deeper layers of frontal cortex. Neurons were much better preserved in JNCL with graded effects on neuron survival between cortical regions, with surviving neurons frequently encircled by GFAP positive processes. In LINCL there was more pronounced neuron loss with blurring of laminar boundaries. GFAP staining revealed markedly differing degrees of astrocytosis between forms of NCL, with more hypertrophied astrocytes present in LINCL vs. JNCL tissue. Indeed, GFAP immunoreactivity was consistently paler in JNCL cases with astrocytes rarely displaying hypertrophy or thickened cell processes. These data suggest that the astrocyte response is either incompletely triggered in JNCL, or that astrocytes are themselves compromised by Cln3 mutation. CD68 and MHC-II immunoreactivity revealed similar contrasting degrees of microglial activation between the forms of NCL. Relatively few CD68 and MHC-II positive microglia were detected in INCL, suggesting that the microglial response had now subsided following neuron loss. In contrast in LINCL, where neurodegeneration is still ongoing, there were many intensely CD68 and MHC-II immunoreactive microglia and brain macrophages present, often appearing in clusters of 2-3 (up to 5) cells, with evidence of engulfment of neuronal debris. Far fewer and less morphologically transformed microglia were evident in JNCL, either reflecting the relatively mild degree of neuron loss, or suggesting that microglia are themselves affected in this form of NCL. Taken together these data highlight the marked differences between the extent and type of glial activation in the three major forms of NCL, highlighting the distinct nature of these responses in JNCL.

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Cerebellar neurobiology of Niemann-Pick type C1 disease

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The lysosomal storage disorder Niemann-Pick type C1 (NPC1) is a neurodegenerative disease. Symptoms include cerebellar ataxia and tremor, due to the loss of Purkinje cells. NPC1 has been considered primarily a cholesterol storage disorder, but recent reports suggest NPC1 is a sphingosine storage disease that causes a lysosomal calcium defect. However, whether sphingosine storage and calcium defects occur in NPC1 brain has not been established.
Using HPLC analysis, we observed that an increase in sphingosine levels is one of the first measurable pathological phenotypes in NPC1 cerebellar tissue. This occurs by the first postnatal week, coinciding with the first reports of cellular pathology, and several weeks before measurable glycosphingolipid storage. No significant cholesterol increase was measured over the lifespan of the NPC1 mouse model. In addition, analysis of lysosomal calcium in cerebellar slice cultures, taken before glycosphingolipid storage, indicates a lysosomal calcium defect in cerebellar neurons. Interestingly, not all Purkinje cells are equally affected in NPC1, with a subset of Purkinje cells surviving up to disease end-point. Immunohistochemical analysis of cerebellar tissue demonstrated no difference in cholesterol or GM1-staining patterns between disease-resistant and disease-susceptible neurons, indicating these lipids are not overtly correlated with neuronal death. However, differential microglial activation and calbindin expression is observed in the distinct lobules disparately affected in NPC1. In conclusion, these data suggest that sphingosine storage is an early pathological event in the NPC1 brain and NPC1 neurons have a lysosomal calcium defect, while distinct cellular and biochemical phenotypes do correlate to differential Purkinje cell pathology.

Anti-retinal antibodies in Juvenile Neuronal Ceroid Lipofuscinosis (JNCL)

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Purpose: An 8 year old female newly diagnosed with genetically confirmed JNCL was noted to have a cellular reaction in the anterior vitreous, suggesting an inflammatory component to the retinal degeneration. Anti-retinal antibody testing was performed.

Methods: Western blots of human retinal protein were probed with serum from the affected index patient and her unaffected sibling. Serum from the index patient was re-evaluated for anti-retinal antibodies 2 months after treatment with posterior subtenon triamcinolone injection in the right eye, followed by oral mycophenolate. An additional 7 samples from patients 10-20 years old with genetically confirmed JNCL were evaluated for anti-retinal antibodies.

Results: Serum from the index patient at initial visit contained antibodies that reacted with 7 bands of 23, 30, 33, 35, 45, 60, and 62kDa. No anti-retinal antibodies were detected in the index patient's unaffected sibling. Of the 7 additional samples, 2 had prominent single bands plus several faint bands, and the other 5 had multiple faint bands. The antiretinal antibody screen in the index patient was negative 2 months after immune suppression. A repository of 76 serum samples from Batten patients and controls has been collected for evaluation.

Conclusions: There may be an inflammatory and/or autoimmune component to the retinal degeneration that occurs in CLN3-associated Batten disease (JNCL). This may parallel the reported neuro-inflammation associated with anti-GAD antibodies that is thought to play a role in the neurodegenerative process. Antiretinal antibodies may be markers of this component, and may offer a clue for treatment strategies. In one patient treated with immunosuppression, the anti-retinal antibodies became undetectable. This anecdotal report prompted us to develop the hypothesis that anti-retinal antibodies may be a marker an inflammatory response that could contribute to the retinal degeneration in these patients. However the lack of a consistent reproducible autoantigen, and the presence of retinal autoantibodies in some unaffected individuals reported in the literature, mean that future studies will be needed to clarify what role, if any, antiretinal antibodies play in the underlying pathophysiology of JNCL.

CLN3 mutation associated with altered dendritic cell phenotype

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Juvenile neuronal ceroid lipofuscinosis (JNCL) is caused by mutation of the *Cln3* gene. The presence of autoantibodies in the blood of JNCL patients and *Cln3* mutant mice suggests the disease has an autoimmune component. Dendritic cells are the professional antigen presenting cells of the body. Processing and presentation of antigen requires proper endocytosis, vesicle trafficking, and lysosomal pH regulation, three functions in which CLN3 has been implicated. Dendritic cells manifest a hyperstimulatory phenotype in several autoimmune diseases associated with the production of autoantibodies, including multiple sclerosis and systemic lupus erythemetosus. We thus sought to characterize the activation process in bone marrow derived dendritic cells from *Cln3* $^{-/-}$ mice compared to cells from wild type mice.

Results: A larger proportion of *Cln3* $^{-/-}$ dendritic cells were activated in the resting state compared to wild type. In addition, *Cln3* $^{-/-}$ cells expressed higher levels of activation markers in both the resting and activated states.

Conclusions: These results suggest that dendritic cells might be hyperstimulatory in JNCL.

Epilepsy in Juvenile Neuronal Ceroid Lipofuscinosis is Usually Characterized by Well-Controlled Generalized Tonic-Clonic Seizures

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Background: Juvenile Neuronal Ceroid Lipofuscinosis (JNCL, Juvenile Batten Disease) is an inherited neurodegenerative disorder characterized by vision loss, motor, cognitive, and behavioral impairment as well as epilepsy. NCLs are commonly considered in the differential diagnosis of treatment-refractory progressive myoclonic epilepsy.
Objectives: We sought to evaluate seizures in a cohort of children with JNCL, including seizure type, treatment, and severity.

Methods: Seizures were assessed by parent report using the Unified Batten Disease Rating Scale (UBDRS). Seizure severity was rated on a scale of 0-58, using frequency, duration of post-ictal period, seizure related injury, hospitalization, and recent medication adjustment as factors.

Results: Of 80 subjects, 85% had ever had a seizure. Of those with seizures, generalized tonic-clonic seizures were most common (89.7%); complex partial or absence seizures had the next highest occurrence (64.7%). Other seizure types included atonic (14.7%), myoclonic (29.4%), and focal seizures (10.3%). Most subjects (57%) experienced only a single seizure type, and on average subjects each used 1.71 medications in seizure management with valproate being the most commonly used anti-epileptic. On the UBDRS seizure sub-scale, mean seizure severity was 7.41 at the time of the most recent evaluation. Mean clinical global impression (CGI) was 2.88 (0-5 scale), representing mild seizure severity. Seizure severity did not correlate with disease duration.

Conclusion: Although myoclonic seizures do occur in JNCL, they are not the most common seizure type. Seizures in JNCL are most commonly generalized tonic-clonic in nature and are well managed with two or fewer anti-epileptic drugs. For most children, seizures are infrequent and are not progressive in nature.

Statistical properties of the jNCL scoring system according to Kohlschütter (1988)

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Problem: The jNCL scoring according to Kohlschütter (1988) is a standardised rating scheme to systematically and quantitatively describe the clinical symptoms of children and adolescents with juvenile neuronal ceroid lipfuscinosis. It was developed to easily and efficiently obtain reliable and valid scores both prospectively and retrospectively. The scoring has now been in clinical use over many years and a large set of data has been generated. Thus, an evaluation of statistical properties of the scoring is now possible and needed.

Methods: In this study data from n = 32 patients with genetically diagnosed CLN3 disease (M = 17.0 years old, SD = 6.19, 56.3% female) were used. Scoring assessments were made retrospectively as well as prospectively in 6-month intervals at the NCL specialty clinic in Hamburg, Germany. Clinical scoring was assessed for vision, intellect, language, and motor function as follows: normal function (3); minor but readily recognized dysfunction (2); severe dysfunction (1); total loss of function (0). To evaluate statistical properties of the collected data methods of classical test theory and item response theory were used.

Results: The intercorrelation of the four items "motor", "language", "intellect" and "vision" is on average r_{Med} = .44. The reliability of the total score, derived by the unweighted sum of item scores, is Cronbachs α = .83. The (corrected) correlations between the four items and the total sore are r_{it} = .79, .72, .75 und .41. A hypothesised ordinal Partical Credit Rasch model showed no significant deviance between modelled and observed data (bootstrap-p = .120) and thus indicate the unidimensionality of the scale. The correlation between the total score and the Clinical Global Impression Severity scale rated by the attending physician is r = -.68.

Conclusions: The results demonstrate sufficient to good properties of the items. The reliability of the total score is sound, indicating that at least 69% of the measured variance is systematic variance. The score is unidimensional even in the strict Rasch-sense and is highly associated with the CGI score. However, the symptomatology is only roughly estimated, because only a few steps of the latent trait are observed. Altogether, the jNCL scoring can be seen as a reliable and valid screen for the evaluation of clinical symptoms in affected children and adolescents.

Experiences of providing a genetic diagnostic service for the neuronal ceroid-lipofuscinoses / Batten disease in the U.K.

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The neuronal ceroid-lipofuscinoses (NCLs) are a group of inherited, neurodegenerative, lysosomal-storage disorders characterised by progressive mental and motor deterioration, seizures, and early death. Visual loss is a feature of most forms. Phenotypes have been characterised clinically by age of onset and order of appearance of the clinical features: infantile neuronal ceroid-lipofuscinosis (INCL, Santavuori-Haltia), late-infantile (LINCL, Jansky-Bielschowsky), juvenile (JNCL, Spielmeyer-Vogt), adult (ANCL, Kuf's disease), and Northern epilepsy (NE, progressive epilepsy with mental retardation). The Molecular Genetics Laboratory, NE Thames Regional Genetics Service at Great Ormond Street Hospital has over 10 years experience in the genetic testing of the NCLs, in particular *CLN1/PPT1*, *CLN2/TPP1*, *CLN3*, *CLN5*, *CLN6*, and *CLN8*. Over 900 patients have been tested since 1999 with approximately 45% being "query affected" and the remainder being for carrier testing and prenatal diagnosis. Approximately 37% of the "query affected" patients had a positive genetic diagnosis of a NCL - we will present data on the spectrum and frequency of mutations in our patient cohort. In addition, we will present two interesting NCL cases in which the affected patients are compound heterozygotes for a mutation in the *CLN8* gene and a large deletion of the 8p23.3 region, which encompasses *CLN8*. As a consequence, we now strongly recommend that chromosomal microarray analysis and testing of parental samples is carried out for any NCL patient who is apparently homozygous for a *CLN8* mutation. The frequency of this large deletion may be of some significance and it may affect the phenotype of a subset of *CLN8*-related NCL patients.

Neuronal Ceroid Lipofuscinosis in Norway

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Background: Neuronal Ceroid Lipofuscinosis (NCL) is a group of lysosomal, autosomal recessive inherited neurodegenerative conditions characterized clinically by blindness, epilepsy, mental retardation, and early death. No curative treatment is available and few therapeutic guidelines have been published regarding symptomatic treatments.

Aim of the study: To get a systematic overview over the medical and behavioural problems the families face, how they have coped with these problems, and their experience with treatments and interventions.

Methods: A questionnaire was sent to all known patients in Norway (total population of 4.9 millions) with this condition during 2009. All were invited for a physical examination and laboratory tests during 2009/2010. During 2011, 19 patients have been followed up with a second examination.

Results: Thirty-two (12 female and 20 male) out of 40 potential responders participated in the study. One additional participant (female) was included later, and 7 patients have died (age 10.1-33.0 years) during this time period.

According to the parents, all but 1 had NCL3 (Spielmeyer-Vogt-Batten disease). Preliminary data from the NCL3 patients (n=32) are presented.

The median age at symptom debut was 5.6 years (range 2.0 – 9.0 years). Median age at diagnosis was 7.7 years (range 3.0-13.0 years). The age at onset of symptoms is described in the table below:

Symptoms (n)	Median (yrs)	Range (yrs)
Reduced vision (32)	6.0	2.0-9.8
Epilepsy (26)	10.5	5.0-16.0
Sleep Disturbance (21)	9.0	0.0-20.0
Reduced Motor Ability (21)	13.0	1.0-19.5
Feeding Difficulties (8)	19.0	14.0-28.0
Psychiatric symptoms (13)	12.5	0.0-18.0
Behavioural problems (21)	6.5	0.0-17.0
Speech Difficulties (25)	12.5	0.0-18.5

Physical examination was performed in 27 patients (10 female and 17 male, median age 15.9 years [range 6.9-35.0 years]). Examination revealed normal speech in 9 patients, and 11 had difficulties being understood. Gait was normal in 3 patients, 8 had difficulties walking and 5 could not walk at all. Echocardiography was performed in 13 patients (5 female and 8 male in the age of 15-35 years) and revealed 2 with left ventricular dysfunction and 4 other patients with left ventricular hypertrophy. Laboratory data and data from the 2011 follow-up will also be presented.

Conclusion: NCL3 is by far the most common type of NCL in Norway. Patients with NCL and their families have to face numerous challenges, in addition to blindness and epilepsy.

Characteristics of neuronal ceroid lipofuscinosis in patients from the West Balkan region

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The aim of this study was to evaluate the frequency of specific types of neuronal ceroid lipofuscinosis (NCL), main clinical characteristics and outcome in patients with NCL in the West Balkan region. **Method**: The study included pediatric NCL patients treated at two tertiary centers: in Belgrade. The study population included patients from Serbia, Montenegro, Bosnia and Herzegovina evaluated in the period from 1991-2011 and diagnosed with NCL by enzyme, genetic, and histopathologic analysis. All enzyme and genetic analyses and some of histopathologic analyses were done abroad, including at the MGH-CHGR Joint Program in NCL Disorders in Boston, MA. The patients were divided in groups according to NCL classification. The initial symptoms, epilepsy presentation, neurophysiological and neuroradiological characteristics, period from onset of disease to definitive diagnoses and outcome were evaluated. **Results**: The study included 17 patients (14 males, 3 females) with different types of NCL treated in two centers for last 20 years. Late infantile form of NCL (LINCL) was diagnosed in 16 (12 classical type, 4 variant), while the juvenile form of NCL (JNCL) was diagnosed in one case. Seizures, febrile or afebrile, were the initial manifestations in all cases with LINCL, while ataxia and mental regression were the first manifestations in the boy with JNCL and two siblings with the Finnish variant of LINCL (CLN5). The mean age of disease onset was 2.8 (range 2-4.5) years in LINCL children, while first symptoms were recognized at 9 years in boy with JNCL. Hyperactivity at the time of disease onset was noticed by parents in the majority of children with LINCL. During the course of disease all patients suffered epileptic seizures (simple and complex focal seizures with/without secondary generalization and myoclonus), ataxia, visual loss, cognitive regression and progressive global brain atrophy on MR/CT. The rate of disease progression was different even in the patients with the same NCL type. The mean age of death in children with LINCL was 10.6 years (7-13). The period from disease onset to definitive diagnoses ranged 1-12 years (mean 4 yrs). With the improvement in education and resources available for diagnosis, a definitive diagnosis was made in 5 cases over the past year, including one case that eluded diagnosis for 12 years. **Conclusion**: The results of our study suggested that LINCL is the most frequent type of NCL in patient from West Balkan region. It could be explained by regional characteristics or, more probably, by poor recognition of other NCL types. The initial manifestations in cases with LINCL were similar: seizures were preceded by hyperactivity in majority of cases and were followed by ataxia and mental regression. Very long period from disease onset to definitive diagnoses was caused by insufficient recognition of disease by professionals, but also by unavailability to make definitive NCL diagnosis in the region. Collaboration with centers of clinical and diagnostic expertise in NCL, including the MGH-CHGR Joint Program in NCL Disorders, have significantly expedited the establishment of a definitive diagnosis in many cases.

Clinical Application of a proposed axial classification system for the Neuronal Ceroid Lipofucinoses

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Objective:
The neuronal ceroid lipofusinoses (NCLs) are progressive degenerative diseases that primarily affect the brain and sometimes the retina. Historically the nomenclature for NCL classification has been clinically led. Recently an axial classification system which takes into account advances in our understanding of the genetic, ultra-structural and biochemical mechanisms causing NCLs has been proposed. We aimed to determine whether this classification could be applied to a cohort of patients seen in our clinical practice.

Methods:
We applied the following classification system to a cohort of 39 patients seen within our NCL clinic, using data extracted from medical records and clinic letters:
Axis 1: Affected gene
Axis 2: Mutation diagnosis
Axis 3: Biochemical phenotype
Axis 4: Clinical phenotype
Axis 5: Ultra structural features
Axis 6: Functionality
Axis 7: Other remarks of possible medical relevance.

Results:
For most Axes, patients could be easily classified from information contained in clinical notes. Axis 4 could be completed for 39/39 patients. Axis 1 could be completed for 37/39, with CLN3 disease most commonly seen (21/39), followed by CLN2 disease (6/39). Axis 2 could be completed for 32/39 patients and Axis 4 31/39 patients. Only 17/39 patients could be classified along Axis 5.

Conclusions:
The proposed Axial classification was easy to apply in a clinical context. The lack of data available for the completion of Axes 5 compared with Axes 2 and 3 is likely to reflect a move away from tissue biopsy with improvements in the elucidation of the genetic and biochemical causes of the NCLs. We have demonstrated the applicability of the classification system in the clinical setting. We believe the new system can provide young people, carers and professionals with a diagnosis that gives information that leads to effective clinical management of symptoms, as well as informing basic scientific as well as clinical research.

Spectrum of mutations in French patients with ceroid lipofuscinosis

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Neuronal ceroid lipofuscinoses (NCL) are inherited neurodegenerative disorders mainly affecting children and characterized by the accumulation of autofluorescent lipopigments in various tissues. Four main NCL forms are usually distinguished according to the age of onset: infantile (INCL), late-infantile (LINCL), juvenile (JNCL) and adult (ANCL), but numerous other forms have been reported. Nine loci (from CLN1 to CLN10) have been found as responsible for these different forms, three of them encoding enzymes : palmitoyl protein thioesterase (CLN1), tripeptidyl-peptidase I (CLN2) and cathepsin D (CLN10).

90 French NCL patients were completely characterized at the enzymatic and/or molecular level. Among them, 69 exhibit a late infantile form of NCL, which is the most common clinical form in France. The CLN2 locus was involved in a majority of patients (60%), as demonstrated by the presence of a tripeptidyl-peptidase I deficiency and of deleterious mutations on the CLN2 gene. Complete sequencing showed the predominance of two previously reported mutations: c.509-1G>C (IVS5-1G>C) and c.622C>T (p.Arg208X) representing 44% of alleles. Thirteen other mutations have been found on the other alleles. The CLN6 and CLN7 genes were involved in variant LINCL patients (6 patients each). The CLN6 patients mainly originated from Portugal and Pakistan and predominantly carried previously reported deletions or insertions. However, two novel point mutations (p.Arg136His and p.Tyr295X) were found in a patient of French extraction. Among the CLN7 patients, some mutations have been previously reported, some were novel, including 3 small deletions and 3 missense (p.Pro92Arg, p.Ser154Leu, p.Arg465Gln). Only one patient carried mutations on the CLN8 gene. To date, no mutation has been found on the CLN5 and CLN10 genes among French patients. The CLN1 gene was responsible for the classical infantile forms (9 patients), as well as for atypical LINCL only present in patients from Spain (Gypsy) and exhibiting the c.541G>T (p.Val181Leu) mutation. Patients with juvenile form of NCL mainly exhibit the common 1 kb deletion on the CLN3 gene (50%), but other mutations have also been characterized.

Our results confirm the heterogeneity of the mutations involved in French NCL patients. The recent identification of these abnormalities has allowed us to offer genetic counselling and prenatal diagnosis to couples at-risk for this devastating disease.

Neuronal Ceroid Lipofuscinosis in Italy: Epidemiological Assessment of Childhood Forms in the Molecular Age

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The neuronal ceroid lipofuscinoses (NCLs) are the commonest neurodegenerative disorders of children, secondary to mutations in 8 CLN genes. The aims of this study were to review the descriptive epidemiology of the NCL in genetic era in Italy, identify the spectrum of mutations in the causative genes, and analyze possible relationship between phenotypes and genotypes. Through CLNet, a nation-wide network of child neurology units, 176 NCL patients were ascertained between 1966 and 2008; 114 of them (from 88 families) were tested for known NCL genes. An incidence of 0.91/100000 births was obtained from 65 NCL patients (from 59 families), born between 1992 and 2004. Classical late infantile NCL (cLINCL) secondary to *CLN2* mutations is the most frequent form (24.6%); altogether, classical and variant LINCL (vLINCL) account for 76.4% of cases. Classical Juvenile NCL patients represent 12.3% of this cohort, which is smaller than what is observed elsewhere in Europe. In our survey 14% of patients remained undiagnosed. Twenty-eight children were affected with the clinically severe cLINCL; gene mutations predicted markedly impaired gene product function in 87.5% of cases. Likewise, in 13 children affected with vLINCL due to mutations in *CLN7*, another form associated with a disabling phenotype, gene mutations predicted severely altered protein in 85.7% of patients. Descriptive epidemiology data indicate a lower incidence of NCL in Italy as compared to other European countries. The increased incidence rate may be accounted for by the introduction of gene testing in clinical practice.

DEM-CHILD - A Treatment-Oriented Research Project of NCL Disorders as a Major Cause of Dementia in Childhood

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The DEM-CHILD project focuses on the main cause for childhood dementia in Europe, the neuronal ceroid lipofuscinoses (NCLs). The NCLs are neurodegenerative diseases characterized by dementia, blindness, epilepsy and physical decline leading to an early death of the patients. Since no cure is currently available, these disorders represent a serious social, medical, and economic challenge.

To date, nine NCL genes have been characterized. There is evidence suggesting that further gene loci remain to be identified. NCLs are under-diagnosed in many countries around the world as there is an overall lack of research, early diagnosis, treatment and expert availability. Furthermore, due to their broad genetic heterogeneity it is difficult to collect large numbers of genetically similar patients. As such, large therapeutic studies required for advances in treatment are difficult to intiate.

The DEM-CHILD project will combine the expertise of (i) recognized **European research teams** with (ii) high-technology **SMEs,** and will (iii) collaborate with Indian experts on the following objectives: (1) High-technology SMEs will develop innovative cost- and time-effective testing and screening methods for all NCLs in order to ensure early diagnosis and thereby prevention; (2) DEM-CHILD will collect the world's largest, clinically and genetically best characterised set of NCL patients in order to study disease prevalence and precisely describe the natural history of the NCLs leading to the development of an evaluation tool for experimental therapy studies; (3) Novel biomarkers and modifiers of NCL will be identified to support the development of innovative therapies; (4) Focussing on the development of therapies for NCL disease caused by mutations in intracellular transmembrane proteins, two complementary therapeutic strategies will be used and compared in eye and brain of mouse models: a) viral-mediated gene transfer and b) neural stem cell-mediated delivery of neuroprotective factors.

The DEM-CHILD project has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 281234.

Symptom Care Planning for young children affected by NCL

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Aim: To develop and pilot a template symptom care plan for use by families, carers and professionals from a variety of multidisciplinary backgrounds

Method: Over a number of months a symptom care plan was evolved and used for a number of children affected by refractory epilepsy in a tertiary neurology hospital setting as part of discharge planning. A disease specific template was then prepared and used for a small number of children affected by NCL and at late stages of disease progression, as part of their routine multidisciplinary clinical care. The care plans were prepared and reviewed alongside parents, and then distributed to members of the hospital and community health teams. Initial informal feedback was very positive, both from family members and professionals. The template has been reviewed and further developed by NCL expert parents.

Results: The template will be presented

Multidimensional Clinical Assessment Tool for juvenile CLN3 Disease

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Aim:
To develop and pilot a standardised quantitative scheme for the clinical assessment of children and young people homozygous for the common CLN3 deletion

Method:
Ethical approval was obtained. Participating children and young adults were recruited through the Batten Disease Family Association (BDFA) and the clinical service at Evelina Children's Hospital (ECH). A schedule for history and examination was devised incorporating existing Batten clinical scoring systems and using items drawn from scoring systems used in other conditions. Children underwent a number of assessments over 24 months (not more often than 6 monthly). The schedule was revised and simplified part way through the project as some items were found to be repeated or time-consuming and not therefore useful.

Results:
9/14 young people with CLN3 disease homozygous for the common 1kb deletion. These 9 underwent 19 assessments. The age range was from 7 years to 26 years.

Conclusion:
Standardised assessments of clinical symptoms and developmental skills can be quantified and be meaningful in a clinical setting. The results of Hamburg scores and UBDRS physical subscale are broadly in line with published data. A number of additional subscales including those of number of prescribed daily medications and a simple mobility score deserve further investigation.

This study was funded by the BDFA via a Jeans 4 Genes grant.

Novel approaches to chaperone therapy of Batten disease

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There is currently no therapy for any form of Batten disease. Some mis-sense mutations in the CLN1 gene (Infantile Batten) result in single amino acid changes (G108R and T75P) residual (<2%) PPT1 enzyme activity and later disease onset, suggesting they could be candidates for chaperone therapy to re-fold misfolded proteins. We synthesized novel chaperones such as AcGDap(Pal)VKIKK (Dap1), which are non-hydrolyzable competitive inhibitors of PPT1 (IC_{50} of 2uM) and are taken up by cells to promote additional hydrolysis of thioester peptides in such CLN1 patients. We observed that Dap1 was uniquely able to promote egress from endosomes to other parts of the cell, including the endoplasmic reticulum where protein folding occurs. A major barrier to treating CNS disorders is delivery across the blood brain barrier. To overcome this problem we have generated coated, intensely fluorescent quantum dots (6nm) to which the peptide is attached through the interaction of Zn and polyhistidine. Microinjection into the spinal cord of 3 day chick embryos revealed that 4 days later the Dap1 (Palm-1) is distributed throughout the spinal cord and developing CNS without any adverse pathology. We believe that this method has the potential to deliver chaperones to the human CNS and will show that greater specificity can be achieved by adding peptides such as RVG to specifically target chaperones to neurons.

We have also used non-specific chaperones to enhance enzyme activity in patients with atypical Late Infantile NCL (tripeptidylpeptidase 1, TPP1) deficiency. Responsive CLN2 patients had later than normal onset resulting from non-active site single amino acid substitutions G509C //T1439>G. Residual activity (2% of control) was enhanced 2-fold by culture of fibroblasts in the presence of Guanabenz (Wytensin), an FDA approved drug which inhibits a phosphatase complex that controls misfolded protein proteostasis in the ER. Smaller effects were observed in R208X/1439 and 1303del/V480G patients cells. Chaperone therapy is in Phase 3 trials for other LSDs (Fabry disease) suggesting that it might be of value for treating Batten disease if the blood brain barrier is overcome.

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Glutamate receptors as a therapeutic target for the treatment of INCL

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The neuronal ceroid lipofuscinoses (NCLs) are the most common form of pediatric-onset neurodegeneration. Of these disorders, infantile NCL (INCL) is one of the most rapidly progressing and earliest onset variants. Effected children experience retinal degeneration leading to blindness that is followed by neurocognitive decline, seizures, and premature death. Previous studies suggest that disruptions in glutamatergic function may contribute to INCL disease progression. Prior investigation of glutamate receptor function in the *Ppt1*$^{-/-}$ mouse model of INCL suggested a decrease in AMPA receptor function and an increase in NMDA receptor function in isolated cerebellar granule cells. As glutamate receptor function is regulated in part by the surface expression level of the receptor, we also examined the surface levels of AMPA and NMDA receptor subunits in the cerebella of four-week-old WT and *Ppt1*$^{-/-}$ mice. This examination showed a significantly lower surface level of the GluR4 AMPA receptor subunit in the *Ppt1*$^{-/-}$ cerebella, providing a plausible explanation for the decreased vulnerability of *Ppt1*$^{-/-}$ cultured CGCs to AMPA-mediated cell death. To determine whether this difference in GluR4 expression is maintained after symptomatic onset, we have recently investigated surface expression levels of AMPA and NMDA receptor subunits in 37-week-old mice. At that advanced point in disease progression, we found significant aberrations in surface expression levels of GluR1 and GluR2 in the aged *Ppt1*$^{-/-}$ cerebellum.

Our previously published results demonstrating AMPA receptor hypo- and NMDA receptor hyper-function in *Ppt1*$^{-/-}$ neurons suggest that glutamate receptor targeted therapeutics may ameliorate some of the symptoms associated with INCL. To test this, we have treated mice with memantine, an NMDA receptor antagonist, and monitored their progress up to two weeks following treatment.

Galactosylceramide (GalCer) as a potential treatment for juvenile neuronal ceroid lipofuscinosis (JNCL)

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JNCL is a neurodegenerative disorder caused by *CLN3* gene defects. These negatively modulate cell growth/apoptosis. The CLN3 protein harbors antiapoptotic motifs and a galactosylceramide (GalCer) lipid raft-binding domain. CLN3p plays a role in GalCer transport to the cell surface, and there is a GalCer deficit in lipid rafts of CLN3-deficient cells. Importantly, CLN3-defective brain/cells have elevated ceramide (Cer). Previous hypotheses suggest that low GalCer in lipid rafts (LR) leads to increased Cer generation in an attempt to overcome the GalCer deficit, and that this leads to neuronal cell death by apoptosis. The $Cln3^{\Delta ex7/8}$ knock-in mouse closely mimics the human disorder exhibiting early onset and progressively accumulating JNCL storage material, gliosis, motor disturbances and a shortened life span. Here, to further test this hypothesis, we have examined Cer levels in $Cln3^{\Delta ex7/8}$ knock-in mouse brain, and found that they were significantly elevated compared to wild-type littermate control brain Cer levels. We have also tested whether exogenous GalCer administered to homozygous $Cln3^{\Delta ex7/8}$ mice would correct the reduced LR/Golgi GalCer ratios in homozygous $Cln3^{\Delta ex7/8}$ mouse brain. Daily intraperitoneal injections of 20 mg/kg GalCer, or vehicle only, were administered to homozygous $Cln3^{\Delta ex7/8}$ mice between the ages of 5 and 17 weeks. GalCer levels, measured by immunostaining with an anti-GalCer antibody, were increased in thalamus, hippocampus and the cerebellar granular layer in the GalCer-treated versus vehicle-treated $Cln3^{\Delta ex7/8}$ mice. Exogenous GalCer also decreased the JNCL hallmark storage material levels, measured by subunit c immunostaining, in selected brain regions and in the peripheral tissues examined. In particular, subunit c storage material was dramatically reduced in the liver of GalCer-treated homozygous $Cln3^{\Delta ex7/8}$ mice versus the vehicle-treated homozygous $Cln3^{\Delta ex7/8}$ mice. Brains from GalCer-treated homozygous $Cln3^{\Delta ex7/8}$ mice also tended to display reduced gliosis, as measured by GFAP and S100 immunostaining. In locomotor activity tests, such as pole climbing, an enhanced benefit for both males and females was observed. It was, however, only statistically significant in males. Finally, addition of GalCer significantly normalized brain ceramide in homozygous $Cln3^{\Delta ex7/8}$ males and females as a group, and this was particularly robust in males.

In conclusion, exogenous GalCer partially corrected the GalCer deficit in lipid rafts in tissues from long-term treated homozygous $Cln3^{\Delta ex7/8}$ mice. Moreover, GalCer treatment resulted in diminished subunit c storage in homozygous $Cln3^{\Delta ex7/8}$ tissues, particularly in liver. Significantly, GalCer supplementation also lowered brain ceramide and improved behavior. Thus, our results support the hypothesis that CLN3 defects lead to impaired GalCer trafficking and increased brain ceramide, which may accelerate neurodegeneration in JNCL. GalCer supplementation will be further explored as a treatment option for JNCL.

Gene therapy in ovine Batten disease – pre-trial vector testing in neuronal cultures

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Naturally-occurring forms of Batten disease in three breeds of sheep have been extensively used to study Batten disease. These models are genetically similar to CLN5 and CLN6 forms of human disease and sheep have the advantage of similar brain structure and disease progression to that of humans. Our previous studies in *CLN6*-deficient South Hampshire sheep have shown that lentiviral vectors, expressing green fluorescent protein, are able to transduce sheep neural cells *in vitro* and *in vivo* (Linterman et al. 2011). Here we generated and tested lentiviral vectors expressing either CLN5 or CLN6 and developed new assays in primary neural cell cultures to test transgene expression and function.

Ovine *CLN5* or *CLN6* coding sequences containing a C-terminal myc-tag were cloned into a lentiviral plasmid containing the constitutively active viral LTR from the myeloproliferative sarcoma virus (MND) (Linterman et. al 2011). Viral particles were packaged using a VSV-G containing envelope and tested in both 293T cells and primary foetal sheep neural cultures.

Overexpressed CLN5-myc was efficiently modified by post-translational glycosylation in the endoplasmic reticulum (ER) and Golgi as demonstrated by EndoH and PNGaseF sensitivity and western blot. In addition, CLN5 was secreted from transduced cells and taken up by non-transduced cells showing the potential for *in vivo* cross-correction. Cultured *CLN5*-deficient neural cells (embyronic day 85) show accumulation of autofluorescent subunit c positive storage bodies over time and possible defects in lysosomal pH.

CLN6-myc was also successfully expressed via lentivirus in *CLN6*-deficient neural cultures. *CLN6*-deficient neural cultures (embryonic day 65) were assayed for lysosomal function using the probe LysoTracker Red. Our results showed an early and statistically significant decrease in lysosomal acidity. We also found diminished immunoreactivity to the ER chaperone protein calnexin suggesting a change in ER structure.

Future studies will utilise the lysosome and ER assays to test the functional correction of deficient cells by lentiviral-mediated gene transfer, and provide a basis for ongoing *in vivo* gene therapy trials in the sheep flocks.

Evaluation of the efficacy of a calpain inhibitor as an anti-neurodegenerative agent in Borderdale sheep with neuronal ceroid lipofuscinosis (CLN5)

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The current study aimed to evaluate the efficacy of a novel macrocyclic calpain inhibitor (CAT0811) in moderating the neurodegenerative cascade in the neuronal ceroid lipofuscinoses. Calpains (Ca^{2+}–dependent proteases) exert both regulatory and catabolic functions in neuron development. Increased intracellular Ca^{2+} is a ubiquitous feature of neurodegenerative diseases and over-activation of the calpain proteolytic system by Ca^{2+} flux is a key event in excitotoxicity leading to pathological neuronal death. The working hypothesis is that calpain-mediated neuronal cell death leads to further excitotoxicity and dysfunction in the neuronal network, and that appropriate inhibition of calpain activity may interrupt the neurodegenerative cascade in Batten disease.

Three key phase studies were carried out:

Phase 1) Cytotoxicity of CAT0811 and the possibility of an adverse reaction to CAT0811 were assessed on neuron cultures and *in vivo* in control sheep. Up to 10 µM CAT0811 in the medium for 3 days treatment had no cytotoxic effect and the lethal concentration (LC50) of CAT0811 was 100 µM. A normal 11 month- old sheep that received three 0.1mg CAT0811 per kg live weight intravenous injections over 24 hrs showed no signs of an acute chronic response and no signs of toxicity at *post-mortem*.

Phase 2) The bioavailability and penetration of CAT0811 were evaluated by mass spectrometery (TOF-MS). When two normal 7 month old sheep received a single 0.1mg CAT0811 per kg live weight intravenous injection, CAT0811 was detected in the plasma at least 16 hours later.

Phase 3) The efficacy of CAT0811 was tested on development of disease in CLN5 affected Borderdale sheep. For this study CAT0811 was administrated to 7-8 months old affected CLN5 sheep (n=3, Treated Group) by intravenous injection (0.1mg CAT0811per kg live weight), twice a week for 4 months, blank formula to age matched CLN5 affected sheep (n=4, Placebo Group). Heterozygous CLN5 sheep were similary housed but untreated and served as controls (n=3, Normal Group). There was no sign of adverse drug reaction to the treated sheep assessed monthly during the 4-month trial epriod. Tissue pathological examination at *post-mortem* also revealed no sign of cytotoxicity.

Behavioural changes in the sheep were recorded and analysed. Changes in brain volume and body compositions of the sheep were examined monthly by computer-tomography (CT) scanning. Changes in brain volume were minimal for two of the treated CLN5 affected sheep compared to the Placebo Group. At the end of the 4-month trial, all sheep were sacrified and brain and other tissues collected for analysis of neuropathology and calpain activities, which is ongoing.

Developing viral vector gene therapy for CLN5 and CLN6 Batten disease in ovine models

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The neuronal ceroid lipofuscinoses (NCLs; Batten disease) are inherited neurodegenerative lysosomal storage diseases with common clinical features of blindness and seizures culminating in premature death. The causative genes code for two classes of proteins, soluble lysosomal proteins or intramembrane proteins. This has consequences for therapies. Intercellular correction can be an important part of therapies in soluble protein deficits, whereas deficits of membrane bound proteins are anticipated to be entirely intracellular.

Possible therapies are best developed through testing in large animal models with large complex human-like brains. Here, we test *in vivo* viral therapies for two forms of Batten disease in sheep, a soluble protein defect, CLN5 in Borderdales, and an intramembrane protein defect, CLN6 in South Hampshires. Different pseudotypes of lentiviral vectors designed to target different cell types and integrate the gene into the host genome were tested in the first study. Specific areas of the brain were surgically targeted, particularly a recognised region of extended neurogenesis to allow for corrected cell replacement of dying affected cells in the intramembrane bound form. Myc- tagged *CLN5* and *CLN6* cDNA have subsequently been cloned into the vectors and transduction monitored by following co-expressed green fluorescent protein and the myc marker protein for each of the corrective genes.

Selected combinations are now being used in a therapy trial in affected lambs (n = 4/ genotype), which started before any disease development. The animals are being monitored for amelioration of developing symptoms of the disease with age, using brain CT scanning, anatomical and neurophysiological measurements. To date, volumetric results show either a steady decline within the realms of that expected for affected animals or plateaux in brain volume loss, whilst the animals exhibited varied disease onsets and progression from 4-8 months post-injection for the Borderdales to 8-10 months post-injection for the South Hampshires. The persistence and distribution of the transduced genes will be studied at *post mortem* along with indicators of the disease process, such as glial activation. The sheep are also being carefully examined for any other systemic pathology, such as tumour growth. These studies will indicate the possibility of therapy in preclinical affected humans for these and similar diseases.

Nonsense-suppression does not rescue the NCL-like phenotype of a zebrafish model of late infantile neuronal ceroid lipofuscinosis

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There is a pressing need to develop a safe and effective cure or treatment for each form of NCL. As nearly 30% of mutations in human LINCL are caused by a nonsense mutation in the *CLN2* gene, it is likely that the use of nonsense-suppressing drugs will result in read-through of the nonsense codon to produce full-length protein. A likely consequence of such a treatment, if successful, would be to halt the progression of the disease. Such non-toxic nonsense-suppressing drugs are currently providing promising results in clinical trials for Cystic Fibrosis and Duchenne Muscular Dystrophy.

In this project we assessed the feasibility of using nonsense-suppressing drugs to treat LINCL by treating a zebrafish model of LINCL (*cln2* mutants with a premature stop codon) using *in vivo* assays. Mutants treated with drugs had increased mortality compared to untreated mutants or normal siblings with drugs. In addition, mutants treated with drugs had no significant phenotypic improvement.

These results and their implications for the use of this class of drug for treating NCL will be discussed.

Supported by Newlife charity

TFEB-mediated lysosomal enhancement as a therapeutic strategy for Batten disease

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The neuronal ceroid lipofuscinoses (NCLs) are autosomal recessive storage diseases with common clinical features, including blindness, seizures, dementia and motor decline. The juvenile form of neuronal ceroid lipofuscinosis (JNCL), also called Batten disease, is caused by mutations in the *CLN3* gene that encodes a ubiquitously expressed protein of unknown function localized to the lysosomal membrane. NCL diseases are marked by two histopathological findings: degeneration of nerve cells, foremost in the cerebral cortex, and accumulation of autofluorescent ceroid-lipopigment in both neural and peripheral tissues. In JNCL, the ultrastructure of the autofluorescent inclusions resembles a fingerprint and the major storage component is identified as mitochondrial ATP synthase subunit c. There is currently no cure for these disorders. We recently identified the transcription factor EB (TFEB) as a master regulator of lysosomal function. Cells overexpressing TFEB have a larger number of lysosomes and an enhanced degradative capability against lysosomal substrates such as glycosaminoglycans and autophagy substrates such as protein aggregates. We and others have recently proposed that lysosomal/autophagic enhancement could be used as a therapeutic strategy for the treatment of LSDs and neurodegenerative diseases. TFEB-mediated lysosomal enhancement may be used to slow, halt or reverse the accumulation of undegraded molecules in affected tissues, towards a therapeutic clearance of pathogenic aggregates or storage deposits that are found in these diseases. Here, we evaluate *in vitro* and *in vivo* the effects of lysosomal enhancement on the clearance of lipofuscin in cells from patients and in a JNCL mouse model. We show improved clearance of the mitochondrial ATP synthase subunit c in mice in which TFEB has been either overexpressed or activated, and also investigate the distribution of neurodegenerative markers in these test mice. Our results provide the proof of principle that lysosomal enhancement may be an effective strategy to counteract pathogenic mechanisms of these diseases, and also provide a framework for future studies focused on its pharmacologic activation.

Exploring the potential of mouse neural stem cell grafts in Infantile NCL

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Whilst there are currently no effective clinical therapies for any form of NCL, several therapeutic approaches have showed some success in animal models and are currently in Phase I trials. These approaches depend upon cross-correction, with therapeutically delivered enzyme diffusing to correct neighboring deficient cells. One means to achieve this goal is to graft neural stem cells (NSCs) into the CNS, an approach that may also potentially replace dead or dying neurons. We have previously shown that human NSCs have a variety of beneficial effects when transplanted into immunodeficient Ppt1 null mutant mouse. Nevertheless, the NOD-SCID background of these mutant mice complicates these studies. Therefore, we revisited the potential of NSCs by transplanting *Ppt1*$^{-/-}$ mice with the MHP36 mouse neural stem cell line that has been thoroughly characterized for therapeutic efficacy in a variety of lesion models. Using a variety of exogenous cell labels we were able to track the migration and post-transplantation fate of MHP36 cells, and assess their impact upon the accumulation of storage material, glial activation and neuron survival in this mouse model of INCL. When transplanted into mice that had begun to show signs of disease, MHP 36 cells preferentially migrated into the neurogenic region of the subventricular zone, but were also found in sites of on-going neurodegeneration. The majority of transplanted MHP36 cells did not appear to adopt a neuronal fate, but did have a positive impact upon neuropathology. Largely this appeared to be by reducing inflammation, as assessed by thresholding image analysis of astrocyte and microglial markers. However, there also appeared to be moderate beneficial effects upon storage material accumulation and neuron survival, even in mice grafted at 5 months of age. Taken together, these data reveal that transplanted mouse NSCs have the tendency to migrate to certain sites within the *Ppt1*$^{-/-}$ CNS, either drawn their by cues released from these sites or perhaps survive preferentially within the potentially less hostile environment of the SVZ. Some of the beneficial effects of these transplanted NSCs are likely to be mediated by their secretion of Ppt1 enzyme, but an indirect impact upon the innate immune system may cause the greatest benefit, as has been proposed in experimental models of stroke. These data confirm that mouse NSCs may have some therapeutic potential, but these effects are not likely to be via cell replacement, but via other indirect mechanisms.

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NCL Foundation – a concept to fight an orphan disease?

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In 2012 the German based NCL Foundation will have its 10th anniversary. The aim of the non-profit foundation is to fight the fatal metabolic disease Neuronal Ceroid Lipofuscinoses (Batten disease) through the following objectives:

- To increase public awareness of NCL in order to promote the early diagnosis of the disease.
- To build an NCL-network of medical specialists to collect and coordinate existing national and international expertise.
- To initiate research and development concerning possible cures. Experts shall be brought together in order to utilize their particular expertise in the fight against NCL.
- To initiate concrete NCL research projects by creating research fellowships or other initiatives.

Since its start in 2002 more than 1,600 pupils in 50 high-schools have been informed about rare diseases in classroom settings. To facilitate early diagnosis, the NCL Foundation has instituted medical education courses for relevant physicians' groups such as ophthalmologists and pediatricians. As such, 53 medical training programs have been organized to date throughout Europe. The foundation also sets up lecture series for established medical conferences in order to engage scientists and clinicians from broad areas of expertise. To extend the scientific research and clinical network, nine national NCL congresses have been organized, as well as the first international JNCL PhD symposium bringing together junior scientists working in the NCL field. Currently the NCL Foundation is the largest promoter of JNCL PhD fellowships, with 10 ongoing projects based in 4 countries.

These international programs are made possible through collaborative efforts with other non-profit partners and universities. Moving forward, these established partnerships along with a focused research initiative will be necessary to expand the NCL network and ultimately eradicate Batten disease.

The Batten Disease Family Association (BDFA)

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The Batten Disease Family Association (BDFA) is a national charity, which aims to support families, raise awareness and facilitate research into Neuronal Ceroid Lipofuscinoses (Batten disease).
The BDFA was formed in 1998, with the help of See Ability and Contact-a-Family, by a small group of parents of children with Batten disease and was granted Registered Charity status in 2001. The BDFA is based in Hampshire, but works with children, young people, families and professionals across the UK.

The BDFA believes that no family should go through the devastating journey of Batten disease alone & aims to provide support for its members and families affected by the disease through the following projects:

- Providing the only UK Helpline dedicated to Batten disease
- Providing a Family Support Officer
- Facilitating a Family Networking Scheme
- Producing information and support resources
- Running professional and carer training workshops

The BDFA aims to facilitate research into all forms of the NCLs to increase knowledge about the disease, potential therapies and ultimately a cure. The charity supports forums for scientists, within the NCL field, to come together to in order to advance NCL research.
In 2007 the research funding programme was launched to directly find research projects, with an emphasis on projects that could lead to therapeutic advances with over £175 000 having been awarded culminating in the funding of a 3-year PhD studentship in 2011.

The BDFA aims to continue to work with all those involved to ensure effective collaboration between Batten Patient Organisations & research foundations, scientists, clinicians, professionals and those affected by Batten disease.

'Working Together' - A Child Development Team's Support to Children with a Rare Neurometabolic Condition

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Background

How do local teams support rare conditions?

Objectives

To describe the pathway through identification, diagnostic assessment, sharing news, symptom management, living with the condition and care and support until death, in five cases of Batten's Disease presenting to a Child Development Centre over 10 years

Methods

Consultants working at a local CDC identified five children diagnosed with Batten's disease over the past 10 years. The notes were reviewed to assess the time between each stage in the care pathway and were compared to the ACT (Association for Children's Palliative Care[1]) standards for managing a life-limiting condition.

Results

Five children were identified and seen by Community Paediatrics and Paediatric Neurologists. Patients were referred with a variety of symptoms. They all had Late Infantile Batten's Disease diagnosed by ERG and biopsy. The average age at diagnosis was 51 months. The average time from initial concerns to diagnosis was 14 months. Each child had a team of around 18 professionals around them who met at Team around the Child meetings. Three children had an end of life plan including a personal resuscitation plan. Two children died during the past 10 years.

Conclusion

We agree with the guidance as set out by ACT, which includes support at diagnosis, support when living with the condition and support at end of life.

We have shown that local care even for rare conditions can follow this guidance with the coordinated support of a large multi-professional team.

Acknowledgements

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Children with juvenile neuronal ceroid lipofuscinosis: how do they present to paediatricans and when? – UK data from the past 16 years

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Objective. To analyse data on children with juvenile neuronal ceroid lipofuscinosis (JNCL) identified via a national prospective study of UK children with progressive intellectual and neurological deterioration (PIND).

Methods. Paediatricians provided data via the British Paediatric Surveillance Unit, January 1995 - July 2011. Clinical details were obtained via questionnaire or clinic visit.
Results. 53 JNCL patients were notified: 32 female and 21 male. Median age at first symptom: 5 years 7 months. Median age at presentation to the reporting paediatrician: 8 years.

Presenting symptoms (not mutually exclusive) -visual failure 46 (87%), developmental delay/learning difficulties 29 (55%), behavioural problems 18 (34%), seizures 13 (25%), memory loss 8 (17 %), extrapyramidal signs 7 (13%) and gait disturbance/ataxia 6 (11%). Seizures and gait disturbance became evident later.

Investigations -MRI brain in 20: atrophy in 6 and subtle white matter changes in 2. EEGs in 27: 23 had abnormalities, usually slow and spike/wave discharges. ERGs in 39 and VEPs in 32: all were abnormal. Genetic results in 53: 37 positive, 36 had CLN3 mutations (29 homozygous, 3 heterozygous, 4 unknown) and 1 had a CLN5 homozygous mutation (compound heterozygote). 21/37 had the characteristic fingerprint or curvilinear inclusions on histological examination of biopsy specimens. No genetic studies in 16: 13 had characteristic histological findings, 3 had characteristic history and vacuolated lymphocytes.

Conclusion. This population-based study provides useful data about the clinical onset and diagnosis of JNCL: the majority presented to ophthalmologists with early visual failure. Children were referred to paediatricians about two years after developing symptoms. Most MRI scans were normal, however ERG/VEP studies were abnormal in all cases with results. Increasingly children are diagnosed by DNA studies resulting in re-classification of cases by genetic abnormality rather than by clinical/biopsy findings.

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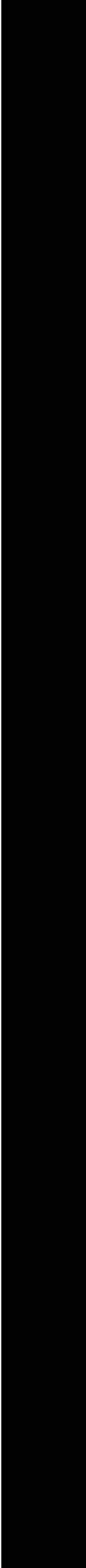
Lists of Participants



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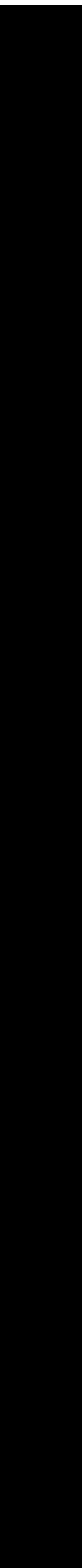


Conference Venue

Meals
All events except some meals and social occasions will be held in the Windsor Conference Centre, with our reception and 'Banquet' held in the Founder's Building. Most accommodation is in Tuke Hall (double rooms) and Reid Hall (single rooms). Breakfast will be served in 'The Hub' and lunches will be in the Windsor Building. Evening meals will be in the Founder's Building or 'The Hub'. Our 'Pub Social' will take place in 'The Medicine Bar', which is also open each evening. There is also a quiet area beneath The Hub, called 'Imagine', which is set aside primarily for families and parents. There is a Campus shop, which sells newspapers and a Bank with ATM machines. If you get lost please follow the signs with the NCL2012 logo on them or ask Conference staff for directions.

Postcode
The site postcode is TW20 0EX

On Site Facilities
There are a number of on-site facilities that, as a delegate, you may wish to take advantage of:

'The Store on Campus'
The on site convenience shop is located in the centre of campus close to the Founder's and Windsor buildings. It is open daily and stocks a wide variety of goods, including groceries, newspapers, stationery, stamps, mobile phone top-up cards as well as College memorabilia.

The College Bookshop
The on site bookshop is located next to the 'The Store on Campus' and stocks a selection of academic and non-academic books.

Natwest Bank
Natwest bank is located in the same area as the College Shop and Waterstones. It provides two ATM machines.

Chapel
There is a beautiful non-denominational chapel located in the Founder's building. It can be used as a place of worship for people of all faiths.

Emergency Contacts for Guests
The Conference Office telephone number to give family and friends in case they need to contact you at the conference is **01784 414149** and the office is open Monday to Friday 9am until 5pm. They can also contact either Andrea West (07841578684) or Ruth Williams (07766254206).

Numerous events can be running simultaneously and therefore it would be very useful if you could give family and friends the name of the event you are attending and the name of the accommodation where you will be staying. By giving them this information we will be able to contact you more quickly.

Outside of these hours, your friends and family will be able to get a message to you contacting Customer Services at the relevant reception listed below:

Accommodation	Customer Service Desk	Telephone:
The Hub, Reid, Runnymede, Gowar, Wedderburn, Williamson, Tuke, Butler	Hub	01784 443285
Founder's Hall	Founder's	01784 443052
Kingswood Hall	Kingswood	01784 435331
Penrose Court, Highfield Court	Highfield	01784 443440

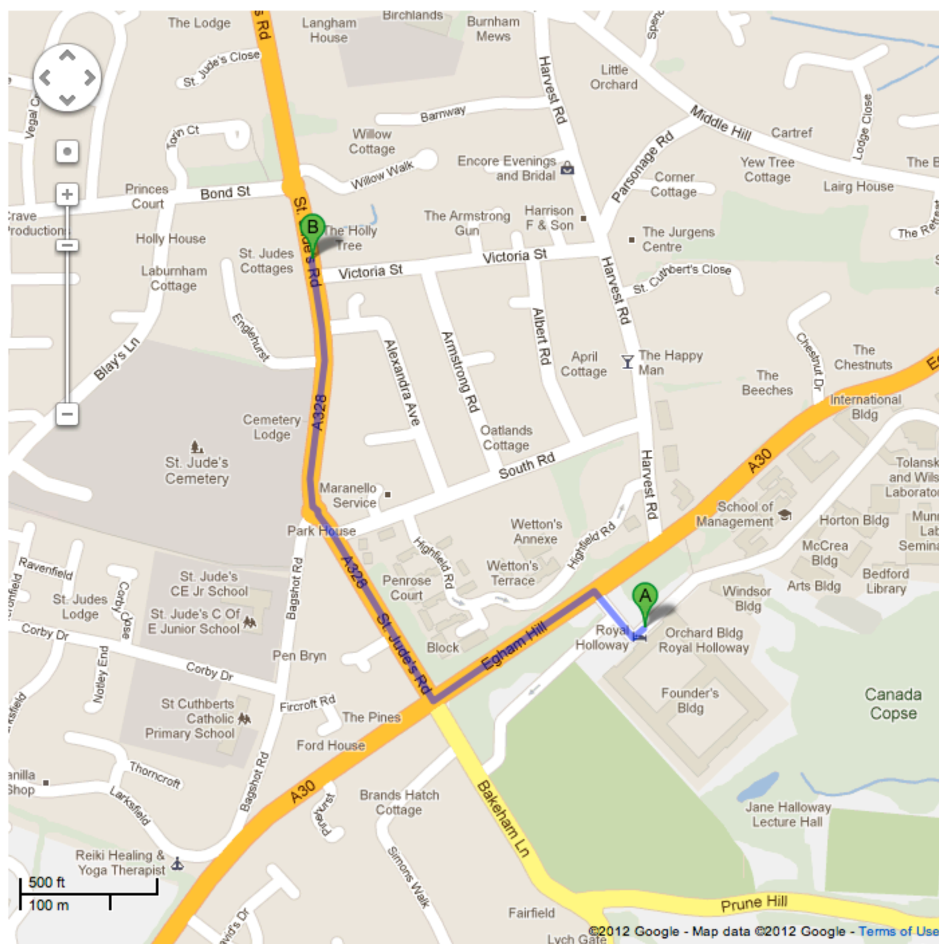
Travel information

Train, bus, coach: http://www.traveline.org.uk/ 0871 200 22 33
Transport for London: http://www.tfl.gov.uk/gettingaround/default.aspx 0843 222 1234
National Rail: http://ojp.nationalrail.co.uk 08457 48 49 50

Taxis:
Area Cars 01784 471001,
Gemini Cars 01784 471111,
Egham Cars 01784 434646,
Windsor Cars 01753 677677

Nearest Pharmacy
Lloyds Pharmacy, 98, St.Judes Rd, Egham, Surrey TW20 0DF
Tel: 01784 432161
Opening Hours: Monday-Friday: 09:00 - 19:00, Saturday: 09:00 - 17:30, Sunday: Closed

Directions are shown on the map below:
A=Royal Holloway, University of London
B=Lloyds Pharmacy



Summary of new classification nomenclature of the NCLs

	Gene symbol	Protein	Diseases
Soluble lysosomal enzyme deficiencies	*CTSD* *CLN10*	Cathepsin D	CLN10 disease, congenital CLN10 disease, late infantile CLN10 disease, juvenile CLN10 disease, adult
	PPT1 *CLN1*	Palmitoyl protein thioesterase 1, PPT1	CLN1 disease, infantile CLN1 disease, late infantile CLN1 disease, juvenile CLN1 disease, adult
	TPP1 *CLN2*	Tripeptidyl peptidase 1, TPP1	CLN2 disease, late infantile CLN2 disease, juvenile
	CTSF *CLN13*	Cathepsin F	CLN13 disease, adult Kufs type B
Non-enzyme deficiencies, (functions of identified proteins generally poorly understood at the current time)	*CLN3*	Transmembrane protein	CLN3 disease, juvenile
	CLN5	Soluble; lysosomal	CLN5 disease, late infantile CLN5 disease, juvenile CLN5 disease, adult
	CLN6	Transmembrane protein; ER	CLN6 disease, late infantile CLN6 disease, adult Kufs type A
	MFSD8 *CLN7*	Major facilitator superfamily domain-containing protein 8 Transmembrane protein; Endolysosomal transporter	CLN7 disease, late infantile
	CLN8	Transmembrane protein; ER, ER-Golgi intermediate complex	CLN8 disease, late infantile CLN8 disease, EPMR
	DNAJC5 *CLN4*	Soluble cysteine string protein α	CLN4 disease, adult autosomal dominant
	GRN *CLN11*	Progranulin	CLN11 disease, adult Heterozygous mutations cause frontotemporal lobar dementia
	ATP13A2 *CLN12*	P-type ATPase	CLN12 disease, juvenile Mutations also cause Kufor-Rakeb syndrome
	KCTD7 *CLN14*	Potassium channel tetramerization domain-containing protein 7	CLN14 disease, infantile Mutation also causes progressive myoclonic epilepsy-3
Others: those whose classification is uncertain because of incomplete diagnostic investigations or absence of confirmed gene/mutation designation, or where NCL is a rare or minor mutation-specific phenotype	*?*	Mutations not yet defined in any gene	Congenital/infantile variants
	?	Mutations not yet defined in any gene	Late infantile variants
	?CLN9?	Mutations not yet defined in any gene	Juvenile variants
	?	Mutations not yet defined in any gene	Late onset/adult variants including some adult Kufs type B
	CLCN6	Mutations not yet found on both disease alleles in human disease	Chloride transport defect, adult onset
	SGSH	Mutations usually cause MPSIIIA	Adult onset

Diseases listed are those now described, and all are autosomal recessive unless noted. It is possible that further cases of later onset e.g. CLN2 disease, adult, or those with atypical progression, e.g. CLN3 disease, juvenile, will yet be recognised.

Clinical Summaries

CLN1 Disease, infantile onset and others

What is the cause?

The gene called CLN1 lies on chromosome 1. CLN1 disease is inherited as an autosomal recessive disorder, which means that both chromosomes carry mutations in the CLN1 gene, and both parents are unaffected carriers. The gene was discovered in 1995. CLN1 normally directs production of a lysosomal enzyme called Palmitoyl protein thioesterase 1 or PPT1. A deficiency of PPT1 results in abnormal storage of proteins and lipids in neurons and other cells and impaired cellular function. The cells cannot function as they should and symptoms develop.

How is it diagnosed?

The diagnosis is usually made by enzyme (PPT1) and genetic (*CLN1*) tests on blood samples. Occasionally a skin biopsy may be necessary. Granular osmiophilic deposits (GRODSs) are the characteristic storage body at the electron microscope level.

Does it have any alternative name?

CLN1 disease was first described in the 1970s in Finland and is sometimes called Haltia-Santavuori Disease, Infantile neuronal ceroid lipofuscinoses, or INCL.

How common is it?

1-2 children are diagnosed with infantile Batten disease each year in the UK. We estimate there are between 15 and 30 affected children in the UK. Children have been diagnosed in many countries although when the condition was first described most cases came from Scandinavian backgrounds

How does the disease progress? Genotype/phenotype correlations

Classical CLN1 disease, infantile

Babies are healthy and develop normally for the first few months of life. Towards the end of the first year, developmental progress starts to slow down. Infants may have difficulty sleeping through the night and may become more restless and irritable during the day. Some infants develop repetitive hand movements and fiddling. They often become floppy and developmental skills such as walking, standing and speech are lost. Children become less able and increasingly dependent during the toddler years. By the age of 2 years, most will have epileptic seizures and jerks. Vision gets worse until they are no longer able to see.

From about the age of three years, children are completely dependent, unable to play, feed themselves, sit independently or communicate. They may need a feeding tube and their arms and legs usually become stiff. Some children get frequent chest infections.

Death usually occurs in early to mid childhood.

CLN1 disease, juvenile

Some children with mutations in CLN1 have a later onset of symptoms and slower disease progression. Occasionally the symptoms resemble those of children with mutations in the CLN3 gene and juvenile onset disease, with onset around 5-6 years of age. They present with behavioural difficulties and early visual deterioration followed by seizures in mid-childhood. Death commonly occurs in the teenage years.

CLN1 disease, variant late infantile and adult types.

A wide variety of age at symptom onset and disease progression is seen with mutations in CLN1.

CLN2 disease, late-infantile

What is the cause?

The gene called CLN2 lies on chromosome 11. CLN2 disease is inherited as an autosomal recessive disorder, which means that both chromosomes carry mutations in the CLN2 gene, and both parents are unaffected carriers. The gene was discovered in 1998. CLN2 normally directs production of a lysosomal enzyme called tripeptidyl peptidase1 or TPP1. A deficiency of TPP1 results in abnormal storage of proteins and lipids in neurons and other cells and impaired cellular function. The cells cannot function as they should and symptoms develop.

How is it diagnosed?

The diagnosis is usually made by enzyme (TPP1) and genetic (*CLN2*) tests on blood samples. Occasionally a skin biopsy may be necessary. Curvilinear bodies (CVB) are the characteristic storage body at the electron microscope level.

Does it have any alternative name?

CLN2 late infantile disease is sometimes called Jansky-Bielschowsky Disease or late infantile NCL (LINCL).

How common is it?

5-6 children are diagnosed with late-infantile Batten disease each year in the UK. We estimate there are between 30 and 50 affected children in the UK. Children have been diagnosed in many countries and from a variety of ethnic backgrounds.

How does the disease progress?

Children are healthy and develop normally for the first few years of life. Towards the end of the second year, developmental progress may start to slow down. Some children are slow to talk. The first definite sign of the disease is usually epilepsy. Seizures may be drops, vacant spells or motor seizures with violent jerking of the limbs and loss of consciousness. Seizures may be controlled by medicines for several months but always recur, becoming difficult to control. Children tend to become unsteady on their feet with frequent falls and gradually skills such as walking, playing and speech are lost. Children become less able, and increasingly dependent. By 4-5 years the children usually have myoclonic jerks of their limbs and head nods. They may have difficulties sleeping and become distressed around this time, often for no obvious reason. Vision is gradually lost. By the age of 6 years, most will be completely dependent on families and carers for all of their daily needs. They may need a feeding tube and their arms and legs may become stiff. Some children get frequent chest infections. Death usually occurs between the ages of 6 and 12 years (but occasionally later).

CLN3 disease, juvenile

What is the cause?

The gene called *CLN3* lies on chromosome 16 and was discovered in 1995. CLN3 disease is inherited as an autosomal recessive disorder, which means that both chromosomes carry mutations in the CLN3 gene, and both parents are unaffected carriers. This gene codes for a transmembrane protein. The nerve cells cannot function as they should and symptoms develop.

How is it diagnosed?

The diagnosis is usually made by genetic (*CLN3*) tests on blood samples. Occasionally a skin biopsy may be necessary.

Does it have any alternative name?

At the beginning of the 20th century Dr Frederick Batten described a group of disorders that now bear his name. Over time it was discovered that there were several types of the disease with similar but distinct features and ages at onset of symptoms: infantile, late infantile, juvenile, and adult. CLN3 disease is often called Batten disease, or Spielmeyer-Sjogren-Vogt disease.

How common is it?

3-4 children are diagnosed with juvenile Batten disease each year in the UK. We estimate there are between 30 and 60 affected children and young adults in the UK. Children have been diagnosed in many countries and from a variety of ethnic backgrounds.

How does the disease progress?

Children are healthy and develop normally for the first few years of life. The first sign of the disease is usually a gradual loss of vision between 4 and 7 years of age. This may be noticed first at nursery or at school. Vision changes rapidly over 6 to 12 months initially but children retain some awareness of colour and light/dark until later. By the end of primary school, children are beginning to show some difficulties with concentration, short-term memory and learning. Many are still able to attend mainstream school but may need extra learning support in the classroom. The next stage of the disease starts with the onset of epileptic seizures (average age of onset of seizures is 10 years). Often the first seizures are motor seizures with violent jerking of the limbs and loss of consciousness. Seizures may be controlled by medicines for several months or years, but always recur, eventually becoming difficult to control completely. The pattern of seizures may change over time and other seizure types may evolve, such as vacant spells and episodes of partial awareness with fiddling and muddled speech.

During the teenage years children tend to slowly become more unsteady on their feet. At around the same time speech may become repetitive and gradually more difficult to understand. Not uncommonly children become anxious and tend to worry. Some feel things, hear voices or see things that are unreal. Teenagers become less able and increasingly dependent. The course of the disease is extremely variable even for children from the same family. The teenagers and young adults are much more able some days than others, especially in terms of mobility, communication and feeding skills. The disease progresses with periods of stability which may last months or years alternating with periods of deterioration lasting several months which may be triggered by intercurrent illness.

Death usually occurs between the ages of 15 and 35 years (but occasionally later).

Variant late infantile onset NCLs: CLN5, CLN6, CLN7 and CLN8 diseases & others

What is the cause?

Late-infantile variant Batten disease is caused by a genetic mistake in one of the Batten disease genes. We now know of at least eight different genes that can cause Batten disease. Those responsible for late infantile variant are usually *CLN1, CLN5, CLN6, CLN7, CLN8 and CLN10*. These genes code for proteins which are either soluble lysosomal proteins or proteins embedded within membranes. These diseases are inherited as autosomal recessive disorders, which means that both chromosomes carry mutations in the disease gene, and both parents are unaffected carriers. The nerve cells cannot function as they should and symptoms develop.

How is it diagnosed?

The diagnosis is usually made by histological and genetic tests on blood samples. A skin biopsy may be necessary and the abnormal storage material takes on a mixed appearance with granular osmiophilic deposits (GRODS), curvilinear bodies (CVB), rectilinear profiles (RLP), and/or fingerprint profiles (FPP). The appearance of the storage material can guide the genetic diagnostic tests in some cases.

Does it have any alternative name?

Several forms of late infantile variants have been recognised since the 1980s and different names have been used: variant late infantile NCL, early juvenile NCL, Finnish variant, Turkish variant, Indian variant, Mediterranean variant NCLs and so on.

How common is it?

1-2 children are diagnosed with late-infantile variant Batten disease each year in the UK. We estimate there are between 10 and 20 affected children in the UK. Children have been diagnosed in many countries and from a variety of ethnic backgrounds.

How does the disease progress?

Children are healthy and develop normally for the first few years of life. Children with late infantile variant NCL can be very different from each other, making the disease course difficult to predict in individual cases. The first symptoms may be apparent within the first few years of life but may ne develop until after school entry. Challenging behaviour is common especially in retrospect. Slowing of developmental progress, epilepsy and later loss of thinking and learning skills should prompt diagnostic investigations, especially where vision may also be deteriorating. Vision is gradually lost at some stage but this is variable. Some children will be completely dependent on their families and carers for all their daily needs by the age of six years whereas others will lose their walking and talking much later.

Death usually occurs in childhood or during the teenage years.

CLN8 Disease, EPMR and late infantile variant

What is the cause?

The gene called CLN8 lies on chromosome 8. CLN8 disease is inherited as an autosomal recessive disorder, which means that both chromosomes carry mutations in the CLN8 gene, and both parents are unaffected carriers. The gene was discovered in 1999. CLN8 normally directs production of a protein that is embedded in internal cell membranes. The cells cannot function as they should and symptoms develop.

How is it diagnosed?

The diagnosis is usually made by histological and genetic (*CLN8*) tests on blood samples. A skin biopsy may be necessary. The characteristic storage bodies at the electron microscope level often show a mixture of fingerprint profiles (FPP) and curvilinear bodies (CVB).

How does the disease progress? Genotype/phenotype correlations

Epilepsy with Progressive Mental Retardation (EPMR) or Northern Epilepsy

This disease is caused by mutations in CLN8 but has seldom been described outside Scandinavian countries. Symptoms usually start between the ages of 5 and 10 years, with seizures. Cognitive decline occurs at around the same time. Seizure frequency increases until puberty. Cognitive deterioration is more rapid during puberty. Behavioural disturbances can occur, eg: irritability, restlessness, inactivity and these features may continue into adulthood. Epilepsy is partially responsive to treatment. The number of seizures decreases spontaneously after puberty, even with no change in treatment, and by the second-third decade they become relatively sporadic. Cognitive decline continues and in some cases loss of speech has been reported. Motor function is also impaired. In a number of cases, visual acuity is reduced (without evidence of retinal degeneration). The disease has a chronic course and survival to the sixth or seventh decade has been reported. EPMR is very unusual amongst the NCLs of childhood onset in this respect.

CLN8 disease, variant late infantile

All children have developmental delay before the onset of symptoms at 2 -7 years of age: myoclonic seizures and an unsteady gait are commonly the initial symptoms; other seizures follow soon after. Cognitive decline and visual impairment usually occur. Behavioural abnormalities are frequent. Rapid disease progression with loss of cognitive skills is observed over two years from the time of diagnosis. By the age of 8-10 years severe deterioration of neurological and cognitive skills is apparent together with medication resistant epilepsy. Spasticity, dystonic posturing, tremors, and other extrapyramidal signs are also observed commonly. In the second decade of life children are unable to walk or stand without support. The life-expectancy of children affected by this disease is not yet known. The eldest patients known are now in their second decade, and their general health remains good.

CLN10 disease, congenital, neonatal and late infantile

This is a very rare form of NCL and only a small number of cases have been written about. There may be undiagnosed cases.

What is the cause?
This disease is caused by mutations in a gene called Cathepsin D also called CLN10, which lies on chromosome 11. CLN10 disease is inherited as an autosomal recessive disorder, which means that both chromosomes carry mutations in the CLN10 gene, and both parents are unaffected carriers. The gene was discovered in 2006. CLN10 normally directs production of a lysosomal enzyme called cathepsin D. If no enzyme is produced, symptoms start very early in life, or even before birth. If some enzyme is working, symptoms develop later and disease progression is slower.

How is it diagnosed?
The diagnosis is usually made by enzyme (CTSD) and genetic (*CLN10*) tests on blood samples. Occasionally a skin biopsy may be necessary. Granular deposits are the characteristic storage body at the electron microscope level.

How does the disease progress? Genotype/phenotype correlations

CLN10 disease, congenital
Seizures occur before birth. In the newborn period the babies have refractory seizures and apnoeas. Babies may die within the first weeks of life.

CLN10 disease, late infantile
Some children with mutations in CLN10 have a later onset of symptoms and slower disease progression, like variant late infantile NCL. Children become unsteady, develop seizures and visual impairment. Later they lose skills.

Adult onset NCLs

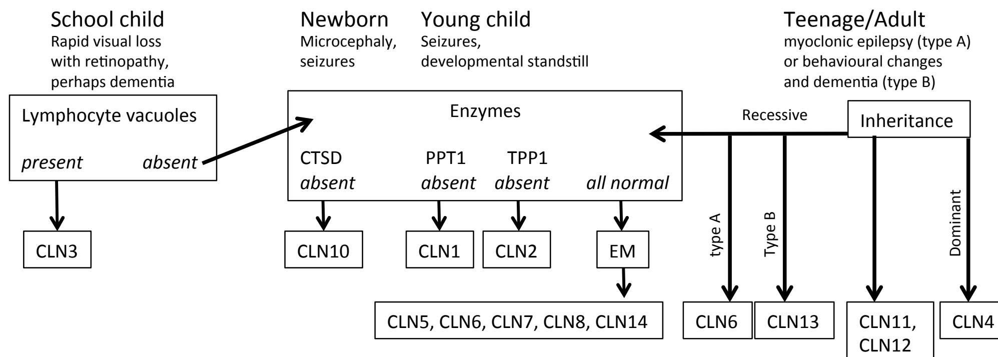
Adult NCL is very rare although affected families have been described from several different countries. Adult NCL has often been called Kufs disease and doctors recognise two main types – called type A and type B. Unlike the childhood NCLs, vision is not affected in either type. In some families inheritance is recessive but in others a dominant inheritance pattern is seen. Until the genetic basis for the adult NCLs is fully understood, diagnosis is usually dependent on a brain biopsy. It is emerging that several genes can cause adult onset NCL diseases, some of which have been presented at this meeting.

Type A presents in early adulthood with a progressive myoclonic epilepsy, ataxia and slow cognitive deterioration over many years.

Type B usually presents with an early dementia or evolving movement disorder.

Mild mutations in childhood NCL genes may also cause NCL disease with delayed age of onset and slow disease progression but vision is generally affected and abnormal storage is seen more reliably in peripheral tissues.

Summary Diagnostic Algorithm for the NCLs



NCL Diagnostic Clinical Description

Axis 1: Affected gene

Axis 2: Mutation diagnosis

Axis 3: Biochemical phenotype. e.g. enzyme deficiency

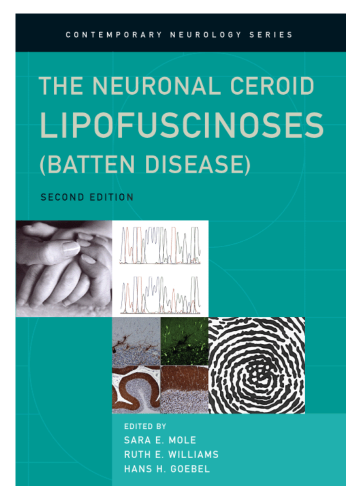
Axis 4: Clinical phenotype. e.g. congenital, infantile, late infantile, juvenile, adult; classic or variant

Axis 5: Ultra-structural features where known

Axis 6: Functionality. e.g. UBDRS, Hamburg score

Axis 7: Other remarks of possible medical relevance eg social factors

Resources



The neuronal ceroid lipofuscinoses (Batten disease)

Editors Mole, Goebel and Williams.

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Book Review in J Child Neurol. 2011 Oct; 26(10):1330

NCL Resource Gateway (Dr Sara Mole, UCL) including NCL Mutation Database

http://www.ucl.ac.uk/ncl

Pediatric Storage Disorders Lab (Prof Jonathan Cooper)

http://tinyurl.com/newpsdl

Previous NCL meetings

International Symposium on Human and Animal Models of Ceroid-lipofuscinosis, Røros, Norway. 1-4 June 1980

Second Congress on the Neuronal Ceroid Lipofuscinosis, Staten Island, USA. 30 May-1 June 1987

Third International Conference on the Neuronal Ceroid Lipofuscinosis, Indianapolis, USA. 30 May-1 June 1990

Fourth International Symposium on the Neuronal ceroid-lipofuscinoses, Hamburg, Germany. 10-13 June 1992

Fifth International Conference on Neuronal Ceroid-Lipofuscinoses. Newark, USA. 19-21 May 1994

Sixth International Congress on Neuronal Ceroid-lipofuscinoses (NCL-96). Gustavelund, Finland. 8-11 June 1996

Seventh International Congress on the Neuronal Ceroid-Lipofuscinoses (NCL-98), Dallas, USA. 13-16 June 1998

NCL-2000. The 8th International Congress on Neuronal Ceroid Lipofuscinses (Batten Disease). Exeter College, Oxford, UK. 20-24 September 2000

9th International Congress on Neuronal Ceroid Lipofuscinosis (Batten Disease). Chicago, USA. 9-13 April 2003

NCL-2005. The 10th International Congress on Neuronal Ceroid Lipofuscinoses. Helsinki, Finland. 5-8 June 2005

NCL2007. 11th International Congress on Neuronal Ceroid Lipofuscinosis (Batten Disease). Rochester, NY, USA . 14-17 July 2007.

NCL2009. 12th International Congress on Neuronal Ceroid Lipofuscinoses. Hamburg, Germany. 3-6 June 2009.

Useful links and contacts

Batten Disease Family Association (UK) www.bdfa-uk.org.uk

Bee for Battens (Ireland) www.BeeForBattens.org

Batten Disease Support and Research Association (USA) www.bdsra.org

Beyond Batten Disease Foundation (USA) www.beyondbatten.org

Beat Batten (Netherlands) www.beatbatten.com

ContactpuntNCL (Belgium) www.contactpuntncl.be

Danish NCL Family Association www.dsvf.dk

"Life" Association against child rare illness (Serbia) www.zivotorg.org

NCL Stiftung (Germany) www.ncl-foundation.com

NCL-Naechstenliebe (Germany) www.ncl-naechstenliebe.de

NCL Gruppe (Germany) www.ncl-deutschland.de

Norwegian NCL Family Association www.nsvf.org

Asociación Española de Familias afectadas por Lipofuscinosis (Spain) www.nsvf.org

Noah's Hope (USA) www.noahshope.com

www.ncl2012.org *www.bdfa-uk.org.uk*

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