Published in final edited form as:

J Child Neurol. 2009 October ; 24(10): 1310–1315. doi:10.1177/0883073809332696.

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

Kym M Boycott, PhD, MD,

Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, Canada

Carsten Bonnemann, MD,

Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, USA

Joachim Herz, MD,

University of Texas Southwestern Medical Center at Dallas, Texas, USA

Stephanie Neuert, MSc,

Department of Medical Genetics, University of Calgary, Calgary, Canada

Chandree Beaulieu, BSc,

Department of Medical Genetics, University of Calgary, Calgary, Canada

James N Scott, MD,

Department of Radiology, Foothills Hospital, Calgary, Canada

Anuradha Venkatasubramanian, MD, and

Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, USA

Jillian S Parboosingh, PhD

Department of Medical Genetics, University of Calgary, Calgary, Canada

Kym M Boycott: kboycott@cheo.on.ca; Carsten Bonnemann: Bonnemann@email.chop.edu; Joachim Herz: joachim.herz@herzlab.org; Stephanie Neuert: Stephanie.Neuert@calgaryhealthregion.ca; Chandree Beaulieu: Chandree.Beaulieu@calgaryhealthregion.ca; James N Scott: James.Scott@calgaryhealthregion.ca; Anuradha Venkatasubramanian: Venkatasubramanian@email.chop.edu; Jillian S Parboosingh: Jillian.Parboosingh@calgaryhealthregion.ca

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.

Address correspondence to: Dr. Kym Boycott Department of Genetics Children's Hospital of Eastern Ontario 401 Smyth Road Ottawa, ON, K1H 8L1 Ph: 613-737-7600 ext 3223 FAX: 613-738-4822, kboycott@cheo.on.ca.

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.¹ in a case report in the 1940s and subsequently adopted as ‘Dysequilibrium syndrome’ (DES) by Hagberg et al.² and Sanner³ 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^{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 2005.⁶ 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.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.⁷ *VLDLR* is a member of the low-density lipoprotein receptor (LDLR) gene family⁸ and part of the reelin signalling pathway⁹, which guides neuroblast migration in the cerebral cortex and cerebellum (reviewed in¹⁰). Very recently, three different and independent loss-of-function mutations in *VLDLR* have been reported in families from Iran¹¹ and Turkey^{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.¹⁴ 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 domain¹⁵; this codon is completely conserved between *VLDLR* and other family members (including *LDLR*, *APOER2*, *LRP1*, *LRP1B*, *LRP2*, and *LRP4*)¹⁶ (Figure 2B). In the *LDL* receptor, sequence alterations in the equivalent position D482 and the neighbouring amino acid W483 invariably result in familial hypercholesterolemia.^{17,18} 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.¹⁹

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.⁶ By contrast, the families reported from Turkey^{12,13} present with a high incidence of quadrupedal locomotion. Ozcelik and coworkers¹² 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.^{13,20}

VLDLR is part of the Reelin signaling pathway⁹, which guides neuroblast migration in the developing cerebral cortex and cerebellum (reviewed in¹⁰). 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)^{21,22}. 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.²³ Human neurodevelopmental defects in *DAB1* or *APOER2* have so far not been found. Turkmen and colleagues²⁴ 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-1²⁵. 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.¹⁶ 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.²⁶ Mutations in *Lrp4* are responsible for a form of polysyndactyly in mice,²⁷ and syndactyly in cattle.^{28,29} 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

We thank the family for their interest. Livija Medne MS, CGC assisted in coordination of the family's participation in this study. This work was supported by a grant from the Alberta Children's Hospital Foundation. JH is supported by grants from the NIH (HL20498, HL63792).

References

1. Alajouanine T, Aubrey M, Nehlil J. Sur une affection familiale caractérisée par un syndrome de déséquilibre avec importantes perturbations vestibulaires centrales. *Rev Neurol* 1943;75:252–254.
2. Hagberg B, Sanner G, Steen M. The dysequilibrium syndrome in cerebral palsy. Clinical aspects and treatment. *Acta Paediat Scand* 1972;61:1–63. [PubMed: 5018574]
3. Sanner G. The dysequilibrium syndrome. A genetic study. *Neuropadiatrie* 1973;4(4):403–413. [PubMed: 4801892]
4. Schurig V, Oram AV, Bowen P. Nonprogressive cerebellar disorder with mental retardation and autosomal recessive inheritance in Hutterites. *Am J Med Genet* 1981;9:43–53. [PubMed: 7246619]
5. Pallister P, Optiz J. Brief clinical report: Disequilibrium syndrome in Montana Hutterites. *Am J Med Genet* 1986;22:567–569. [PubMed: 4061489]
6. Glass H, Boycott K, Adams C, et al. Autosomal recessive cerebellar hypoplasia in the Hutterite population: a syndrome of nonprogressive cerebellar ataxia with mental retardation. *Dev Med Child Neurol* 2005;47:691–695. [PubMed: 16174313]
7. Boycott KM, Flavelle S, Bureau A, et al. Homozygous deletion of the very low density lipoprotein receptor gene causes autosomal recessive cerebellar hypoplasia with cerebral gyral simplification. *Am J Hum Genet* 2005;77(3):477–483. Epub 2005 Jul 22. [PubMed: 16080122]
8. Herz J, Bock HH. Lipoprotein receptors in the nervous system. *Annu Rev Biochem* 2002;71:405–434. [PubMed: 12045102]
9. Trommsdorff M, Gotthardt M, Hiesberger T, et al. Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* 1999;97(6):689–701. [PubMed: 10380922]
10. Tissir F, Goffinet AM. Reelin and brain development. *Nat Rev Neurosci* 2003;4(6):496–505. [PubMed: 12778121]
11. Moheb LA, Tzschach A, Garshasbi M, et al. Identification of a nonsense mutation in the very low-density lipoprotein receptor gene (*VLDLR*) in an Iranian family with dysequilibrium syndrome. *Eur J Hum Genet* 2008;16(2):270–273. [PubMed: 18043714]
12. Ozcelik T, Akarsu N, Uz E, et al. Mutations in the very low-density lipoprotein receptor *VLDLR* cause cerebellar hypoplasia and quadrupedal locomotion in humans. *Proc Natl Acad Sci U S A* 2008;105(11):4232–4236. [PubMed: 18326629]
13. Turkmen S, Hoffmann K, Demirhan O, et al. Cerebellar hypoplasia, with quadrupedal locomotion, caused by mutations in the very low-density lipoprotein receptor gene. *Eur J Hum Genet* 2008;16(9):1070–1074. [PubMed: 18364738]
14. Sakai J, Hoshino A, Takahashi S, et al. Structure, chromosome location, and expression of the human very low density lipoprotein receptor gene. *J Biol Chem* 1994;269(3):2173–2182. [PubMed: 8294473]
15. Springer TA. An extracellular beta-propeller module predicted in lipoprotein and scavenger receptors, tyrosine kinases, epidermal growth factor precursor, and extracellular matrix components. *J Mol Biol* 1998;283(4):837–862. [PubMed: 9790844]
16. May P, Herz J, Bock HH. Molecular mechanisms of lipoprotein receptor signalling. *Cell Mol Life Sci* 2005;62(1920):2325–2338. [PubMed: 16158188]
17. Day IN, Whittall RA, O'Dell SD, et al. Spectrum of LDL receptor gene mutations in heterozygous familial hypercholesterolemia. *Hum Mutat* 1997;10(2):116–127. [PubMed: 9259195]

18. Graham CA, McClean E, Ward AJ, et al. Mutation screening and genotype:phenotype correlation in familial hypercholesterolaemia. *Atherosclerosis* 1999;147(2):309–316. [PubMed: 10559517]
19. Hobbs HH, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. *Hum Mutat* 1992;1(6):445–466. [PubMed: 1301956]
20. Herz J, Boycott KM, Parboosingh JS. “Devolution” of bipedality. *Proc Natl Acad Sci USA* 2008;105(21):E25. [PubMed: 18487453]
21. Hiesberger T, Trommsdorff M, Howell BW, et al. Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron* 1999;24(2):481–489. [PubMed: 10571241]
22. D'Arcangelo G, Homayouni R, Keshvara L, et al. Reelin is a ligand for lipoprotein receptors. *Neuron* 1999;24(2):471–479. [PubMed: 10571240]
23. Hong SE, Shugart YY, Huang DT, et al. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat Genet* 2000;26(1):93–96. [PubMed: 10973257]
24. Turkmen S, Demirhan O, Hoffmann K, et al. Cerebellar hypoplasia and quadrupedal locomotion in humans as a recessive trait mapping to chromosome 17p. *J Med Genet* 2006;43(5):461–464. [PubMed: 16371500]
25. Park TJ, Curran T. Crk and Crk-like play essential overlapping roles downstream of disabled-1 in the reelin pathway. *J Neurosci* 2008;28(50):13551–13562. [PubMed: 19074029]
26. Kantarci S, Al-Gazali L, Hill RS, et al. Mutations in LRP2, which encodes the multiligand receptor megalin, cause Donnai-Barrow and facio-oculo-acoustico-renal syndromes. *Nat Genet* 2007;39(8):957–959. [PubMed: 17632512]
27. Simon-Chazottes D, Tutois S, Kuehn M, et al. Mutations in the gene encoding the low-density lipoprotein receptor LRP4 cause abnormal limb development in the mouse. *Genomics* 2006;87(5):673–677. [PubMed: 16517118]
28. Johnson EB, Steffen DJ, Lynch KW, et al. Defective splicing of Megf7/Lrp4, a regulator of distal limb development, in autosomal recessive mulefoot disease. *Genomics* 2006;88(5):600–609. [PubMed: 16963222]
29. Duchesne A, Gautier M, Chadi S, et al. Identification of a doublet missense substitution in the bovine LRP4 gene as a candidate causal mutation for syndactyly in Holstein cattle. *Genomics* 2006;88(5):610–621. [PubMed: 16859890]

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

Family	Hutterite	Iranian	Turkish ^b	Family A	Family D ^b	Family C
Ethnicity	Hutterite	Iranian	Turkish	Turkish	Turkish	Caucasian
Reference	Boycott et al. ⁷	Moheb et al. ¹¹	Turkmen et al. ¹³	Ozcelik et al. ¹²	Ozcelik et al. ¹²	This report
Mutation - DNA	Homozygous Deletion ^a	c.[1342C>T]+[1342C>T]	c.[2339delT]+[2339delT]	c.[769C>T]+[769C>T]	c.[2339delT]+[2339delT]	c.[1561G>C]+[1711_1712dupT]
Mutation - Protein	Absent	p.[R448X]+[R448X]	p.[I780TfsX3]+[I780TfsX3] ^c	p.[R257X]+[R257X]	p.[I780TfsX3]+[I780TfsX3]	p.[D521H]+[Y571fsX7]
Clinical Features						
Truncal ataxia	Severe	Present	Severe	Severe	Severe	Present
Peripheral ataxia	Present	NI	Present	Minimal	No	Present
Ambulation	Delayed	No	Delayed-Q	Delayed-Q	Delayed-Q	Delayed
Speech	Dysarthric	NI	Dysarthric	Dysarthric	Dysarthric	NI
Strabismus	Present	Present	NI	Present	Present	Absent
Seizures	Present	Absent	NI	Rare	Absent	Absent
Mental retardation	Moderate-Profound	Moderate-Severe	Moderate-Severe	Profound	Profound	Developmental Delay
Neuroimaging						
Inferior cerebellum	Hypoplasia	NI	Hypoplasia	Hypoplasia	Hypoplasia	Hypoplasia
Inferior vermis	Absent	NI	Absent	Absent	Absent	Absent
Pons	Small	NI	Small	Small	Small	Small
Cortical gyri	Mild simplification	NI	Mild simplification	Mild simplification	Mild simplification	Mild simplification

Abbreviations : NI, no information; Q, quadrupedal.

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

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

^cThe mutation reported by Turkmen et al.¹³ was reported as c.2339delT, p.I779fsX3 which we believe should be p.I780TfsX3.