UCL

Inherited Neuropathies Consortium

UNIVERSITY OF MIAMI
MILLER SCHOOL OF MEDICINE
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for HUMAN GENOMICS

A MUTATION IN ALFA-B-CRYSTALLIN CAUSING CMT2

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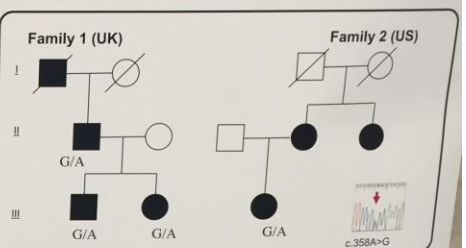
BACKGROUND

Mutations in Alpha-B-crystallin (CRYAB) gene have been associated with myofibrillar myopathy, dilated cardiomyopathy and cataracts.
CRYAB, also known as HSPB5, is a member of a family of 10 small heat-shock proteins that are molecularly defined by the presence of a highly conserved Alpha-B-crystallin domain and are functionally involved in chaperone-mediated autophagy. Of interest, mutations in two other members of this family, HSBP1 and HSPB8, have been associated with a combined neuromuscular disorder, which includes motor neuropathy and myofibrillar myopathy. However, as opposed to these, peripheral neuropathy has never been reported to be a feature of CRYAB-related disease.

AIM

To report two unrelated families with CMT2, associated with congenital cataracts in the majority of cases, and a pathogenic c.358A>G;p.Arg120Gly mutation in CRYAB

PEDIGREE

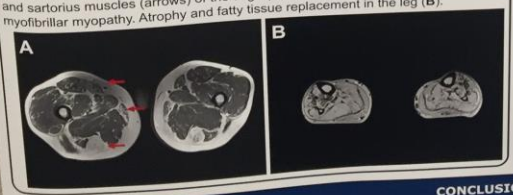


CLINICAL FEATURES

	UK-I-1	UK-II-1	UK-III-1	UK-III-2	US-III_1
Age, Gender	Deceased, M	52, M	28, M	26, F	47, F
Development	Normal	Normal	Normal	Normal	Normal
Past medical history	-	Congenital cataracts	Congenital cataracts	Congenital cataracts	Congenital cataracts
Age of onset	50	40	26	25	40
Symptom at onset	Walking difficulties	Walking difficultes, slaps feets, falls	Cramps in dominant hand	Finds harder to dance	Walking difficulties
Neurologic Examination					
Gait	Not available	High stepping gait, heels/toes not possible. Uses AFOs	Normal	Normal	Not available
Weakness	Not available	Moderate to severe distally. Hip flexion and dyaphragm	Mild weakness of instrinsic hand muscles	Mild distally, knee flexion and dyaphragm	Not available
Sensation	Not available	Reduced vibratory sensation to knees	Normal	Normal	Not available
Reflexes	Not available	present	present	present	Not available
Associated features					
Congenital cataracts	Not reported	Yes	Yes	Yes	Yes
Myopathic features	Not reported	Dyaphragmatic weakness	No	Dyaphragmatic weakness	Not reported
Heart involvement	Not reported	Absent	Absent	Absent	Not reported

Muscle MRI of the lower limbs

Lower limb MRI of **UK-II-1**: Selective involvement of the semi-tendinous, gracilis and sartorius muscles (arrows) of the thigh (**A**), as often reported in CRYAB-related myofibrillar myopathy. Atrophy and fatty tissue replacement in the leg (**B**).



Nerve conduction study

Motor and sensory axonal neuropathy

	UK-II-1	UK-III-1	UK-III-2
Motor NCS			
Ulnar	5.5 mV; 48 m/s	5.5 mV; 48 m/s	7.9 mV; 58 m/s
Peroneal	**1.6 mV; 48 m/s**	**1.6 mV; 48 m/s**	4.1 mV; 48 m/s
Sensory NCS			
Radial	19 μV; 51 m/s	19 μV; 51 m/s	62 μV; 56 m/s
Sural	**Not recordable**	**Not recordable**	13 μV; 36 m/s
EMG	Normal	Normal	Normal

CONCLUSIONS

Our report expands the phenotype of CRYAB-related disorders to encompass CMT2. It also adds further evidence to the continuous spectrum of expressivity from myopathic to neuropathic and mixed forms, which seems to characterise mutations in a growing number of genes involved in protein degradation and chaperone-

Expanding concepts of heredity in CMT (Stephan Zuchner)

- CMT1 : > 90% mendelian diagnosis
  CMT2 : 50% cases are genetically undiagnosed
  Phenotype variance : genes and environment
- Genetic modifying variations may contribute to the phenotypic variability of CMT1A patients. Heretability studies indicated 60% of effect is inherited
- Gene-based rare variant analysis : comparison of number of variants in patients and in controls. If more variants in controls = burden➔ gene might be a modifying gene
- EXOC4 : involved in vesicle transport and membrane tethering. Expressed in Schwann cells ➔ risk gene candidate for CMT
- SIPA1L2 : GTPase expressed in peripheral nerve and brain. Part of a SOX10-regulated myelination gene network ➔ risk gene for CMT1a severe phenotype

Modeling reveals TARS as a candidate gene for axonal neuropathy (Rebecca Mayer)

- Amynoacyl-tRNA-Synthetase. 37 ARS
- Loss of function recessive diseases
- Dominant diseases : CMT, dHMN, dSMA-V (GARS : CMT2D)
  Loss of function, toxicity
- Some mutations in TARS in worm models provokes similar phenotype
- Parallel testing TARS variant in the general population

Mutation in HSPB1 impairs its mitochondrial role (Elias Adriaenssens)

- HSPB1 causes axonal CMT2F
- Small HSP form dynamic oligomers
- Imports proteins in the mitochondria
- Mutations in alpha crystallin domain increase mitochondrial residence
- Other mutations decrease mitochondrial import

Mutations in the CADM family cause CMT (Andrea Cortese)

- CADM (nectin-like) : axon-glia interaction in the internode
- WES identified a CADM3 mutation in 3 families with CMT2
  - 2 isolated cases, 1 dominant
  - Moderate to severe atrophy and weakness in distal muscles
  - Upper limb predominant weakness
  - Brisk reflexes
  - Preserved sensitivity
  - The mutation disrupts disulfide bonds connectivity od CADM3
  - Mutant CADM3 is retained in the ER and actiavtes the UPR
  - Mutant CADM3 shows reduced instability
- WES identified a variant in CADM4 in one AD CMT2 patient
  - Onset in the 5th decade
  - Distal numbness, paresthesia, weakness
  - Intermediate velocities and sensory motor neuropathy

NGS reveals new gene responsible for recessive motor neuropathies (Stephano Previtali)

- HMN/CMT2 (>motor). Families with at least 2 siblings affected and healthy parents (cosanguious or not)
  . Known genes for CMT excluded
  ➔ 15 families
- Possible candidate gene for 12 families. In 8/12 families the candidate gene has already been described in neuromuscular diseases
- ARHGEF28 : described in ALS
  - Onset 1 year. Axonal motor neuropathy. CK elevation
- GNE : described in myopathy and recently in ALS
  - 34yo, reduced pallesthesia, proximal weakness at 50y. EMG : first chronic denervation then myopathic features. IBM andgrouping on muscle biopsy
- Agrin : described in CMS
  - - Onset 2y, distal hypopallesthesia, also impairment of neuromuscular junction
- PNKP : described in AOA4
  - Onset 30y, CK elevation, ± ataxia. Axonal motor neuropathy
- KBTBD13 : described in nemaline myopathies, promotes protein ubiquitination
- SIGMAR1 : described in family ALS.
  - Onset 18/10y
  - Pure motor neuropathy

Preliminary Phase 2 results for ACE-083 in patients with CMT1 and CMTX
(Florian Thomas)

- ACE-083 : fllistatin binding myostatin and muscle regulators
- Administrated in TA every 3 weeks
- No serious adverse events
- Mean percent change in total muscle volume at day 106: 12.6% (±2.9). Mean change in fat fraction from baseline : -1.7% (±1.2)

Pivotal Phase III study of PXT3003 for CMT1A (Rene Goedkoop)

- Multicenter randomized placebo controlled (12/2015)
- PXT3003 : baclofen + nalterxone + sorbitol
- Oral 2 per day during 15 months
- Outcome : ONLS at month 12 and 15

Therapeutic effects of HDAC6 in models of inherited and acquired neuropathies
(Ludo Van de Bosch)

- Inherited models : HSPB1, GRAS
- Acquired models : Vincristine induced
- Models showed decreased acetylation of alpha-tubulin, which affects axonal transport
- Does inhibition of HDAC6 restore acteylation and axonal transport?
- HDAC6 inhibition has a therapeutic effect: motor performance, motor and sensory conduction, neuromuscular junction (decreased number of demyelinated NJM)
- HDAC6 inhibitors also have an additionnal anti-cancer effect

Serum protein biomarker & a potential role of complement int CMT (Matthew Jennings)

- Proteomic approach
- Biotinidase is increased in CMT1 and correlated with severity
  Biotinidase maintains free biotin for fatty acid synthesis, involved in myelination
- Complement system is elevated in CMT1 and CMT2, but does not correlate with severity
- Juneja et al. found the C3 CR2 increased in CMT2 patients lymphoblasts
- Complement is involved in the NMH in CMS and ALS

A novel molecular mechanism causing hereditary neuropathy with liability to pressure palsies

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HELSE SØR-ØST

Introduction

Hereditary neuropathy with liability to pressure palsies (HNPP) is usually caused by a deletion of the PMP22 gene on chromosome 17p11.2 while duplication of PMP22 causes Charcot-Marie-Tooth disease type 1A (CMT1A). Point mutations in PMP22 may result in HNPP or CMT1A. HNPP has autosomal dominant inheritance.

HNPP is characterized by repeated focal pressure neuropathies such as carpal tunnel syndrome and peroneal palsy with foot drop. The first attack usually occurs in the second or third decade. Recovery from acute neuropathy is often complete; when recovery is not complete, the resulting disability is usually mild. Some affected individuals also have signs of a mild to moderate peripheral neuropathy.

Materials and methods

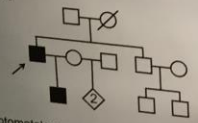
An adult with rather typical symptomatology of HNPP was, after exclusion of the *PMP22* deletion, investigated for variants in known peripheral neuropathy genes by Next-generation sequencing (NGS). His son with similar symptomatology was examined by Sanger sequencing.

Methods

1. Clinical investigation of willing family members
2. Targeted capture and DNA sequencing
3. Sequence analysis and Sanger verification
4. Variant interpretation according to international guidelines

Results

- Clinical features
  - Pedigree



- Symptomatology

Proband (father)

Symptoms

51 years of age, previously treated for lung sarcoidosis

Onset at 22 years of age with right-sided dropfoot lasting for months after squatting

Proband (father) *(cont.)*

Symptoms

Sometimes loses items, e.g. his cup when drinking, and sometimes more difficult to use keys

Reduced hand function most pronounced during winter

Right hand paresis gradually ameliorated last months

Numbness in feet and periodically tendency to step over in ankles

Findings

Slight muscular atrophy in hands and feet

Reduced sensibility to pinprick both 5th fingers and ulnar part of right 4th finger and generally reduced vibration sense

NCVs in arms around 41 m/s and in legs 31 m/s or absent

EMG revealed chronic neurogenic changes

Son

Symptoms

14 years of age

Age at onset at 10 years of age with paresis of right arm lasting for weeks to months and later same year right-sided dropfoot

Still problems playing soccer

Findings

Absent biceps reflexes

NCV at 10 years of age 45 m/s in right motor median nerve and probable conduction block right peroneal nerve at knee level

MRI of columna at 10 years of age was normal

- Genetic analyses
  - Multiplex Ligation-dependent probe Amplification, P405-A1 and P143-C1, was normal, i.e. rsa *PMP22,MPZ,MFN2*)x2,(*GJB*)x1
  - Targeted NGS for hereditary neuropathy genes and Sanger sequencing identified a heterozygous sequence variant in *PMP22*, i.e. NM_153322.2:c.1A>C p.(?)
- Variant interpretation
  - Novel variant probably causing loss of one allele due to loss of start codon. Bioinformatics reveal no alternative start codon nearby.
  - *PMP22* is not expressed in cells from blood.
  - RNA assays have not yet been performed since we have only cells from blood (and no nerve biopsies / other tissue).

Conclusion

Loss of *PMP22* start codon as a novel molecular mechanism causing HNPP

Pentanucleotide Repeat Expansion is a frequent cause of late-onset sensory ataxic neuropathy (Andrea Cortese)

- CANVAS : non-length dependent sensory neuropathy, cerebellar dysfunction, bilateral vestibulopathy ± cough/dysautonomic symptoms
- Occasionaly reported in siblings, WES unsuccessful
- Non parametric AR linkage analysis identified a unique 1.5Mb locus associated with the disease. No coding or splicing variants in WES.
- WGS showed absent coverage of a simple repeat ➔ deletion or expansion
- Repeat primed PCR approach confirms the presence of repeat expansion in familial and sporadic patients (all familial cases and 24 sporadic cases), but none of 280 controls
- Allelic carrier frequency in the control population was 5/560 chromosomes (1%)
- 8 to 15kb expansion : 6000-2000 repeats
- Age of onset 54±8, disease duration 13
  Sensory neuropathy 100%, bilateral vestibular impairment 71%, cerebellar syndrome 78%, cough 37%, autonomic involvement 5%
  cerebellar atrophy 84%

Functional studies : non relevant gene