

Brown-Vialetto-Van Laere Syndrome, a Ponto-Bulbar Palsy with Deafness, Is Caused by Mutations in *C20orf54*

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Brown-Vialetto-Van Laere syndrome is a rare neurological disorder with a variable age at onset and clinical course. The key features are progressive ponto-bulbar palsy and bilateral sensorineural deafness. A complex neurological phenotype with a mixed picture of upper and lower motor neuron involvement reminiscent of amyotrophic lateral sclerosis evolves with disease progression. We identified a candidate gene, *C20orf54*, by studying a consanguineous family with multiple affected individuals and subsequently demonstrated that mutations in this gene were the cause of disease in other, unrelated families.

Brown-Vialetto-Van Laere syndrome (BVVLS [MIM 211530]) was first reported by Brown in 1894¹ as familial amyotrophic lateral sclerosis (ALS [MIM 105400]) with onset in infancy. Following the reports by Vialetto, in 1936,² and Van Laere, in 1966,³ the name BVVLS was adopted. The observation of recurrences in siblings in some families suggested that the condition was probably autosomal recessive. However, the variable age at onset and clinical course raised the possibility that it may be etiologically heterogeneous and that some cases may be caused by a new dominant mutation.⁴

BVVLS is a rare disorder characterised by progressive ponto-bulbar palsy and bilateral sensorineural hearing loss. The disease presents with VII, IX, X, XI, and XII cranial nerve palsies, which develop over a relatively short period of time in a previously healthy individual. Sensorineural hearing loss may precede the neurological signs.^{1–5} The course is invariably progressive, but the rate of decline is variable within and between families. With disease evolution, long tract signs, lower motor neuron signs, cerebellar ataxia, and lower cranial nerve (III–VI) palsies develop, giving rise to a complex picture resembling amyotrophic lateral sclerosis.^{5,6,7,8} Diaphragmatic weakness and respiratory compromise are some of the most distressing features, leading to recurrent chest infections and respiratory failure, which are often the cause of patients' demise.^{5,6}

Fifty-eight cases have been documented in the literature. Sathasivam provided a comprehensive review of the published reports, showing a female to male prevalence of 3:1, with an apparently more severe clinical course in males.⁵ The age at onset ranged from infancy to early in the third decade, with the majority presenting in the

second decade. Hearing loss preceded the onset of neurological signs in most cases. The neurological picture, severity, and disease duration were variable.⁵

The early-onset cases tend to have a much more rapid course, lasting between 6 and 18 mo,⁶ but survival is prolonged with active management (personal observation). The early motor milestones are usually normal. On detailed questioning, a history of increased susceptibility to chest infections can be elicited in some cases (personal observation), and occasionally, the disease onset may follow an episode of viral infection.⁷ The age at onset and clinical presentation may vary within the same family.⁶ Also, childhood- and adult-onset disease have been observed in the same sibship (personal observation).

Appropriate informed consent was obtained from the patients and guardians of the participants in this study, in compliance with the UK National Research Ethics Service regulation on research in human subjects. The clinical details of the patients included in this report are presented in Table 1.

Initial work was undertaken on family 1. The proband (case 1) came from a multiply consanguineous family with several similarly affected children, who died in infancy, strongly suggesting involvement of an autosomal-recessive gene. We were able to identify a stored muscle biopsy sample from a deceased cousin (case 2), which enabled us to undertake this study. The pedigree could not be included for reasons of confidentiality.

Case 4 was born with complex congenital heart disease. He developed the condition at the age of 9 mo and died 18 mo later. He had a similarly affected sister whose DNA was not available for testing.

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Table 1. Clinical Features of Patients with BVLS

Case	Origin	Consanguinity	Pre-Morbid Status	Age at Onset	Sex	Initial Presentation	Deafness, Age at Diagnosis	Respiratory Compromise, Age	Associated Features	Disease Duration
Family 1										
Case 1	Arabic	Yes	Recurrent chest infections since age 4 mo	13 mo	M	Sub-acute encephalopathy	13 mo	17 mo	Hypotonia, cerebellar signs, brisk reflexes in the lower limbs, persistent ankle clonus, stridor, EMG: bulbar palsy, anterior horn involvement; phrenic nerve denervation, auditory neuropathy	Alive by age 2 yr; required respiratory support
Case 2	Arabic	Yes	Not known	6 mo	M	Hypotonia, bulbar palsy	Not known	6 mo	Hypotonia, bulbar palsy, respiratory difficulties	Died, age 13 mo
Family 2										
Case 3	European ancestry	No	Normal	16 mo	F	Progressive bulbar palsy	16 mo	Ventilated, over last few mo	Anterior horn neuropathy; Intact cognitive development	Died, age < 3yr
Family 3										
Case 4	European/Asian	No	Normal	9 mo	M	Breathing problems	Yes	2 yr	Multiple cranial nerve involvement	Died, age 2 yr
Family 4										
Case 5	Pakistani	Yes	Normal	12 yr	F	VII nerve palsy, deafness	12 yr	Stridor on exercise, age 28 yr	Progressive muscle weakness	Alive, age 36 yr
Case 6	Pakistani	Yes	Normal	12 yr	F	Deafness, Tongue wasting and fasciculations	12 yr, cochlear implant, age 15 yr	Stridor on exercise, age 21 yr	Poor balance, proximal muscle group weakness	Alive, age 29 yr
Family 5										
Case 7	Pakistani	Yes	Normal	5 yr	F	Tongue fasciculations, facial palsy,	7 yr, aided	13 yr	Progressive muscle weakness and wasting, external ophthalmoplegia	Died, age 14 yr
Family 6										
Case 8	European	No	Normal	3 mo	F	Breathing difficulties, weakness	Yes	3 mo	General weakness, flaccid	Died, age 11 mo
Family 7										
Case 9	European	No	Normal	Second decade	M	Multiple peripheral neuropathy	Yes	No	Progressive weakness, muscle wasting and truncal ataxia	Alive, age 57 yr

Table 2. Mutations Found in Patients with BVVLS

Case	Ethnic Origin	Consanguinity	Number of Affect Cases	Nucleotide Change	Amino Acid Change	Location
1, 2	Arabic	Y	2	c.1048T>A c.1325_1326 delTG	p.L350M p.L442RfsX35	Exon 3 Exon 5
3	European ancestry	N	1	c.211G>T	p.E71X	Exon 2
4	European/Asian	N	1	c.639C>G	p.Y213X	Exon 3
5, 6	Pakistani	Y	2	c.394C>T	p.R132W	Exon 2
7	Pakistani	Y	1	c.670T>C	p.F224L	Exon 3
8	European	N	1	c.1371C>G	p.F457L	Exon 5
9	European	N	1	c.106G>A, c.1237T>C	p.E36K, p.V413A	Exon 2, Exon 5

Cases 5 and 6 are two siblings of a consanguineous Pakistani couple, who presented in early childhood on the background of previously normal motor and cognitive development. Careful exploration of the previous medical history, however, raised the possibility of mild early respiratory difficulties upon exercise. They both have had a slow progressive course and are alive in their late twenties and thirties, respectively.

Case 7 is one of four siblings of a consanguineous Pakistani family, who were reported by Dipti et al. in 2005.⁶

Patient 9 developed the condition in his early twenties. He is now in his fifties and is still able to walk a short distance with a stick. He has significant muscle wasting, truncal ataxia, and progressive sensorineural deafness, but he does not have respiratory compromise. There is a similarly affected brother who has not been available for testing at this stage. Two sisters died in childhood and had a clinical picture consistent with BVVLS.

DNA from the proband and the affected relative was used for autozygosity mapping with the use of the Illumina Human CNV370 array. Only one region of shared homozygosity longer than 1 Mb was identified: a 2.25 Mb region on chromosome 20p13, containing 44 genes (172128–2459030).

Several genes from the candidate region were sequenced by Sanger dideoxy sequencing. We elected to first analyze *NRSN2* (MIM 610666), *TCF15* (MIM 601010), *SNPH* (MIM 604942), *SDCBP2*, and *NSFL1C* because of their high expression in the brain, followed by sequencing of the remaining exons within the target region. A homozygous 2 bp frameshift mutation was identified in exon 5 of *C20orf54* (Table 2). The mutation was also homozygous in the affected cousin and was heterozygous in the proband's parents and healthy sibling. The mutation was predicted to cause a frameshift resulting in a mutant protein built of 504 amino acids, 35 residues longer than the wild-type protein (Figure S1, available online). A second change was also found in these two patients (c.1048T>A [p.L350M]). The change is not a known SNP but is unlikely to be pathogenic, because it is predicted to be tolerated by SIFT⁹ and L350 is not highly conserved, even among mammalian orthologs.

DNA from an additional seven patients (cases 3–9, Table 1) was sequenced for the entire *C20orf54*. Homozygous

nonsense mutations were found in cases 3 and 4 (Table 2), whereas both siblings from family 4 (cases 5 and 6) and cases 7 and 8 harbored homozygous missense mutations (Table 2). Conservation of the residues involved in missense mutations across a range of species is shown in Figure 1. Evidently, these three residues are highly conserved (although R132 can also be Q). Residue R132 is predicted to occur in an intracellular loop, F224 is predicted to be part of transmembrane helix 6, and F457 is predicted to occur in the extracellular tail. All three are predicted to be deleterious by SIFT.

Case 9 was found to carry two heterozygous missense mutations (Table 2). p.E36K is predicted to be deleterious by SIFT, whereas the other, p.V413A, is predicted to be tolerated. This would be in keeping with the somewhat milder phenotype in this patient.

With the exception of case 9 (compound heterozygote), all patients reported here were homozygous for their respective mutations. Cases 1, 2, 3, 5, 6, and 7 were also homozygous for all 18 polymorphisms covered by the sequenced regions, suggesting a common origin for each mutation (as expected for the consanguineous families 1, 4, and 5). However, cases 4 and 8 were heterozygous for one or more SNPs, indicating that the same mutation has occurred on more than one background (Table S1).

None of the mutations identified in our cohort was found in 210 control chromosomes, demonstrating that they are uncommon in the general population (allele frequency below 1%, 80% power¹⁰).

C20orf54 is a 5 exon gene located in 20p13 within our candidate region. It comprises 11 predicted transmembrane helices and is therefore likely to be a transmembrane protein. *C20orf54* is highly conserved in evolutionary terms, sharing 59% identity with chicken and zebrafish and 36% with *C. elegans* (homologene).

Evidence of a possible function for this protein has recently come from a study by Yamamoto et al., who speculate that the rat ortholog (rRFT2) is a transporter of riboflavin in the small intestine.¹¹ The human ortholog, *C20orf54*, shares 83% similarity with rRFT2; hence, they suggest renaming the gene *RFT2*.

Riboflavin deficiency has a clinical picture very different from that of BVVLS; therefore, the postulated function of the protein is unlikely to play a substantive role in disease

Homo sapiens	--MAFLMHLVLCVFGMGSWVTINGLWVLEPLLVMEPLPGWYLPSTLVVVIQLANIGPLLV	58
Pan troglodytes	--MAFLMHLVLCVFGMGSWVTINGLWVLEPLLVMEPLPGWYLPSTLVVVIQLANIGPLLV	58
Macaca mulatta	--MAFLMHLVLCVFGMGSWVTINGLWVLEPLLVMEPLPGWYLPSTLVVVIQLANIGPLLV	58
Canis familiaris	--MAFLIHLVCTFAMGSWVAINGLWVLEPLLVTEPLPGWYLPSTLVVVIQLANIGPLLV	58
Equus caballus	--MALLTHLLVCTFAMGSWVAINGLWVLEPLLVTEPLPGWYLPSTLVVVIQLANIGPLLV	58
Rattus norvegicus	--MAFLTHLLVLCVFGMGSWVAINGLWVLEPLLVTKPLPGWYLPSTLVVVIQLANTGPLLV	58
Mus musculus	--MAFLTHLLVLCVFGMGSWVAINGLWVLEPLLVTEPLPGWYLPSTLVVVIQLANIGPLLV	58
C.elegans	MKTFLETFCLVAIFGSSWIGTNSVMMELSLTAKLEPGWNLPSYLSAIVQIACLGPLYI	60
Homo sapiens	TLHHFRPSCSEVPIIFTLLGVGTVTCIIFAFLWNMTSWVLDGHSIAFLVLTFFLALV	118
Pan troglodytes	TLHHFRPSCSEVPIIFTLLGVGTVTCIIFAFLWNMTSWVLDGHSIAFLVLTFFLALV	118
Macaca mulatta	TLHHFRPSCSEVPIIFTLLGVGTVTCIIFAFLWNMTSWVLDGHSIAFLVLTFFLALV	118
Canis familiaris	TLHHFRPSCSEVPIIFTLLGVGTACTLFAFLWNMTSWVLDGHSIAFMVLTFFLALV	118
Equus caballus	TLHHFRPSCSDVPVIFTLGVGTVTCIIFAFLWNMTSWVLDGHSIAFMVLTFFLALV	118
Rattus norvegicus	TLHHRFRPCLSEVPIFLLCVGTACILLFAFLWSMTSWIQGQHSVAFIVLTFPLALV	118
Mus musculus	TLHHRFRPCLSEVPIFLLCVGTACILLFAFLWSMTSWIQGQHSVAFIVLTFPLALV	118
C.elegans	SIHKGKIMTIPVPLIFIFMVLACIQGLGCFWDDTYIFGAIRSWPLVLLFLGLAIV	120
Homo sapiens	DCTSSVTFLPFMSRLPTYLYLTFVFGEGLSGLLPALVALAQSGSLTTCVNVTEISDSVPS	178
Pan troglodytes	DCTSSVTFLPFMSRLPTYLYLTFVFGEGLSGLLPALVALAQSGSLTTCVNVTEISDSVPS	178
Macaca mulatta	DCTSSVTFLPFMSRLPTYLYLTFVFGEGLSGLLPALVALAQSGSLTTCVNVTEISDSVPS	178
Canis familiaris	DCTSSVTFLPFMSRLPAHYLTFVFGEGLSGLLPALVALAQSGSLTTCVNVTEISDSVPS	178
Equus caballus	DCTSSVTFLPFMSRLPTYLYLTFVFGEGLSGLLPALVALAQSGSLTTCVNVTEISDSVPS	178
Rattus norvegicus	DCTSSVTFLPFMSRLPTYLYLTFVFGEGLSGLLPALVALVQSGSLTTCVNVTEISDSVPS	178
Mus musculus	DCTSSVTFLPFMSRLPTYLYLTFVFGEGLSGLLPALVALVQSGSLTTCVNVTEISDSVPS	178
C.elegans	DAISSVFLPFMAQPHPSFLNAYFVGMGLSALISLSSLIQSGTSNYWCDDNK-----	172
Homo sapiens	PVPTRETDIAQGVPRALVSALPGMEAPLHLESRYLPAHFSPLVFLLSVMMACCLVAF	238
Pan troglodytes	PVPTRETDIAQGVPRALVSALPGMEAPLHLESRYLPAHFSPLVFLLSVMMACCLVAF	238
Macaca mulatta	PVPTRETDIAQGVPRALVSALPGMEAPLHLESRYLPAHFSPLVFLLSVMMACCLVAF	238
Canis familiaris	PETTRKTVPQASSTFVSDLMETTRSAVHLESRYLPAHFSPLVFLLSVMMACCLVAF	238
Equus caballus	PVTTTGMTTQGDNSILMSTLPGTAPSAVHLESRYLPAHFSPLVFLLSVMMACCLVAF	238
Rattus norvegicus	PVTTMETSITQR---TLSPSLT---PLTWLESRYLAPRFSPLVFLLSVFLMGCCLVAF	232
Mus musculus	---TMEPTITQG---NLSPSLP---SPSWHQESRYLAPRFSPLVFLLSVFLMGCCLVAF	229
C.elegans	-----TPHYYPFRFS-VSMFLINIFFTFCAAVAA	200
Homo sapiens	FVLQRPQRCWEASVEDLLNDQVTLHSIRPRENDLGPAGTVDDSSQGG---YLEEKAAFC	295
Pan troglodytes	FVLQRPQRCWEASVEDLLNDQVTLHSIRPRENDLGPAGTVDDSSQGGHLEEKAAFC	298
Macaca mulatta	FVLQRPQRCWEASVEDLLNDQVTLHSIRPRENDLGPAGTVDDSSQGG---HLEEKAAFC	295
Canis familiaris	FLQRPQRCWEASVEDLLNDQVTLHSIRPRENDLGPAGTVDDSSQGG---HPEKATSD	295
Equus caballus	FFLQRPQRCWEASVEDLLNDQVTLHSIRPRENDLGPAGTVDDSSQGG---HLEEKAAFC	295
Rattus norvegicus	FLQRPQRCWEASVEDLLNDQVTLHSIRPRENDLGPAGTVDDSSQGG---SVEVSAVL	289
Mus musculus	FLQRPQRCWEASVEDLLNDQVTLHSIRPRENDLGPAGTVDDSSQGG---SVEVSAVL	286
C.elegans	FLVLYKIGAHKNSQVEPEPKHSIQIQGDSSTVNEVNTSSPQETS---SIPDSSA	256
Homo sapiens	CPAHLAFIYTLVAFVNALTNGLVPSVQTYSCLSYGPVAYHLAATLSIVANPLASLVSMLF	355
Pan troglodytes	CPAHLAFIYTLVAFVNALTNGLVPSVQTYSCLSYGPVAYHLAATLSIVANPLASLVSMLF	358
Macaca mulatta	CPAHLAFIYTLVAFVNALTNGLVPSVQTYSCLSYGPVAYHLAATLSIVANPLASLVSMLF	355
Canis familiaris	HPAYLAFIYTLVAFVNALTNGLVPSVQTYSCLSYGPVAYHLAATLSIVANPLASLVSMLF	355
Equus caballus	HLAHLAFIYTLVAFVNALTNGLVPSVQTYSCLSYGPVAYHLAATLSIVANPLASLVSMLF	355
Rattus norvegicus	RPAQLAFIYSVVAFVNALTNGLVPSVQTYSCLSYGPVAYHLAATLSIVANPLASLVSMLF	349
Mus musculus	RPAQLAFIYSVVAFVNALTNGLVPSVQTYSCLSYGPVAYHLAATLSIVANPLASLVSMLF	346
C.elegans	TGARLAFILLLTALVNAQMNGIVTSVQSYATLVYQNTYHYAVLTSNIVSIPLASLVSMLF	316
Homo sapiens	PNRSLLFLGVLVSLGTFCFGYNNMAMAVMSPCLLQGHGWGGEVLIVASWVLFSGCLSYVK	415
Pan troglodytes	PNRSLLFLGVLVSLGTFCFGYNNMAMAVMSPCLLQGHGWGGEVLIVASWVLFSGCLSYVK	418
Macaca mulatta	PNRSLLFLGVLVSLGTFCFGYNNMAMAVMSPCLLQGHGWGGEVLIVASWVLFSGCLSYVK	415
Canis familiaris	PHRSLLFLGVLVSLGTFCFGYNNMAMAVMSPCLLQGHGWGGEVLIVASWVLFSGCLSYVK	415
Equus caballus	PNRSLLFLGVLVSLGTFCFGYNNMAMAVMSPCLLQGHGWGGEVLIVASWVLFSGCLSYVK	415
Rattus norvegicus	PNRSLLFLGVLVSLGTFCFGYNNMAMAVMSPCLLQGHGWGGEVLIVASWVLFSGCLSYVK	409
Mus musculus	PNRSLLFLGVLVSLGTFCFGYNNMAMAVMSPCLLQGHGWGGEVLIVASWVLFSGCLSYVK	406
C.elegans	KIRSLPIALFLTLCSSLTAVIYLAALSPNWFNSSETAGTIISIASLLAAGLHSLYLRV	376
Homo sapiens	MLGVILRD--LSRSALLWCGAAVQLGSLGALLMFPVNVNLRLESSADFCNLHCPA	469
Pan troglodytes	MLGVILRD--LSRSALLWCGAAVQLGSLGALLMFPVNVNLRLESSADFCNLHCPA	472
Macaca mulatta	MLGVILRD--LSRSALLWCGAAVQLGSLGALLMFPVNVNLRLESSADFCNLHCPA	469
Canis familiaris	MLGVILRD--RSRSALLWCGAAVQLGSLGALLMFPVNVNLRLESSADFCNLHCPA	469
Equus caballus	MLGMILRD--RSRSALLWCGAAVQLGSLGALLMFPVNVNLRLESSADFCNLHCPA	469
Rattus norvegicus	MLGVILRD--RSRSALLWCGAAVQLGSLGALLMFPVNVNLRLESSADFCNLHCPA	463
Mus musculus	MLGVILRD--RSRSALLWCGAAVQLGSLGALLMFPVNVNLRLESSADFCNLHCPA	460
C.elegans	MFAALLREGNQKESRLEFWCGAFIQVGLVVGKFNLYSLGI-----	417

Figure 1. Multiple Alignment of *C20orf54* Protein Sequence

The *C20orf54* protein sequence with the six missense mutations (p.E36K, p.R132W, p.F224L, p.L350M, p.V413A, and p.F457L) is shown in eight orthologs. The mutations p.E36K and p.V413A are found in case 9 (Table 1), who is a compound heterozygote. The alignment was performed with ClustalW2, and the GenBank accession numbers are NP_212134.3, XP_001152229.1, XP_001112427.1, XP_542938.2, XP_001916183.1, NP_001032275.1, NP_081448.2, and NP_001033515.

The clinical similarity between BVVLS syndrome and ALS has long been recognized, suggesting that the two conditions may be part of the same spectrum. On the basis of the limited evidence that *C20orf54* product is involved in the synthesis of molecules with pivotal role in cell energy metabolism, it is tempting to speculate that these conditions share a common pathway. It is conceivable that some cases of early-onset ALS, especially of the familial type, may be due to mutations in *C20orf54*, and it is worthwhile exploring this hypothesis by testing an appropriate cohort of patients. A locus on 20p13 designated as ALS7 (MIM 608037), which was identified by studying a large dominant family with multiple affected individuals,¹⁵ overlaps with *C20orf54*. However, the absence of any symptomatic carriers in our cohort makes it less likely that heterozygous mutations in this gene are associated with dominant ALS.

mechanism. Riboflavin is essential for synthesis of Flavin Adenine Dinucleotide (FAD) and Flavin Mononucleotide (FMN). Both operate as cofactors for a number of biological processes involved in energy metabolism. Flavoproteins have been implicated as having signal transduction roles in apoptosis¹² and DNA repair.¹³ Flavins contribute to oxidative stress through their ability to produce superoxide, as well as in the reduction of hydroperoxides by forming parts of multi-redox-center enzymes, such as the succinate and NADH dehydrogenases, xanthine oxidase/dehydrogenase, cytochrome P450 systems, and nitric oxide synthase.¹⁴ It is plausible that the protein has a maintenance function in the nervous system and that the disease is precipitated by exhaustion of a pathway tightly regulated by this protein.

In conclusion, we have identified a gene that is highly conserved across the evolutionary time scale. The function of this gene in the nervous system is as yet unknown. We have demonstrated that recessive mutations give rise to BVVLS. The methodology used here to locate the causative gene confirms the power of autozygosity mapping with targeted sequencing in identifying genes in rare recessive disorders for which consanguineous families are available.

Supplemental Data

Supplemental Data include two figures and one table and can be found with this article online at <http://www.ajhg.org>.

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Web Resources

The URLs for data presented herein are as follows:

ClustalW2, <http://www.ebi.ac.uk/Tools/clustalw2/index.html>

Homologene, <http://www.ncbi.nlm.nih.gov/sites/entrez>

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>

SIFT, <http://sift.jcvi.org/>

TMHMM-2.0, <http://www.cbs.dtu.dk/services/TMHMM-2.0/>

Translate, <http://www.expasy.ch/tools/DNA.html>

Accession Numbers

The GenBank accession numbers for the *C20orf54* orthologous sequences reported in this paper are NP_212134.3, XP_001152229.1, XP_001112427.1, XP_542938.2, XP_001916183.1, NP_001032275.1, NP_081448.2, and NP_001033515.1.

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