PNPLA6 mutations cause Boucher-Neuha¨user and Gordon Holmes syndromes as part of a broad neurodegenerative spectrum

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Boucher-Neuha¨user and Gordon Holmes syndromes are clinical syndromes defined by early-onset ataxia and hypogonadism plus chorioretinal dystrophy (Boucher-Neuha¨user syndrome) or brisk reflexes (Gordon Holmes syndrome). Here we uncover the genetic basis of these two syndromes, demonstrating that both clinically distinct entities are allelic for recessive mutations in the gene.
PNPLA6. In five of seven Boucher-Neuha¨user syndrome/Gordon Holmes syndrome families, we identified nine rare conserved and damaging mutations by applying whole exome sequencing. Further, by dissecting the complex clinical presentation of Boucher-Neuha¨user syndrome and Gordon Holmes syndrome into its neurological system components, we set out to analyse an additional 538 exomes from families with ataxia (with and without hypogonadism), pure and complex hereditary spastic paraplegia, and Charcot–Marie–Tooth disease type 2. We identified four additional PNPLA6 mutations in spastic ataxia and hereditary spastic paraplegia families, revealing that Boucher-Neuha¨user and Gordon Holmes syndromes in fact represent phenotypic clusters on a spectrum of neurodegenerative diseases caused by mutations in PNPLA6. Structural analysis indicates that the majority of mutations falls in the C-terminal phospholipid esterase domain and likely inhibits the catalytic activity of PNPLA6, which provides the precursor for biosynthesis of the neurotransmitter acetylcholine. Our findings show that PNPLA6 influences a manifold of neuronal systems, from the retina to the cerebellum, upper and lower motor neurons and the neuroendocrine system, with damage of this protein causing an extraordinarily broad continuous spectrum of associated neurodegenerative disease.

Keywords: ataxia; recessive ataxia; hypogonadism; retinal degeneration; spastic ataxia; early onset ataxia; spasticity; genetics; hereditary spastic paraplegia

Abbreviations: ARCA = autosomal recessive cerebellar ataxia; CMT = Charcot–Marie–Tooth disease; EST = phospholipid esterase domain

Introduction

Autosomal recessive cerebellar ataxias (ARCA) are a clinically and genetically heterogeneous group of spinocerebellar diseases, often associated with additional non-cerebellar features (Fogel and Perlman, 2007; Anheim et al., 2012). Two clinically defined syndromes combine early-onset ARCA with hypogonadotropic hypogonadism: (i) Boucher-Neuha¨user syndrome (MIM 215470), which is associated with choriotinal dystrophy (Boucher and Gibberd, 1969; Neuhauser and Opitz, 1975; Limber et al., 1989); and (ii) Gordon Holmes syndrome (MIM 212840), which is additionally associated with brisk reflexes (Holmes, 1907). Despite various descriptions of familial occurrence, the genetic basis of these syndromes has remained elusive. Moreover, it has remained unclear whether these syndromes present clinically and genetically distinct entities or, alternatively, phenotypic clusters on a phenotypic continuum of neurodegenerative diseases caused by mutations in the same gene.

Here we demonstrate that Boucher-Neuha¨user and Gordon Holmes syndromes are indeed allelic diseases and reveal the major disease gene for these clinical disease entities. By using the significant genetic variants identified in Boucher-Neuha¨user and Gordon Holmes syndrome families as a seed we also analysed >500 exomes from patients with hereditary ataxia and/or spasticity syndromes and establish that variants in the new Boucher-Neuha¨user syndrome/ Gordon Holmes syndrome disease gene are not specific to these particular hypogonadism syndromes, but rather cause these presentations as part of a wider spectrum of neurodegenerative disease.

Materials and methods

Whole exome sequencing of index patients with Boucher-Neuha¨user syndrome and Gordon Holmes syndrome

Whole exome sequencing was performed on two index patients with familial Boucher-Neuha¨user syndrome and one index patient with sporadic Gordon Holmes syndrome. Informed consent was obtained from all individuals and the Institutional Review Boards of the participating medical centres approved the study. The SureSelect Human All Exon 50 Mb kit (Agilent) was used for in-solution enrichment and exome sequencing was performed using the HiSeq2000 instrument (Illumina). Paired-end reads of 100-bp length were produced. BWA and GATK software packages (Li and Durbin, 2009; McKenna et al., 2010; DePristo et al., 2011) were used to align sequence reads to the reference and call variant positions, respectively. All data were then annotated and imported into Genomes Management Application (GEM.app), a web-based tool for next generation sequencing data analysis (Gonzalez et al., 2013) (genomics.med.miami.edu). An average of 73 609 687 sequence reads was produced per sample, 98.8% of which could be aligned to the targeted sequence. Mean coverage was 69-fold; 71% of the targeted sequence was covered by at least 20 reads.

Assuming a common cause for Boucher-Neuha¨user and Gordon Holmes syndromes, we used the GEM.app analysis module ‘Genes Across Families’ to filter for non-synonymous homozygous or compound heterozygous variants, with low frequency in public databases (minor allele frequency in dbSNP137 and NHLBI ESP6500 < 0.5%), moderate conservation (GERP score > 2 OR PhastCons score > 0.6) and moderate genotype quality (GATK quality index > 30 and genotype quality GQ > 30) in genes shared across the three families. Only one gene, PNPLA6 (NCBI reference NM_001166111.1), remained as a candidate gene between the Boucher-Neuha¨user and Gordon Holmes syndrome index patients with segregating variants that were conserved, rare, and predicted to be damaging by at least three different in silico algorithms (MutationTaster; MutationAssessor; Likelihood Ratio Test; and PolyPhen2).

Sanger sequencing of the candidate gene in additional Boucher-Neuha¨user syndrome families

To confirm the significance of PNPLA6 mutations in the pathogenesis of ataxia-hypogonadism syndromes, we screened the PNPLA6 gene in index patients from four additional Boucher-Neuha¨user syndrome families by conventional Sanger sequencing. Oligonucleotide sequences are available upon request.
PNPLA6 causes Boucher-Neuha¨user and Gordon Holmes syndrome

Results

PNPLA6 causes Boucher-Neuha¨user syndrome and Gordon Holmes syndrome

By intersecting the identified variants in whole exomes from two index patients with Boucher-Neuha¨user syndrome and one index patient with Gordon Holmes syndrome under recessive inheritance models, only PNPLA6 remained as a candidate gene. All variants identified in these cases were conserved, had low frequency in the general population, and were predicted to be damaging by at least three different in silico algorithms (Table 2; for coverage see Supplementary Fig. 5). We identified a homozygous missense mutation (c.3173C > T);[3173C > T], p.[Thr1058Ile];[Thr1058Ile]) in Family IHG25190, compound heterozygous splice/misssense mutations in Family ARCA_05 (c.[2212-1G > C];[3328G > A], p.[Val738Glnfs*98];[Val1110Met]), and compound heterozygous frameshift/misssense mutations (c.[3084_3085insGCCA];(c.[4084C > G], p.[Arg1031Glufs*38];[Arg1362Gly]) in Family IHG25330. The splice mutation in Family ARCA_05 destroys a known splice acceptor site in intron 19-20, most likely resulting in skipping of exon 20 (76 bp) and a consecutive shift of the reading frame; in silico analysis did not indicate activation of cryptic splice sites (Divina et al., 2009) (Table 2; for pedigrees and electropherograms see Supplementary Figs 1 and 2).

To confirm the significance of PNPLA6 mutations in the pathogenesis of ataxia-hypogonadism syndromes, index patients from four additional independent Boucher-Neuha¨user syndrome families were screened for PNPLA6 mutations by conventional Sanger sequencing. We identified two additional Boucher-Neuha¨user syndrome families with PNPLA6 mutations: (Family IHG25357: c.[3134C > T]; [3365C > T], p.[Ser1045Leu];[Pro1122Leu]; and Family IHG25353: c.[1732G > T]; [3197T > C], p.[Gly978Trp];[Phe1066Ser]) (Table 2; for pedigrees and electropherograms, see Supplementary Figs 1 and 2).

PNPLA6 causes a wide spectrum of neurodegenerative disease

To explore the possibility that Boucher-Neuha¨user syndrome and Gordon Holmes syndrome are only clusters on a wider spectrum of neurodegenerative disease, PNPLA6 was used as a ‘seed’ in the analysis of exome data from 538 unrelated patients with non-Boucher-Neuha¨user syndrome/Gordon Holmes syndrome neurodegenerative diseases (non-Boucher-Neuha¨user syndrome/ Gordon Holmes syndrome early-onset ataxia; non-Boucher-Neuha¨user syndrome/Gordon Holmes syndrome hereditary spastic paraplegia; CMT type 2) where family history was compatible with recessive disease. This analysis identified a spastic ataxia patient (Subject IHG2617), who carried significant compound heterozygous changes (c.[3084_3085insGCCA]; [3299T > G], p.[Arg1031Glufs*38];[Val1100Gly]) and a case with hereditary spastic paraplegia and mild motor neuropathy (Subject IHG26041) with compound heterozygous variants (c.[4084C > G], p.[Arg1031Glufs*38];[Arg1362Gly]) in Family IHG25330. The splice mutation in Family ARCA_05 destroys a known splice acceptor site in intron 19-20, most likely resulting in skipping of exon 20 (76 bp) and a consecutive shift of the reading frame; in silico analysis did not indicate activation of cryptic splice sites (Divina et al., 2009) (Table 2; for pedigrees and electropherograms see Supplementary Figs 1 and 2).

Clinical assessment

All index patients carrying two pathogenic PNPLA6 variants as well as their affected siblings received a systematic clinical assessment for disturbances in multiple neurological systems (Table 1). In all subjects, routine MRI was performed.
<table>
<thead>
<tr>
<th>Family identifier</th>
<th>Subject</th>
<th>Origin</th>
<th>Gender</th>
<th>Phenotypic syndrome</th>
<th>Age of onset of first symptom (years)</th>
<th>Gait ataxia</th>
<th>Hypogonadotropic hypogonadism</th>
<th>Chorioretinal dystrophy</th>
<th>Spasticity LL/extensor plantar response</th>
<th>Distal muscle wasting</th>
<th>Tendon reflexes</th>
<th>PTR/ATR</th>
<th>Cognitive impairment</th>
<th>Imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHG25190 1</td>
<td>B</td>
<td>F</td>
<td>BNS</td>
<td>2, visual impairment</td>
<td>56, +, since age 6 years</td>
<td>+</td>
<td>(including primary amenorrhoea)</td>
<td>+</td>
<td>+/−/−</td>
<td>+</td>
<td>↓/↓</td>
<td>Mild</td>
<td>Atrophy cerebellum and pons; small pituitary</td>
<td></td>
</tr>
<tr>
<td>2 B M BNS</td>
<td>+, since age 6 years</td>
<td>+</td>
<td>+</td>
<td>−/−</td>
<td>+</td>
<td>+/−/−</td>
<td>+</td>
<td>↓/↓</td>
<td>Mild</td>
<td>Atrophy cerebellum and pons; small pituitary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 B F BNS</td>
<td>1, visual impairment and ataxia</td>
<td>2−3, visual impairment</td>
<td>+ (including primary amenorrhoea)</td>
<td>+</td>
<td>+/−/−</td>
<td>+</td>
<td>↓/↓</td>
<td>Mild</td>
<td>Atrophy cerebellum and pons; small pituitary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARCA-05 1</td>
<td>I</td>
<td>F</td>
<td>BNS</td>
<td>6, gait ataxia</td>
<td>44, +, since age 6</td>
<td>+</td>
<td>(including primary amenorrhoea)</td>
<td>+</td>
<td>+/−/−</td>
<td>+</td>
<td>↑/Ø</td>
<td>Mild</td>
<td>Atrophy cerebellum</td>
<td></td>
</tr>
<tr>
<td>2 I F BNS</td>
<td>without retinal dystrophy</td>
<td>6, gait ataxia</td>
<td>+ (including primary amenorrhoea)</td>
<td>+</td>
<td>+/−/−</td>
<td>+</td>
<td>↓/↓</td>
<td>Mild</td>
<td>Atrophy cerebellum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHG25353 1</td>
<td>B</td>
<td>M</td>
<td>BNS</td>
<td>6, gait ataxia</td>
<td>61, +, since age 6</td>
<td>+</td>
<td>+</td>
<td>−/−/−</td>
<td>n/Ø</td>
<td>N</td>
<td>↑/↓</td>
<td>N</td>
<td>Atrophy cerebellum</td>
<td></td>
</tr>
<tr>
<td>2 B M BNS</td>
<td>+, since age 6 years</td>
<td>+</td>
<td>+</td>
<td>−/−/−</td>
<td>+</td>
<td>+/−/−</td>
<td>+</td>
<td>↑/↓</td>
<td>N</td>
<td>Atrophy cerebellum</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>IHG25357 1</td>
<td>V</td>
<td>M</td>
<td>BNS</td>
<td>14, delayed puberty; visual impairment</td>
<td>26, +, since age 20</td>
<td>+</td>
<td>+</td>
<td>−/−/−</td>
<td>n/Ø</td>
<td>N</td>
<td>↑/↑</td>
<td>N</td>
<td>Atrophy cerebellum; empty sella</td>
<td></td>
</tr>
<tr>
<td>IHG25330 1</td>
<td>F</td>
<td>M</td>
<td>GHS</td>
<td>14, delayed puberty</td>
<td>63, +, since age 30</td>
<td>+</td>
<td>+</td>
<td>+/−/−</td>
<td>↑/↑</td>
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<td>↑/↑</td>
<td>N</td>
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<td></td>
</tr>
<tr>
<td>IHG26041 1</td>
<td>G</td>
<td>F</td>
<td>HSP</td>
<td>20, spasticity</td>
<td>54, −</td>
<td>−</td>
<td>−</td>
<td>+/−/−</td>
<td>↑/↑</td>
<td>N</td>
<td>↑/↑</td>
<td>N</td>
<td>Atrophy cerebellum</td>
<td></td>
</tr>
<tr>
<td>IHG26117 1</td>
<td>G</td>
<td>M</td>
<td>spastic ataxia</td>
<td>4, gait ataxia</td>
<td>48, +, since age 4</td>
<td>−</td>
<td>−</td>
<td>+/−/−</td>
<td>↑/↑</td>
<td>N</td>
<td>↑/↑</td>
<td>N</td>
<td>Atrophy cerebellum</td>
<td></td>
</tr>
</tbody>
</table>

M = male; F = female; N = normal; B = Brazilian; I = Italian; F = French; G = German; V = Venezuelan; BNS = Boucher-Neuhäuser syndrome; GHS = Gordon-Holmes syndrome; HSP = hereditary spastic paraplegia; Ø = absent; LL = lower limbs; PTR = patellar tendon reflex; ATR = achilles tendon reflex.

Cognitive impairment was rated by clinical impression.
Table 2  PNPLA6 mutations identified in this study

<table>
<thead>
<tr>
<th>Family ID</th>
<th>Phenotype</th>
<th>cDNA change</th>
<th>Protein change</th>
<th>GVS function</th>
<th>GERP</th>
<th>PhastCons</th>
<th>phyloP</th>
<th>dbSNP137 MAF</th>
<th>NHLBI EVS MAF</th>
<th>GEM.app MAF</th>
<th>MT</th>
<th>MA</th>
<th>LRT</th>
<th>PP2</th>
<th>SIFT</th>
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<tbody>
<tr>
<td>ARCA_05</td>
<td>BNS</td>
<td>c.2212-1G &gt; C</td>
<td>p.Val738Glnfs*98</td>
<td>Splice-site</td>
<td>4.8</td>
<td>0.83</td>
<td>2.20</td>
<td>np</td>
<td>np</td>
<td>np</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARCA_05</td>
<td>BNS</td>
<td>c.3328G &gt; A</td>
<td>p.Val1110Met</td>
<td>Missense</td>
<td>3.93</td>
<td>0.994</td>
<td>2.04</td>
<td>np</td>
<td>np</td>
<td>np</td>
<td>D</td>
<td>L</td>
<td>N</td>
<td>D</td>
<td>0</td>
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<tr>
<td>IHG26117</td>
<td>sATX</td>
<td>c.3084_3085insGCCA</td>
<td>p.Arg1031Gufs*38</td>
<td>Frameshift</td>
<td>4.04</td>
<td>1</td>
<td>-0.07</td>
<td>np</td>
<td>0.00032</td>
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<tr>
<td>IHG26117</td>
<td>sATX</td>
<td>c.3299G &gt; A</td>
<td>p.Val1000Gly</td>
<td>Missense</td>
<td>4.97</td>
<td>1</td>
<td>1.88</td>
<td>np</td>
<td>0.00033</td>
<td>D</td>
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<td>HS</td>
<td>c.787G &gt; A</td>
<td>p.Val263Ile</td>
<td>Missense</td>
<td>5.2</td>
<td>0.998</td>
<td>2.44</td>
<td>np</td>
<td>np</td>
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<td>N</td>
<td>D</td>
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<tr>
<td>IHG25353</td>
<td>BNS</td>
<td>c.329G &gt; A</td>
<td>p.Val1100Met</td>
<td>Missense</td>
<td>4.66</td>
<td>1</td>
<td>2.59</td>
<td>np</td>
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<td>D</td>
<td>P</td>
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<td>BNS</td>
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<td>p.Pro1122Leu</td>
<td>Missense</td>
<td>4.98</td>
<td>0.661</td>
<td>2.32</td>
<td>np</td>
<td>np</td>
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<td>D</td>
<td>D</td>
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</tr>
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<td>p.Arg1031Gufs*38</td>
<td>Frameshift</td>
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<td>p.Gly578Trp</td>
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<td>5.17</td>
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<td>np</td>
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<tr>
<td>IHG25353</td>
<td>BNS</td>
<td>c.3197G &gt; T</td>
<td>p.Phe1066Ser</td>
<td>Missense</td>
<td>3.94</td>
<td>0.994</td>
<td>2.04</td>
<td>np</td>
<td>np</td>
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<td>p.Arg1031Gufs*38</td>
<td>Frameshift</td>
<td>4.04</td>
<td>1</td>
<td>0.07</td>
<td>np</td>
<td>0.00032</td>
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<td>c.4084C &gt; G</td>
<td>p.Arg1362Gly</td>
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<td>0.996</td>
<td>2.12</td>
<td>np</td>
<td>np</td>
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<tr>
<td>IHG25353</td>
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<td>p.Gly781Glu</td>
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<td>4.98</td>
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<td>1.93</td>
<td>np</td>
<td>np</td>
<td>np</td>
<td>D</td>
<td>M</td>
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</table>

BNS = Boucher-Neuha¨user syndrome; sATX = sporadic ataxia; HS = hereditary spastic paraplegia; GHS = Gordon Holmes syndrome; MAF = minor allele frequency; MT = MutationTaster; MA = MutationAssessor; LRT = Likelihood Ratio Test; PP2 = PolyPhen2; SIFT = Sorting Tolerant From Intolerant; D = damaging; L = low probability of damaging effect; H = high probability of damaging effect; B = benign; M = medium probability of damaging effect; N = neutral; P = probably damaging; U = unknown; np = not present.

GERP = Genomic Evolutionary Rate Profiling; GVS = Genome Variant Server. SIFT scores < 0.05 represent damaging effect.

PNPLA6 causes Boucher-Neuha¨user and Gordon Holmes syndrome. Brain 2014: 137; 69–77

Structural in silico protein modelling

The majority of identified PNPLA6 mutations clustered within a short stretch of the EST domain. This domain has been shown to de-esterify phosphatidylcholine, a major component of biological membranes, into its constituent fatty acids and glycerophosphocholine (Strickland et al., 2003). The majority of identified PNPLA6 mutations clustered within a short stretch of the EST domain. This domain has been shown to de-esterify phosphatidylcholine, a major component of biological membranes, into its constituent fatty acids and glycerophosphocholine (Strickland et al., 2003). The majority of identified PNPLA6 mutations clustered within a short stretch of the EST domain. This domain has been shown to de-esterify phosphatidylcholine, a major component of biological membranes, into its constituent fatty acids and glycerophosphocholine (Strickland et al., 2003). The majority of identified PNPLA6 mutations clustered within a short stretch of the EST domain. This domain has been shown to de-esterify phosphatidylcholine, a major component of biological membranes, into its constituent fatty acids and glycerophosphocholine (Strickland et al., 2003). The majority of identified PNPLA6 mutations clustered within a short stretch of the EST domain. This domain has been shown to de-esterify phosphatidylcholine, a major component of biological membranes, into its constituent fatty acids and glycerophosphocholine (Strickland et al., 2003).
Clinical findings

We aggregated clinical data from 12 affected subjects belonging to seven families. All four index subjects with Boucher-Neuha¨user syndrome presented with the classical triad: visual impairment, ataxia and delayed puberty (Fig. 2B–D). Symptoms in the individuals with Boucher-Neuha¨user syndrome started before the age of 8 years and were slowly progressive in all affected individuals with variable progression rates both between and within families, leading to wheelchair-dependency in the most severely affected subjects. In line with the original publications of Boucher-Neuha¨user and Gordon Holmes syndromes (Holmes, 1907; Neuha¨user and Opitz, 1975), two of four subjects with Boucher-Neuha¨user syndrome as well as the subject with Gordon Holmes syndrome showed clinical signs of upper motor neuron disease (spasticity, positive extensor plantar reflex and/or brisk reflexes), including a spastic Boucher-Neuha¨user syndrome phenotype (Family ARCA_05). Achilles tendon reflexes were reduced or absent in all subjects with Boucher-Neuha¨user syndrome, providing clinical evidence that peripheral neuropathy is commonly associated with Boucher-Neuha¨user syndrome in our subjects. Neuropathy was of sensorimotor axonal type in those subjects with Boucher-Neuha¨user syndrome where nerve conduction studies were available. Affected siblings within Boucher-Neuha¨user syndrome families showed broadly similar phenotypes and age of onset (Table 1). However, the initial symptom of disease differed between and within Boucher-Neuha¨user syndrome families: whereas disease started with visual impairments in some subjects with Boucher-Neuha¨user syndrome, it presented with gait ataxia or delayed puberty in others (Table 1). Moreover, in Boucher-Neuha¨user syndrome Family ARCA_05 one of the affected siblings (Subject 2) did not show evidence for chorioretinal dystrophy.
indicating this as a non-obligate feature. This is further supported by the findings that the cases with hereditary spastic paraplegia and spastic ataxia showed neither retinal dystrophy nor hypogonadism (Table 1), thus demonstrating that hypogonadism is not an obligate feature of disease. Disease in the cases with hereditary spastic paraplegia and spastic ataxia also started before the age of 20 years, corroborating the notion that early-onset neurodegeneration is a common denominator across all affected subjects.

Discussion

To date, the genetic basis of Boucher-Neuhäuser and Gordon Holmes syndromes has not been identified. Here we use exome sequencing to uncover the genetic cause of these two clinical entities and demonstrate that they are allelic diseases, both caused by recessive PNPLA6 mutations. PNPLA6 seems to be a major cause of Boucher-Neuhäuser syndrome with four of six Boucher-Neuhäuser syndrome families carrying PNPLA6 mutations. As not all subjects with Boucher-Neuhäuser syndrome carried PNPLA6 mutations, the genetic basis of Boucher-Neuhäuser syndrome might either be heterogeneous or caused by PNPLA6 mutations not detected by routine sequencing procedures (e.g. deletions or intronic mutations). Further heterogeneity of ataxia-hypogonadism syndromes is also supported by the recent observation that mutations in RNF216 and the combination of mutations in RNF216 and OTUD4 (Margolin et al., 2013) cause ataxia-hypogonadism syndromes complicated by dementia. In contrast to RNF216/OTUD4-associated ataxia-hypogonadism, Boucher-Neuhäuser syndrome and Gordon Holmes syndrome are not typically accompanied by dementia.

Figure 2 The continuous spectrum of PNPLA6-associated disease (A) and the classical clinical trias of the Boucher-Neuhäuser syndrome (B–D). (A) The clinical spectrum of PNPLA6 mutations unfolds along four different neurological key features: ataxia, chorioretinal dystrophy, hypogonadotropic hypogonadism and motor neuron disease (upper motor neuron disease with or without additional lower motor neuropathy). Accordingly, Boucher-Neuhäuser syndrome (BNS) and Gordon Holmes syndrome (GHS) are not distinct entities, but clusters on a continuous spectrum of PNPLA6-associated disease, extending from Boucher-Neuhäuser syndrome via Gordon Holmes syndrome to spastic ataxia (sATX) and pure hereditary spastic paraplegia (HSP). The phenotype of complicated hereditary spastic paraplegia, which has been considered the most prominent phenotype of PNPLA6 so far (Rainier et al., 2008), is only the ‘tip of the iceberg’ of this broad disease spectrum. (B) Photograph of the full body of a male patient with Boucher-Neuhäuser syndrome (Subject IHG 25357) at age 26 years illustrating incomplete secondary sex characteristics with lack of body hair and gynecomastia. (C) Exemplary fundus photography of the Boucher-Neuhäuser syndrome Patient ARCA_05 showing chorioretinal degeneration, characterized by diffuse atrophy of choroidal vessels and the retinal pigment epithelium with pigment clumps. The optic nerve shows no signs of atrophy. (D) Sagittal T2-weighted MRI of this subject with Boucher-Neuhäuser syndrome shows marked cerebellar atrophy.
Complicated hereditary spastic paraplegia has been considered the prominent phenotype of PNPLA6 thus far (Rainier et al., 2008). However, here we show that complicating hereditary spastic paraplegia represents only a relatively special case along a multidimensional PNPLA6-associated spectrum of neurodegenerative disorders. This spectrum includes at least four clinical key features: ataxia, motor neuron disease (upper motor neuron disease with or without additional lower motor neuropathy), hypogonadism and chorioretinal dystrophy (Fig. 2A). Although these clinical features appear to be frequent in PNPLA6 disease none of them is an obligate feature of the disease (Table 1 and Fig. 2A). We postulate that new phenotypic combinations on this PNPLA6 associated disease spectrum will be identified as more patients representing broader phenotypes will be screened for mutations (Fig. 2A). PNPLA6 therefore appears to be another representative of a growing number of disease-related proteins that have been shown to cause a spectrum or even a multitude of seemingly unrelated phenotypic expressions (Ho and Lammerding, 2012; Nilius and Voets, 2013). Driven by the more comprehensive screening capabilities of next-generation sequencing-based approaches, we will likely uncover a more complex structure of phenotype/genotype correlations in the coming years. On a functional level, proteins like PNPLA6, on which different phenotypes converge, may represent functional platforms or hubs that connect a diversity of pathways with multiple biological functions. These ‘hub proteins’, intersection nodes in phenotypic as well as cellular disease networks, might offer promising opportunities for therapeutic intervention for a number of diseases.

PNPLA6 belongs to a protein family of nine patatin-like phospholipase domain-containing proteins. The phospholipid esterase domain (EST) is altered by intoxication of organophorous compounds (Rainier et al., 2008; Richardson et al., 2013). These compounds are used in industry, agriculture, suicide attempts, chemical warfare, terrorist incidents (1995 Tokyo subway incident) and adulterated alcoholic beverages (Jamaica ‘Ginger Jake’ during the Prohibition era) (Rainier et al., 2008; Richardson et al., 2013). Structural analysis of the various PNPLA6 mutations indicated that all mutations identified here harbour the potential to seriously affect the enzymatic activity of the EST domain. Based on current knowledge there are two main pathways of action for PNPLA6. Firstly, PNPLA6 de-esterifies phosphatidylcholine, a major component of biological membranes, into its constituent fatty acids and glycerophosphocholine (Strickland et al., 1995; Atkins et al., 2002; van Tienhoven et al., 2002; Zaccheo et al., 2004). Glycerophosphocholine serves as a precursor for the biosynthesis of acetylcholine, a key neurotransmitter involved in mediating cellular signalling in the nervous system. This may lead to disturbance of development and maintenance of synaptic connections in a variety of neuronal networks. Secondly, PNPLA6—which possesses lysophospholipase activity (van Tienhoven et al., 2002)—has recently been suggested to catalyse 2-arachidonoyl lysophosphatidylglycerol, which brings it into close functional relationship with other hereditary spastic paraplegia genes (DDHD1/SPG28, CYP2U1/SPG49) (Tesson et al., 2012). Thus, PNPLA6 disease might need to be added to this rapidly increasing list of genes involved in lipid metabolism associated with neurodegenerative disease (Schuurs-Hoeijmakers et al., 2012; Tesson et al., 2012; Boukhris et al., 2013; Martin et al., 2013). Our PNPLA6 findings demonstrate that the phenotypic spectrum of these neurodegenerative phospholipid diseases is much broader than previously thought, extending from Boucher-Neuhäuser syndrome/Gordon Holmes syndrome to spastic ataxia and hereditary spastic paraplegia with or without motor neuropathy.

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Supplementary material
Supplementary material is available at Brain online.

References
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