Mutations in VLDLR as a Cause for Autosomal Recessive Cerebellar Ataxia with Mental Retardation (Dysequilibrium Syndrome)

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Abstract

Dysequilibrium syndrome (DES) is a genetically heterogeneous condition that combines autosomal recessive, non-progressive cerebellar ataxia with mental retardation. Here we report the first patient heterozygous for two novel mutations in VLDLR. An 18-month old girl presented with significant hypotonia, global developmental delay, and truncal and peripheral ataxia. MR imaging of the brain demonstrated hypoplasia of the inferior cerebellar vermis and hemispheres, small pons, and a simplified cortical sulcation pattern. Sequence analysis of the VLDLR gene identified a nonsense and missense mutation. Six mutations in VLDLR have now been identified in five families with a phenotype characterized by moderate-to-profound mental retardation, delayed ambulation, truncal and peripheral ataxia and occasional seizures. Neuroanatomically, the loss-of-function effect of the different mutations is indistinguishable. VLDLR-associated cerebellar hypoplasia is emerging as a panethnic, clinically and molecularly well-defined genetic syndrome.

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Web Resources: NCBI Reference sequences (http://www.ncbi.nlm.nih.gov/sites/entrez; Build 36.3) - VLDLR: NP_003374.3; APOER2: NP_150643; LDL: NP_000518; LRP2: NP_004516.2; LRP4: NP_002325.2; LRP1: NP_002323.2; LRP1B: NP_061027.2
Keywords

VLDLR; Cerebellar hypoplasia; Dysequilibrium syndrome

Introduction

The term ‘syndrome de déséquilibration’ was probably first employed by Alajouanine et al.\textsuperscript{1} in a case report in the 1940s and subsequently adopted as ‘Dysequilibrium syndrome’ (DES) by Hagberg et al.\textsuperscript{2} and Sanner\textsuperscript{3} to classify what we now know as a spectrum of genetically heterogeneous conditions that combine autosomal recessive, non-progressive cerebellar ataxia with mental retardation. Dysequilibrium syndrome was described in the endogamous North American Hutterite population in the 1980s\textsuperscript{4,5} and further characterized as a nonprogressive syndrome of moderate-to-profound mental retardation, delayed ambulation, and predominantly truncal ataxia in a review of 12 patients in 2005.\textsuperscript{6} Additional features included strabismus and pes planus in the majority of patients and seizures in 40% and short stature in 15% of patients. Magnetic resonance imaging (MRI) demonstrated inferior cerebellar hypoplasia and mild cortical gyral simplification.\textsuperscript{6} An identity-by-descent mapping approach localized the gene for DES in the Hutterites to 9p24 and a 199 kb homozygous deletion encompassing the entire very low density lipoprotein receptor (VLDLR) coding region, including upstream promoter and downstream sequences, was present in all affected individuals.\textsuperscript{7} VLDLR is a member of the low-density lipoprotein receptor (LDLR) gene family\textsuperscript{8} and part of the reelin signalling pathway\textsuperscript{9}, which guides neuroblast migration in the cerebral cortex and cerebellum (reviewed in\textsuperscript{10}). Very recently, three different and independent loss-of-function mutations in VLDLR have been reported in families from Iran\textsuperscript{11} and Turkey\textsuperscript{12,13} confirming the central role of VLDLR in some forms of DES. This report describes the first compound heterozygous patient who carries two novel mutations in VLDLR and reviews this emerging clinically and molecularly well-defined syndrome.

Case Report

The patient is a 26-month-old girl seen for neurogenetic consultation at 18 months of age because of an abnormal MRI and developmental delay. She is the product of a dizygotic twin pregnancy complicated by maternal diabetes, well controlled with insulin therapy. Delivery at 36 weeks gestation was uneventful. Her birth weight was 5 pounds, 6 ounces and her Apgar scores were normal. Family history is unremarkable. Her twin sister and older sister are normal. The parents are nonconsanguineous; the mother of Irish-German descent and the father of Scottish-German descent.

Developmental concerns were raised at four months of age when hypotonia and motor delay were noted. Gross motor milestones include rolling at 13 months of age, rolling in both directions at 15 months of age and using rolling to reach a point in the room at 17 months of age. She was able to maintain a sitting position when propped up for about 30 seconds and stand in a standing frame with locked knees at 18 months of age. By 26 months of age she was able to sit without support for prolonged periods, get to sitting, commando crawl and pull to a kneeling position. She developed a pincer grasp at 17 months and could bang toys together at 18 months of age. At 26 months of age expressive and receptive language is delayed; she babbles and understands a few commands. She is now beginning to wave ‘bye-bye’. At 26 months she was assessed and fine motor and social skills were at approximately a 12-month old level, gross motor skills were scattered between 7 and 10 months and language skills were between 6 and 9 months, consistent with global developmental delay with more significant impairment in gross motor and language skills.
Examination at 18 and 26 months revealed a well-appearing small child. Her weight at 26 months was 9.6 kg (<5th percentile), height was 76 cm (<5th percentile), and head circumference was 45.5 cm (5-10th percentile). She had full extraocular movements, normal reactive pupils, normal facial strength and tongue was midline without fasciculations. She had some lower facial hypotonia with drooling. Her saccades were hypometric and smooth pursuit was abnormal. Muscle tone was overall decreased. Deep tendon reflexes were symmetric and brisk, plantar responses were flexor and there was no clonus. Her grasp was immature. Truncal unsteadiness was evident and she had dysmetria with reaching.

MRI of the brain revealed hypoplasia of the inferior portion of the cerebellar vermis as well as the hemispheres (Figure 1). The brainstem was small, particularly the pons. The sulcation pattern was simplified with minimally thickened but uniform cortex without a clear anteroposterior gradient. The MRI findings were identical to those seen in Hutterite patients with a homozygous VLDLR deletion.

**Molecular Methods**

Sequence analysis was performed following PCR amplification of each of the 19 exons of the VLDLR gene from genomic DNA from the patient and a control. Primers (sequences available upon request) were located within flanking introns allowing for the detection of splicing mutations. Patient sequences were compared to control sequences and to the Genbank sequence (Accession NT_008413.17) using Mutation Surveyor software (SoftGenetics). Sequence analysis of VLDLR exon 11 and 12 was performed in DNA from the patient’s parents to identify phase of the variants. Analysis of exon 11 in 100 individuals was performed to detect the VLDLR p.D521H variant.

**Results**

Sequencing of VLDLR revealed a paternal missense mutation (c.1561G>C, p.D521H) in exon 11 and a maternal frameshift mutation (c.1711_1712dupT, p.Y571LfsX7) in exon 12 of the VLDLR gene in the affected patient (Figure 2A). The frameshift mutation alters codons 571-576 before introducing a premature stop codon, resulting in the partial loss of the YWTD domain and loss of the O-linked sugar, transmembrane and cytoplasmic domains. The p.D521H mutation is predicted to be disease-causing. Sequence analysis in 100 control individuals did not detect p.D521H. It is located within a highly conserved region in the extracellular β-propeller domain; this codon is completely conserved between VLDLR and other family members (including LDLR, APOER2, LRP1, LRP1B, LRP2, and LRP4) (Figure 2B). In the LDL receptor, sequence alterations in the equivalent position D482 and the neighbouring amino acid W483 invariably result in familial hypercholesterolemia. The functional loss associated with mutations at these positions is likely a result of misfolding and impaired export of the receptors to the cell surface.

**Discussion**

DES is a descriptive term that encompasses a spectrum of genetically heterogeneous clinical conditions that combine autosomal recessive, non-progressive cerebellar ataxia with mental retardation. VLDLR-associated cerebellar hypoplasia is emerging as a clinically and molecularly well-defined subgroup of DES. Including this report, six mutations in VLDLR have now been identified in five families (Table 1) (Figure 2C). The phenotype is characterized by moderate-to-profound mental retardation, delayed ambulation, truncal and peripheral ataxia, dysarthric speech, strabismus and occasional seizures. Neuroanatomically, the effect of the different mutations is indistinguishable and the characteristic brain malformation is distinct and easily recognizable. The MRI findings include hypoplasia of the inferior portion...
of the cerebellar vermis and hemispheres, simplified gyration of the cerebral hemispheres with minimally thickened but uniform cortex without a clear anteroposterior gradient and small brainstem, particularly the pons.

Patients with this form of DES typically learn to walk very late (after the age of 6 years) or else never walk at all. Individuals with DES who ambulate independently demonstrate a wide-based ataxic gait, which has been well documented in the Hutterite population. By contrast, the families reported from Turkey present with a high incidence of quadrupedal locomotion. Ozcelik and coworkers proposed that the presence of quadrupedal locomotion in these individuals is evidence for ‘human devolution’ or ‘reverse evolution’. However, this is not supported by clinical, evolutionary or molecular evidence; quadrupedal locomotion is more readily and easily explained as a behavioural adaptation to the severe orthostatic instability caused by the profound cerebellar hypoplasia. The emergence of this clinically spectacular trait and its perpetuation into adulthood is most likely promoted by environmental conditions during adolescence, such as uneven surfaces in rural areas and lack of useful adaptive devices, which would favour a habitual lowering of the body’s center of gravity to avoid injury.

VLDLR is part of the Reelin signaling pathway, which guides neuroblast migration in the developing cerebral cortex and cerebellum (reviewed in ). In an evolutionarily highly conserved pathway, Reelin (RELN) engages two lipoprotein receptors, VLDLR and Apolipoprotein E receptor-2 (APOER2), which results in phosphorylation of the intracellular adaptor protein Disabled-1 (DAB1). This phosphorylation step is the ‘master switch’ that activates an intracellular signalling cascade that allows neuroblasts to receive the critical positional cues required to form ordered cortical layers. Loss-of-function mutations in the human RELN gene are the cause of an autosomal recessive syndrome consisting of severe cerebellar hypoplasia combined with lissencephaly. Human neurodevelopmental defects in DAB1 or APOER2 have so far not been found. Turkmen and colleagues have identified a 7 Mb minimal region between markers D17S1866 and D17S960 on chromosome 17 as the likely location for another autosomal recessive mutation that causes a similar form of cerebellar hypoplasia and a high incidence of preferred quadrupedal locomotion in consanguineous Turkish pedigrees. We have identified a plausible candidate gene in this region, CRK, a member of a family of intracellular adapter proteins that participate in the intracellular propagation of Reelin signalling downstream of Disabled-1. Verification or exclusion of CRK has to await sequencing of this promising candidate locus in the affected individuals.

In addition to lipoproteins, an increasing number of ligands have been reported for the LDL receptor-related proteins and essential signal transduction and modulator functions in embryonic development, synaptic transmission and in the maintenance of vascular integrity are now becoming apparent. DES secondary to mutations in VLDLR represents the first human lipoprotein receptor malformation syndrome. More recently, mutations in LRP2 (megalin) have been identified in patients with Donnai-Barrow syndrome, which is characterized by agenesis of the corpus callosum, congenital diaphragmatic hernia, facial dysmorphism, ocular anomalies, sensorineural hearing loss and developmental delay. Mutations in Lrp4 are responsible for a form of polysyndactyly in mice, and syndactyly in cattle. It is likely that additional malformation syndromes caused by mutations in other members of the LDLR family will be identified in the future.

In summary, we report the first patient born to nonconsanguineous parents with two deleterious VLDLR mutations and review the reported cases to date. DES resulting from VLDLR deficiency is a distinct and recognizable syndrome characterized by nonprogressive congenital ataxia, moderate-to-profound mental retardation, occasional seizures, and inferior cerebellar
hypoplasia with mild simplification of cortical gyri. We propose that VLDLR-deficiency in Dysequilibrium syndrome be referred to as VLDLR-associated cerebellar hypoplasia.

Acknowledgments

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References

Figure 1.
MRI of the brain demonstrating typical neuroimaging findings of VLDLR-associated cerebellar hypoplasia in this patient. (A) Sagittal T1W and (B) Coronal T2W images demonstrating hypoplasia of the inferior vermis and cerebellar hemispheres (A and B) and a small pons (A). (C) Axial T1W image demonstrating mild simplification of the sulcation pattern, a slightly thickened cerebral cortex and lack of clear anteroposterior gradient. (D) Coronal T1W demonstrating horizontal hippocampi with no evidence of malrotation.
Figure 2.
Identification and characterization of the mutations causing VLDLR-associated cerebellar hypoplasia in this patient. (A) Comparison of the normal and mutant sequences obtained for the patient and a control. The patient is a compound heterozygote for the p.D521H and p.Y571LfsX7 mutations. (B) Sequence alignment of homologous YWTD domains in members of the low density lipoprotein receptor (LDLR) family. The highlighted (red) aspartic acid, p.D521, in the VLDLR protein, mutated in this patient, is invariant amongst family members. Abbreviations: APOER2, apolipoprotein E receptor 2; LDLR, low density lipoprotein receptor; LRP, low density lipoprotein related protein. (C) Localization of mutations known to cause VLDLR-associated cerebellar hypoplasia relative to exon position and functional domains. Mutations leading to a premature stop codon are in black; the missense mutation is in blue. The VLDLR deletion present in the Hutterite patients is not shown. Abbreviations: EGFR, epidermal growth factor repeat motif; OLSD, O-linked sugar domain; TM, transmembrane domain; CD, cytoplasmic domain.
## Table 1
Clinical and Molecular Characteristics of Families with VLDLR-Associated Cerebellar Hypoplasia

<table>
<thead>
<tr>
<th>Family</th>
<th>Ethnicity</th>
<th>Reference</th>
<th>Mutation - DNA</th>
<th>Mutation - Protein</th>
<th>Clinical Features</th>
<th>Neuroimaging</th>
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<td></td>
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<tr>
<td>Hutterite</td>
<td>Hutterite</td>
<td>Boycott et al.</td>
<td>c.[1342C&gt;T]+[1342C&gt;T]</td>
<td>p.[R448X]+[R448X]</td>
<td>Severe</td>
<td>Hypoplasia</td>
</tr>
<tr>
<td>Iranian</td>
<td>Iranian</td>
<td>Moheb et al.</td>
<td>c.[2339delT]+[2339delT]</td>
<td>p.[I780fsX3]+[I780fsX3]</td>
<td>Present</td>
<td>NI</td>
</tr>
<tr>
<td>Turkish</td>
<td>Turkish</td>
<td>Turkmen et al.</td>
<td>c.[769C&gt;T]+[769C&gt;T]</td>
<td>p.[R257X]+[R257X]</td>
<td>Severe</td>
<td>Hypoplasia</td>
</tr>
<tr>
<td></td>
<td>Turkish</td>
<td>Turkmen et al.</td>
<td>c.[2339delT]+[2339delT]</td>
<td>p.[I780fsX3]+[I780fsX3]</td>
<td>Minimal</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>Turkish</td>
<td>Ozecelik et al.</td>
<td>c.[1561G&gt;C]+[1711_1712dupT]</td>
<td>p.[D521H]+[Y571fsX7]</td>
<td>No</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>This report</td>
<td>c.[1561G&gt;C]+[1711_1712dupT]</td>
<td>p.[D521H]+[Y571fsX7]</td>
<td>No</td>
<td>Absent</td>
</tr>
</tbody>
</table>

**Clinical Features**
- **Truncal ataxia**: Severe, Present, NI, No, Delayed-Q, No, Delayed-Q, Delayed
- **Peripheral ataxia**: Present, NI, Present
- **Ambulation**: Delayed, No, Delayed-Q, Delayed-Q, Delayed
- **Speech**: Dysarthric, Dysarthric, Dysarthric, Dysarthric, Dysarthric, NI
- **Strabismus**: Present, NI, Present
- **Seizures**: Present, Absent, Present, Absent
- **Mental retardation**: Moderate-Profound, Moderate-Severe, Moderate-Severe, Profound, Developmental Delay
- **Seizures**: Present, Absent, Absent

**Neuroimaging**
- **Inferior cerebellum**: Hypoplasia, Absent, Absent, Absent, Absent
- **Inferior vermis**: Absent, Absent, Absent, Absent, Absent
- **Pons**: Small, Small, Small, Small, Small
- **Cortical gyri**: Mild simplification, NI, Mild simplification, Mild simplification, Mild simplification

**Abbreviations**: NI, no information; Q, quadrupedal.

*a* The deletion present in the Hutterite population is NC_000009.g.2479657_2678818del (NCBI Build 36.3), encompassing approximately 199 kb of DNA.

*b* The family reported by Turkmen et al.13 and Family D reported by Ozecelik et al.12 are likely the same family based upon having the same ethnic background, mutation and additional details provided in the papers.

*c* The mutation reported by Turkmen et al.13 was reported as c.2339delT, p.I779fsX3 which we believe should be p.I780fsX3.