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Review Article

Sequence Variants of Genes PRKN of 6q26 and ERMARD, DLL1 and TBP of 6q27 Associated To Neurological Disorders Providing Relevant Clues for Evaluation of Genotype-Phenotype Correlation of Chromosome 6q Terminal Deletions

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Abstract

We describe here relevant pathogenic sequence variants of genes PRKN of 6q26 and ERMARD, DLL1 and TBP of 6q27 associated with neurological disorders. Variants of PRKN and ERMARD not described before support the morbid association of both genes, whereas those of DLL1 and TBP increase the small number of known similar pathogenic mutations and knowledge on phenotypic association. The presented data are particularly useful for the evaluation of genotype-phenotype correlations of chromosome 6q terminal deletions and the justified search for prominent genes responsible for associated complex syndromes.

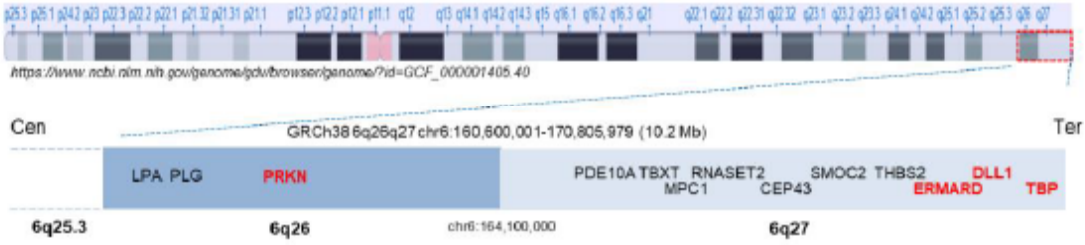
Keywords: Neurological disorders; Sequence variants; PRKN; ERMARD; DLL1; TBP

Introduction

Terminal deletions of chromosome 6q, of q26-q27 region, have been repeatedly observed in patients with phenotypes with remarkable neurological features. Further, despite considerable differences in both deletion size and phenotypic pictures, the concept of a 6q terminal deletion syndrome was suggested [1] and introduced, being used for example in the Orphanet database (ORPHA:75857) to describe a rare partial deletion of the long arm of chromosome 6 characterized by a variable clinical phenotype that includes a characteristic craniofacial dysmorphism (including microcephaly), global development delay (GDD), intellectual disability (ID), and varied occurrence of congenital malformations, muscular hypotonia, seizures, retinal anomalies and brain abnormalities. Besides, and in the manner of contiguous gene syndromes due to recurrent deletions or duplications, speculations for critical 6q terminal genes have been raised and, according to selected data from genomic arrays, a few genes in minimal deletion intervals have been pointed out, mainly DLL1, THBS2, PHF10 and ERMARD in 6q27, considered plausible candidates for causation of structural brain abnormalities, thus reiterating the suggested relevance of 6q27 genes in the development of brain and brain anomalies [2]. The paucity of consistent data of genomic arrays CNVs varied both in size and associated phenotypes, would

however preclude clear genotype-phenotype correlations, so that 6q27 deletions might be considered a complex syndrome with variable expressivity and incomplete penetrance [3, 4], lacking compelling data for one or a few main contributing genes. In this regard, descriptions of a morbid association for point mutations including exon deletions/duplications and sequence variants in isolated genes of 6q26 and 6q27 would be very helpful. Cases of this type have unfortunately only been rarely described, to our knowledge restricted to exon or whole deletions of PRKN and most recently to DLL1 sequence variants in 15 patients with ID, autism spectrum disorder (ASD), seizures, variable brain malformations, muscular hypotonia and scoliosis, as main features, reported in the fundamental and so far unique work of Fischer-Zirnsak et al. [5] concluding that heterozygous pathogenic DLL1 variants are the cause of a variable phenotype including neurodevelopmental and multi-systemic features, due to haploinsufficiency, that would reinforce the importance of DLL1 in human brain development [5].

To contribute to knowledge of 6q terminal genes, we hereby report on a few sequence variants in three genes, including one in PRKN in two patients of different families, two distinct variants in ERMARD, one in DLL1 and also one in TBP in two patients, as presented in Figure 1 and Table 1. In Figure 1 with an overview of q26-q27 gene content highlighting the morbid genes, particularly those of cases presented here involved in neurological disorders, whereas in Table 1 with main clinical data associated to variants identified. Of them, those of PRKN and ERMARD have not been described earlier; that of DLL1 increases the small number of known pathogenic variants and is distinguished by association to a very mild phenotype despite noteworthy brain abnormalities; and last, TBP variant, a short expansion of exon 3 CAG/CAA sequence, pathogenic although probably not due to haploinsufficiency but regardless presented for a comprehensive evaluation of the involvement of 6q terminal genes in neurodevelopmental disorders as well as adult neurogenetic maintenance and degenerative diseases.



6q terminal (q26-q27)

78 genes

13 disease-associated (OMIM): *LPA, PLG, PRKN, PDE10A, TBXT, MPC1, RNASET2, CEP43, SMOC2, THBS2, ERMARD, DLL1 & TBP*

8 associated to *neurological disorders*: *PRKN, PDE10A, TBXT, MPC1, RNASET2, ERMARD, DLL1 & TBP*

Gene	Description	OMIM Phenotype (MIM number)	Inheritance
PRKN	parkin RBR E3 ubiquitin protein ligase	Parkinson disease, juvenile, type 2 (#600116) **DD, ASD and other psychiatric disorders** (*candidate*[1,2])	AR AD
PDE10A	phosphodiesterase 10A	Dyskinesia, limb and orofacial, infantile-onset (#616921) Striatal degeneration, autosomal dominant (#616922)	AR AD
TBXT	T-box transcription factor T	Neural tube defects, susceptibility to (#182940)	AD
MPC1	mitochondrial pyruvate carrier 1	Mitochondrial pyruvate carrier deficiency (#614741)	AR
RNASET2	ribonuclease T2	Leukoencephalopathy, cystic, without megalencephaly (#612951)	AR
ERMARD	ER membrane associated RNA degradation	Periventricular nodular heterotopia 6 (#615544; *candidate*[3]) **DD with/without ASD** (*candidate*[4])	AD AD
DLL1	delta like canonical Notch ligand 1	**Neurodevelopmental disorder with nonspecific brain abnormalities and with/without seizures** (#618709; *candidate*[5,6])	AD
TBP	TATA-box binding protein	**Spinocerebellar ataxia 17** (#607136), **Multiple System Atrophy**[7,8] Parkinson disease, susceptibility to (#168600)	AD AD

Figure 1: q26-q27 region of chromosome 6, of ~10.2 Mb, repeatedly involved in "6q terminal deletions" varied in size and phenotypic features. It includes 78 genes, of them 13 are morbid and described in OMIM database, and 8 associated with neurological disorders are detailed with associated phenotypes and additional data in the prominent genes described hereby (PRKN, ERMARD, DLL1, TBP).

Superscripts: (1) PRKN: https://gene.sfari.org/database/human-gene/PRKN; (2) Two cases in present paper carriers of variant c.155del of PRKN; (3) Conti V et al. Periventricular heterotopia in 6q terminal deletion syndrome: role of the C6orf70 gene. Brain 2013;136:3378; (4) Two cases in present paper of ERMARD variants c.1369_1373dupGAAGA and c.486delT; (5) Fischer-Zirnsak B et al. Haploinsufficiency of the Notch ligand DLL1 causes variable neurodevelopmental disorders. Am J Hum Genet 2019;105:631; (6) Case in present paper with variant c.486delT of DLL1; (7) Toyoshima Y et al. Spinocerebellar ataxia type 17. 2005 Mar 29 [updated 2022 Jul 28]. In: Adam MP, Everman DB, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2022; (8) Two cases in present paper with 42 CAG/CAA repeats in TBP exon 3 polyglutamine sequence.

LOCUS	6q26	6q27			
GENE	*PRKN* (NM_004562.3)	*ERMARD* (NM_018341.3)		*DLL1* (NM_005618.4)	*TBP* (NM_003194.5; exon 3 CAG/CAA sequence)
Variant	P1&P2: **c.155delA**	P3: **c.1369_1373dup**	P4: **c.483delT**	P5: **c.170_173dup**	P6 & P7: **42 CAG/CAA repeats**
Methods	P1&P2: *clinical* exome[1]	*clinical* exome[1]	exome *WES*[2]	exome *WES*[2]	PCR, TP/PCR & Sanger sequencing[3]
Derivation	P1&P2: asymptomatic mother	asymptomatic mother	asymptomatic father	*de novo*	P6 & P7: ND / ND
PHENOTYPE					
Onset	P1&P2: early infantile	early infantile	early infantile	early infantile	P6 & P7: adult; onset age: ND; analysis age: ~70 y.
Main features	P1&P2: GDD, ASD P1&P2: no seizures	GDD, ASD no seizures	mild GDD infantile spasms dyslexia hypotonia divergent strabismus left forearm and testicle agenesis *In adulthood* (now 47 years): clumsy gait without EMG and histological findings of a myopathy chronique fatigue syndrome sleep apnea-hypopnea syndrome	mild GDD with good evolution no seizures	P6 & P7: gait ataxia, dysarthria
EEG	P1&P2: without alterations	without alterations	without alterations	without alterations	P6 & P7: ND
Brain MRI	P1&P2: no relevant findings	no relevant findings	no relevant findings	right temporo-occipital polymicrogyria corpus callosum dysgenesis PNH	P6: cerebellar atrophy / P7: ND P6: cortical cerebral atrophy / P7: ND
Genomic array	P1&P2: no relevant CNVs	ND	ND	no relevant CNVs	P6 & P7: ND

Table 1: Main clinical data in carriers of pathogenic variants in genes RKN of 6q26 and ERMARD, DLL1 and TBP of 6q27. GDD: global developmental delay; ASD: Autism Spectrum Disorder; ND: No Data (unknown); *de novo*: not detected in DNA from blood cells of parents; PNH: Periventricular Nodular Heterotopia, also known as subependymal grey matter heterotopia.

Superscripts: (1) *TruSight One clinical exome* and (2) *WES* (*Human Whole Exome Sequencing* with IDT probes) with reagents, instruments (mainly *NextSeq 500* sequencer) and software (*Variant Interpreter*) provided by Illumina (https://www.illumina.com); (3) PCR and TP/PCR (triplet repeat primed) following the procedures of Oda M et al. (Arch Neurol 2004;61:209) and Warner JP et al. (J Med Genet 1996;33:1022) with minor modifications; *Sanger* sequencing for confirmation.

A PRKN haploinsufficiency variant in two young children with GDD and ASD

Variant c.155del of gene PRKN was identified in two young patients, a boy (P1) and a girl (P2), with an early infantile onset of a neurological disorder including GDD and ASD (as presented in Table 1 and Figure 2).

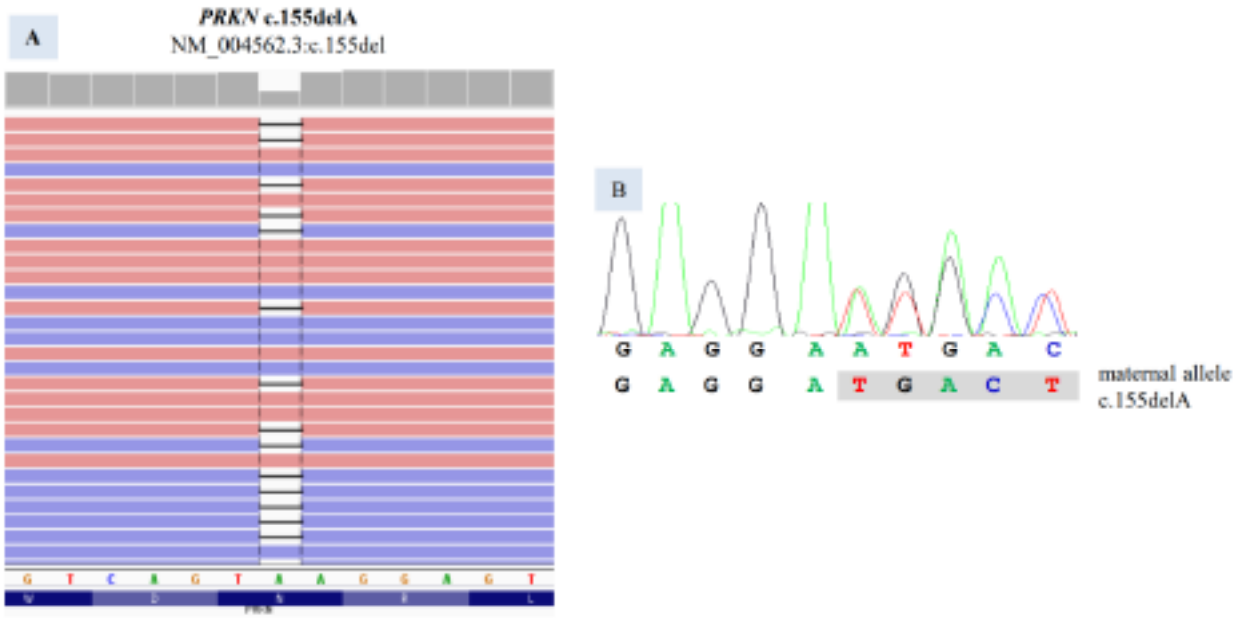


Figure 2: Variant c.155delA (p.Asn52MetfsTer29) of gene PRKN identified in Patients 1 and 2 under evaluation of an early infantile onset of a neurodevelopmental disorder with GDD (global developmental delay) and ASD (autism spectrum disorder), submitted to a clinical exome analysis with use of reagents (*TruSight One Sequencing Panel*), instruments (*NextSeq 500*) and software (*Variant Interpreter*) of Illumina (https://www.illumina.com). A and B: Variant details in Patient 1 data, from (A) the NGS BAM file viewed under IGV (https://software.broadinstitute.org) and (B) Sanger sequencing confirming variant occurrence in the patient also in the mother but not in the father (not shown). Same findings and pictures in Patient 2.

PRKN (parkin RBR E3 ubiquitin protein ligase), formerly described as PARK2, is a gene of locus 6q26 associated after autosomal recessive inheritance to early-onset, juvenile, Parkinson's disease (PD), in genotypes with biallelic pathogenic mutations of varied nature, including sequence variants, deleterious in many cases, as well as exon deletions and duplications. In addition, monoallelic PRKN pathogenic variants, among them c.155del, have been observed in many PD patients, raising the suspicion of monoallelic PRKN variants as a genetic susceptibility factor for PD and therefore of a dominant association of PRKN to PD. In particular, and as it is summarized by Brüggemann and Klein [6], this possibility is suggested by finding in some heterozygous carriers of multimodal neuroimaging and electrophysiology studies disclosing latent nigrostriatal impairment, compensatory hypertrophy of the putamen and pallidum, and increased iron deposition in the substantia nigra, and moreover cases compatible with pseudo-dominant inheritance (i.e., one of the parents affected) in families with autosomal recessive PRKN pathogenic variants [6].

Further, results of studies with genomic arrays provide evidence supporting that CNVs affecting exclusively PRKN, deletions but also duplications, contribute to a genetic etiology of a proportion of cases of neurodevelopment disorders with variable expressivity and incomplete penetrance (as described, for example, by [7] and [8]). It has particularly been reported in cases of ASD collected in the SFARI database (https://gene.sfari.org/database/human-gene/PRKN) with proposal of PRKN as a gene of class #2 risk (strong candidate) of autism, according to 17 studies reporting 27 variants (16 deletions, 11 duplications), 24 of them being exon-disrupting CNVs. PRKN copy number variants were clearly more frequently observed in ASD patients than in controls, predominantly inherited, and displayed incomplete penetrance with ASD. A similar enrichment of PRKN CNVs has also been described in patients with major depressive disorder (MDD), a condition that is otherwise associated with PD [9].

Variant c.155del of PRKN presented hereby, found without additional noteworthy genomic findings in two young children of unrelated families, could thus be considered an etiologic risk factor despite its derivation from asymptomatic progenitors. It would not be questioned by the lack of previous reports on the association of PRKN sequence variants to neurological disease, just considering haploinsufficiency of c.155del that according to its nature and location in exon 2 predicts frameshift change p.Asn52MetfsTer29 being equivalent to a whole gene deletion or a null allele.

Two ERMARD pathogenic variants associated with neurodevelopmental and one of them also with multisystem involvement

Here we also report on two novel deleterious sequence variants of gene ERMARD (in patients P3 and P4, presented in Table 1 and Figure 3) associated with neurological (in P3 and P4) and additional multisystem (in P4) affectation.

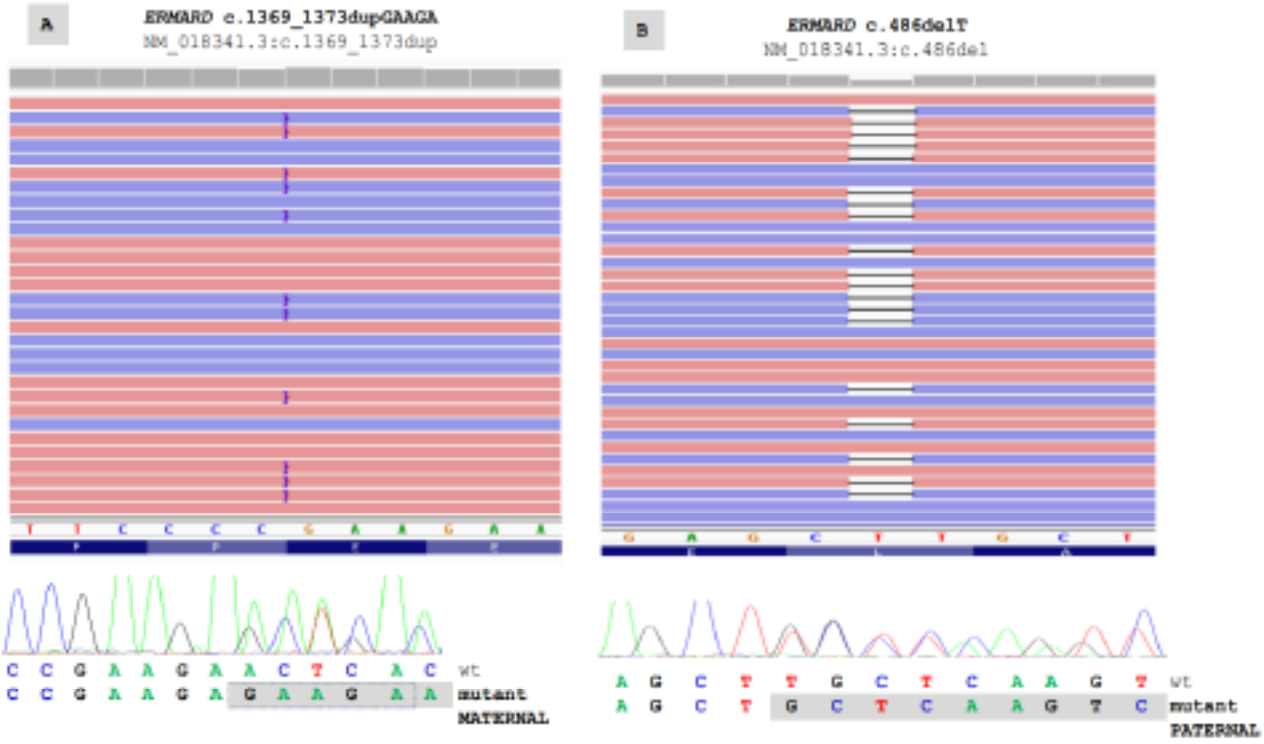


Figure 3: ERMARD variants c.1369_1373dupGAAGA and c.486delT in patients 3 and 4, respectively, found with a genomic sequencing analysisundertaken with reagents (for WES, with IDT probes), instruments (including the *NextSeq 500*) and software (*Variant Interpreter*) of Illumina(https://www.illumina.com). Details from NGS BAM files viewed under IGV (https://software.broadinstitute.org) and at the bottom of Sanger sequencing for confirmation studies in the patients and parents. **(A)**: ERMARD exon 14 variant c.1369_1373dupGAAG (NM_018341.3:c.1369_1373dup) that predicts frameshift change p.Leu459LysfsTer10inherited from the mother**. (B)**: ERMARD exon 5 variant c.486delT (NM_018341.3:c.486del) predicting frameshift mutation p.Ala162LeufsTer8 in the paternal allele.

ERMARD (ER membrane associated RNA degradation), previously designated C6orf70, is a gene of a molecule with 2 transmembrane domains near the C-terminus located in the endoplasmic reticulum, with a proposed morbid association together with other 6q27 genes to a 6q terminal deletion syndrome according to data of several studies with genomic arrays particularly that of Conti et al. [10]. These authors described in 12 patients a ~1.2 Mb minimal critical deleted region of 6q27 containing six genes (THBS2, WDR27, PHF10, TCTE3, C6orf70 [ERMARD] & DLL1) associated with brain developmental anomalies variably combining periventricular nodular heterotopia (PVH), corpus callosum dysgenesis, colpocephaly, cerebellar hypoplasia and polymicrogyria, and clinical manifestations of DD, facial dysmorphism, epilepsy, joint laxity, and ataxic or clumsy gait. In particular, the occurrence of PVH in 9 of 12 patients caught the attention of the authors and as proof of C6orf70 relevance they provided impressive experimental results. Firstly, silencing of C6orf70 in the developing rat neocortex by the in utero RNA interference-mediated knockdown approach [11, 12] led to PVH that was prevented by concomitant expression of wild-type human C6orf70 protein. By contrast, silencing of the contiguous PHF10 or DLL1 genes led only to a slight delay in neuronal migration without PVH. In addition, with WES-based sequencing studies, they identified a de novo missense variant of C6orf70 in a patient with PVH, developmental delay and epilepsy. That variant corresponds to I377N (NM_018341.3:c.1130 T>A) and I251N (NM_001278532.2:c.752T>A) in the updated sequences of isoforms due to alternative splicing described by the authors, of the large canonical (RefSeq NM_018341.3) and a shorter transcript molecules, with 679 and 553 residues respectively. In transfected

HEK293T cells, the variant was associated with reduced expression of the two isoforms and altered cytoplasmic vesicular distribution of C6orf70 protein, from a punctate-like to a diffuse pattern. These elegant results justified the authors' proposal of C6orf70 [ERMARD] as a gene with a major role in the control of neuronal migration, associated with PVH, by gene haploinsufficiency due to whole gene deletions and also probably to heterozygous sequence variants, as it has been widely considered and propose provisionally in OMIM (https://www.omim.org/entry/615544).

A primordial association of ERMARD with PVH needs however further confirmation taking into account that PVH is not always present in cases of 6q terminal deletions including those of our two patients with ERMARD frameshift mutations. PVH has been otherwise found in 2 of 16 patients with neurodevelopmental disorders carriers of DLL1 pathogenic variants, one described by Fischer-Zirnsak et al. [5], the other in our present communication. However, most important, PVH is a brain malformation associated with multiple genes, frequently with FLNA, occasionally with ARFGEF2, DCHS1, ERMARD, FAT4, INTS8, MAP1B, MCPH1, and NEDD4L, as well as several chromosomal abnormalities [13]. Therefore, and as concluded by Conti et al. [10], the complex brain phenotype and additional clinical features observed in patients with 6q27 deletions likely resulted from the combined haploinsufficiency of contiguous genes in the locus, a conclusion that would be also pertinent for PVH.

ERMARD variants hereby reported raise some considerations of potential interest. As the unique apparent relevant findings of the clinical exome analysis in the patients, frameshift mutations c.1369_1373dupGAAGA (p.Leu459LysfsTer10) and c.486delT (p.Ala162LeufsTer8) are considered primordial genetic factors associated with disorders of (P3 and P4) patients with variable penetrance, as inherited from no affected parents. The great difference in the clinical picture of P3 and P4 patients might reside in the mutation location. Thus, c.1369_1373dupGAAGA (p.Leu459LysfsTer10) of exon 14 would yield a shorted molecule that may retain some functions like that of some ERMARD transcripts (described in the Ensembl genome database project, http://www.ensembl.org). As a peculiarity, in addition to GDD, this mutation is associated with ASD not described before in patients of 6q27 deletions, although GDD and ASD are frequent comorbidities in neurological disorders. On the other hand, variant c.486delT of exon 5 predicts the yield of very short molecules (p.Ala162LeufsTer8) equivalent to a complete ERMARD deletion or a null allele. The (P4) patient with this mutation exhibits a complex phenotype, with some features described in patients with 6q27 deletions, such as epilepsy (infantile spasms), language problems (dyslexia), hypotonia, strabismus and clumsy gait [2, 10]. Besides, left testis and forearm agenesis would be uncommon findings associated with ERMARD increasing the spectrum of testicular abnormalities and agenesis of 6q27 deletions, such as hypospadias and rib agenesis [10]. As regards limb agenesis, it would also be considered an example of the known recurrent association of PNH and distal limb deficiency, rising speculations of a common causative mechanism [14]. Alternatively, it may reflect a broad morbid ERMARD effect, as suggested by the case of two siblings with a congenital heart defect (transposition of the great arteries) carriers of a duplication of ERMARD and part of neighbor TCTE3 gene inherited from their phenotypically normal mother [15]. ERMARD mutation might also be responsible for later manifestations of the patient P4 without excluding the possibility of comorbidity, in any case with a plausible preeminent role of the obstructive sleep apnea-hypopnea syndrome that includes psychological and intellectual features (difficulty in attention-concentration, nervous depression, and decreased libido), emotional lability, anxiety, headache, and alterations of the metabolic syndrome (such as dyslipidemia) referred in the patient. It would explain the adult patient complex disorder without the need to resort to proposed but not proven diagnoses of myopathy, chronic fatigue, and/or central sensitivity syndrome.

A novel haploinsufficiency variant of DLL1 associated with brain malformations but very mild DGG and an excellent evolution

DLL1 (delta like canonical Notch ligand 1) is a gene of 6q27 of a human homolog of the Notch Delta ligand member of the delta/serrate/jagged family, involved in the Notch regulatory pathway following interaction with Notch 2 receptor encoded by the NOTCH2 gene. It is linked according to autosomal dominant inheritance to a disorder escribed in OMIM as "Neurodevelopmental disorder with nonspecific brain abnormalities and with or without seizures" (https://www.omim.org/entry/618709) entirely described based on the analysis by Fischer-Zirnsak et al. [5] of a cohort of 15 patients from 12 unrelated families carriers of 10 distinct heterozygous DLL1 mutations, of nonsense, frameshift, splice site and missense type, as well as one whole gene deletion, of de novo occurrence or inherited from a mild affected parent. The most common features in that cohort were DD/ID, brain abnormalities, ASD and seizures, less frequent muscular hypotonia, and scoliosis. A genotype-phenotype correlation was however not observed inclusive for non-specific brain abnormalities that included hydrocephalus, ventriculomegaly, anomalies of the corpus callosum, cortical dysplasia, a small cerebellum/pons, and PNH in one patient. The authors concluded that their data support the association of heterozygous DLL1 pathogenic variants to a variable neurodevelopmental phenotype and multi-systemic features, due to a mechanism of haploinsufficiency, as well as the importance of DLL1 in human brain development.

In addition to the 16 distinct DLL1 pathogenic mutations so far described in the ClinVar database, we present the novel variant c.170_173dupGGAC (NM_005618.4:c.170_173dup; as shown in Figure 4) identified in a 2-year-old girl under evaluation by mild GDD, without relevant CNVs in a CGH (750 K) array, but with impressive cerebral MRI findings, including right temporo-occipital polymicrogyria, corpus callosum dysgenesis and PNH. The identified DLL1 variant, heterozygous and de novo in occurrence (not found in DNA of blood samples of the healthy parents), that predicts frameshift change p.Phe59AspfsTer68, is considered pathogenic like other DLL1 deleterious mutations. Moreover, by its disposition in exon 2 it would yield very short truncated and fully defective molecules, being thus equivalent to a null allele or a complete gene deletion. Despite it, such a clear DLL1 haploinsufficiency and the remarkable brain abnormalities do not fit with the evolution of the patient now at 5 years old with excellent behavior at home and school and with very good academic results.

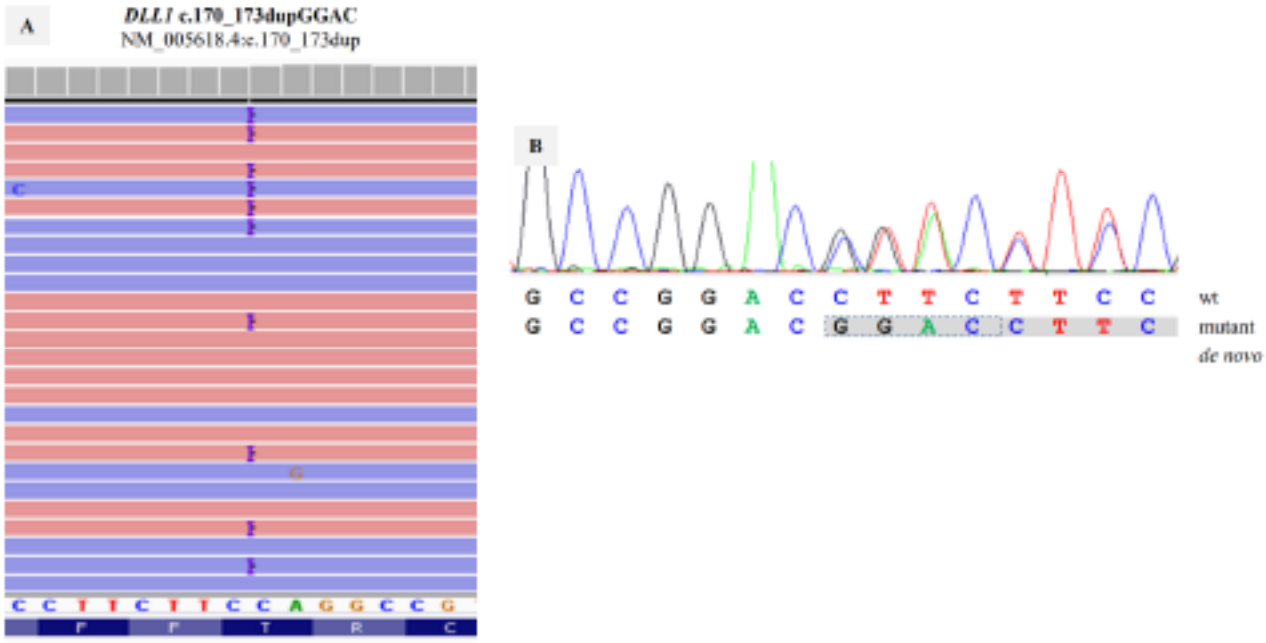


Figure 4: DLL1 exon 2 variant c.170_173dupGGAC (NM_005618.4:c.170_173dup) that predicts frameshift change p.Phe59AspfsTer68, identified with a genomic sequencing analysis (WES, with IDT probes) according to Illumina (https://www.illumina.com). **A** and **B**: Details from NGS BAM files viewed under IGV (https://software.broadinstitute.org) and Sanger sequencing for confirmation studies in the patient and parents of the mutation, de novo (not inherited) in occurrence.

A short expansion of exon 3 polyQ sequence of gene TBP, of 42 CAG/CAA repeats, associated to a neurodegenerative disease

To examine the relevance of the 6q terminal region in neurological disorders we also consider the morbid association of TBP (TATA-box binding protein), a gene of 6q27 that encodes the core molecule of the DNA-binding multiprotein and general transcription factor TFIID. A distinctive feature of TBP is a long string of glutamines [polyQ tract due to a sequence of consecutive CAG/CAA codons] in the N-terminus, a region of the protein that modulates the DNA binding activity of the C terminus and thereby the rate of transcription complex formation and initiation of transcription (https://www.ncbi.nlm.nih.gov/gene/6908). Pathological expansions of the CAG/CAA sequence [with >49 and 41-49 repeats in full- and reduced-penetrance alleles, respectively] are associated according to dominant inheritance to SpinoCerebellar Ataxia Type 17 (SCA17), a neurodegenerative disorder characterized by ataxia, dementia, and involuntary movements, including chorea and dystonia, also commonly with psychiatric symptoms, pyramidal signs, rigidity, and variable atrophy of the cerebrum, brain stem, and cerebellum (see [16] for details). The clinical features of SCA17 correlate with the length of the polyglutamine expansion, but are not absolutely predictive

of the clinical course. TBP alleles with short expansions of CAG/CAA sequence are characterized by decreased penetrance and late presentation, as reported for 10 SCA17 cases associated to expansions of 41 and 42 CAG/CAA repeats, 5 cases of each, with onset as early as 23 and 32 years (as reviewed in [17]. Even shorter alleles, with >38 CAG/CAA repeats, are now described significantly associated with increased Multiple System Atrophy (MSA) risk, suggesting a possible genetic overlap of MSA with SCA17 [18]. The number of SCA17 or even MSA cases due to short TBP CAG/CAA repeat expansions are anyway increased with our two patient's carriers of one allele with 42 repeats and disease onset at adult age (Table 1 and Figure 5).

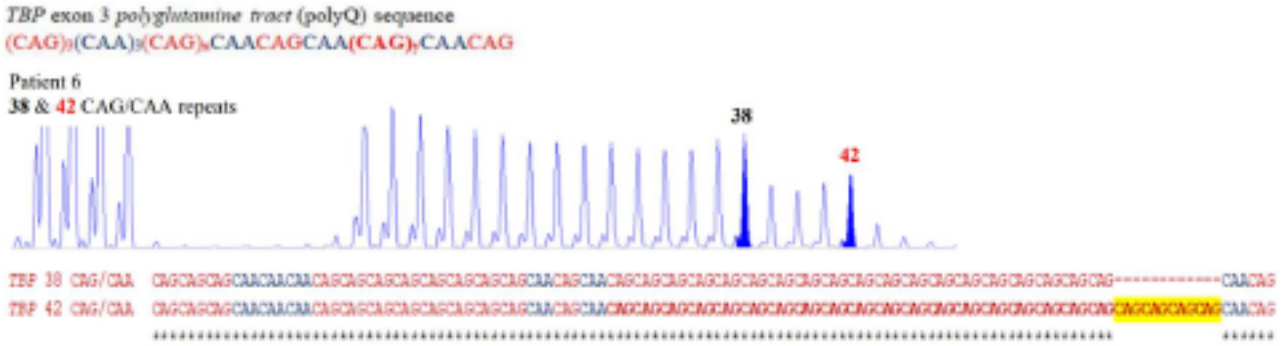


Figure 5: Short expansion, of 42 CAG/CAA repeats, of exon 3 polyglutamine (polyQ) tract sequence of TBP gene identified in two adult patients with neurodegenerative features suggestive of SpinoCerebellar Ataxia type 17 (SCA17) or Multiple System Atrophy (MSA). The sequence was analyzed by the TP/PCR procedure (according to Warner JP et al. J Med Genet 1996;33:1022, with minor changes) and Sanger sequencing following PCR-amplification (after Oda M et al. Arch Neurol 2004;61:209). As indicated in the TP/PCR electropherogram and nucleotide sequences, Patient 6 was carrier of alleles with 38 (normal) and 42 (pathologic) CAG/CAA repeats, the increase in size of the latter occurring at the (CAG)y element of the polyQ sequence. Patient 7was a carrier of TBP alleles with 37 (normal) and 42 (pathologic) CAG/CAA repeats.

In addition to the established neurodegenerative association to SCA17 and MSA, TBP might be considered a gene involved in neurodevelopmental disease association due to haploinsufficiency taking into account the proposal of TBP as candidate gene for mental retardation in patients with subtelomeric 6q deletions [4, 19], of particular interest being a case of neurodevelopmental disorder in an 8-year-old boy carrier of a small 6q27 deletion affecting most three terminal genes of 6q27: PSMB1, TBP, and PDCD2 [20]. TBP might thus be endowed in neurologic development as well as in neurogenetic maintenance and associated degeneration, due to mutations of different or even the same type. In any case, it would be a relevant gene to be considered in the evaluation of cases of multigene 6q terminal deletions and duplications.

Concluding Remarks

The results we describe here are distinguished by the novelty of the genes variants presented, pathogenic neurogenetics mutations of prominent chromosome 6q terminal genes implicated in neuronal development and/or maintenance, namely PRKN of 6q26 and ERMARD, DLL1 and TBP of 6q27. In PRKN and ERMARD, novel deleterious variants with a reasonable claim for consideration as pathogenic mutations, triggers of the neurological disorders of the patients, whereas in DLL1 and TBP variants increase the small number of known similar pathogenic mutations and their phenotypic association.

The described variants are of particular interest taking into account the variable gene content and associated phenotype of 6q terminal deletions, and the goal of delineating a genotype-phenotype correlation. However, and despite its unquestionable value, our data would not define a pivotal implication of affected genes (PRKN, ERMARD, DLL1 and TBP) in phenotypes of multigene 6q deletions. Resembling those, single gene variants exhibit a varied phenotype and penetrance, a finding that would be open to a number of speculations although presided by the possible implication of multiple forces (genes) in neurological development as well as in neurogenetic maintenance and associated degeneration, merely considering the broad genetic heterogeneity of neurological disorders associated to numerous chromosomal loci. A contribution of several genes of a region or locus, such as terminal 6q and 6q27, rendering a cooperative additive or synergistic effect, suggesting a "position effect" (3), would be possible although not necessary. On the other hand, phenotypes

of single genes, such as those associated to ERMARD and DLL1, would also possibly be due to a modulator effect of additional genes even without pathogenic variants although affected by a certain degree of differential regulation and expression. In this way, for a more precise identification of gene variants of different types in multiple genes involved in neurological disorders, the fundamental procedure for genomic studies of neurological disorders would be an exome (WES) sequencing analysis that now enables identification of CNVs without need for genomic arrays that occasionally would be useful for confirmation studies.

References

1. Bertini V, De Vito G, Costa R, Simi P, Valetto A (2006) Isolated 6q terminal deletions: an emerging new syndrome. Am J Med Genet A; 140: 74-81.
2. Peddibhotla S, Nagamani SC, Erez A (2015) Delineation of candidate genes responsible for structural brain abnormalities in patients with terminal deletions of chromosome 6q27. Eur J Hum Genet; 23: 54-60.
3. De Cinque M, Palumbo O, Mazzucco E (2017) Developmental coordination disorder in a patient with mental disability and a mild phenotype carrying terminal 6q26-qter deletion. Front Genet; 8: 206.
4. Hanna MD, Moretti PN, P de Oliveira C (2019) Defining the critical region for intellectual disability and brain malformations in 6q27 microdeletions. Mol Syndromol; 10: 202-8.
5. Fischer-Zirnsak B, Segebrecht L, Schubach M (2019) Haploinsufficiency of the Notch ligand DLL1 causes variable neurodevelopmental disorders. Am J Hum Genet; 105: 631-9.
6. Brüggemann N, Klein C (2001) Parkin type of early-onset Parkinson disease. In: Adam MP, Everman DB, Mirzaa GM, et al., eds. GeneReviews®. Seattle (WA): University of Washington, Seattle; April 17, 2001. Accessed: October 2022
7. Yin CL, Chen HI, Li LH (2016) Genome-wide analysis of copy number variations identifies PARK2 as a candidate gene for autism spectrum disorder. Mol Autism; 7: 23.
8. Palumbo O, Palumbo P, Leone MP (2016) PARK2 microduplication: clinical and molecular characterization of a further case and review of the literature. Mol Syndromol; 7: 282-6.
9. Zhang X, Abdellaoui A, Rucker J (2019) Genome-wide burden of rare short deletions is enriched in major depressive disorder in four cohorts. Biol Psychiatry; 85: 1065-73.
10. Conti V, Carabalona A, Pallesi-Pocachard (2013) Periventricular heterotopia in 6q terminal deletion syndrome: role of the C6orf70 gene. Brain; 136: 3378-94.
11. Bai J, Ramos RL, Ackman JB, Thomas AM, Lee RV, LoTurco JJ (2003) RNAi reveals doublecortin is required for radial migration in rat neocortex. Nat Neurosci; 6: 1277-83.
12. Carabalona A, Beguin S, Pallesi-Pocachard E, Buhler E, Pellegrino C, et al. (2012) A glial origin for periventricular nodular heterotopia caused by impaired expression of Filamin-A. Hum Mol Genet; 21: 1004-17.
13. Cellini E, Vetro A, Conti V (2019) Multiple genomic copy number variants associated with periventricular nodular heterotopia indicate extreme genetic heterogeneity. Eur J Hum Genet; 27: 909-18.
14. De Wit MC, de Coo IF, Schot R (2010) Periventricular nodular heterotopia and distal limb deficiency: a recurrent association. Am J Med Genet A; 152A: 954-9.
15. Sanchez-Castro M, Eldjouzi H, Charpentier E (2016) Search for Rare Copy-Number Variants in Congenital Heart Defects Identifies Novel Candidate Genes and a Potential Role for FOXC1 in Patients With Coarctation of the Aorta. Circ Cardiovasc Genet; 9: 86-94
16. Toyoshima Y, Onodera O, Yamada M (2005) Spinocerebellar ataxia type 17. 2005 Mar 29 [Updated 2022 Jul 28]. In: Adam MP, Everman DB, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2022. Accessed: October 2022
17. Paolini Paoletti F, Prontera P, Nigro P (2021) Small-expanded allele spinocerebellar ataxia 17: imaging and phenotypic variability. Neurol Sci; 42: 4309-15.
18. Wernick AI, Walton RL, Soto-Beasley AI (2021) Frequency of spinocerebellar ataxia mutations in patients with multiple system atrophy. Clin Auton Res; 31: 117-125.
19. Rooms L, Reyniers E, Scheers S, van Luijk R, Wauters J, et al. (2006) TBP as a candidate gene for mental retardation in patients with subtelomeric 6q deletions. Eur J Hum Genet; 14: 1090-6.
20. Eash D, Waggoner D, Chung J, Stevenson D, Martin CL (2005) Calibration of 6q subtelomere deletions to define genotype/phenotype correlations. Clin Genet; 67: 396-403.