Journal of the Neurological Sciences xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Short communication

Novel mutations in the *L1CAM* gene support the complexity of L1 syndrome

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ARTICLE INFO

Article history:
Received 30 November 2009
Received in revised form 31 March 2010
Accepted 31 March 2010
Available online xxxx

Keywords:
Hydrocephalus
L1CAM gene
Mutation screening
Spastic paraplegia

ABSTRACT

X-linked hydrocephalus, MASA syndrome, X-linked complicated Spastic Paraplegia Type I and X-linked partial agenesis of the corpus callosum are the four rare diseases usually referred to L1 syndrome, caused by mutations in the *L1CAM* gene. By direct sequencing of *L1CAM* in 16 patients, we were able to identify seven mutations, five of which were never described before. Patients' phenotype evaluation revealed a correlation between the number of clinical features typical of L1 syndrome and the chance to find causative mutation. Our findings support that *L1CAM* mutations are associated with widely heterogeneous phenotypes, however the occurrence of several clinical features remains the best criterion for planning molecular testing both in familial and apparently sporadic cases.

1. Introduction

L1 syndrome comprises a wide variety of X-linked inherited neurodevelopmental disorders which are all caused by mutations in the *L1CAM* gene (MIM#308840). The *L1CAM* gene is located on the long arm of X-chromosome (Xq28) and is comprised of 28 coding exons spanning about 16 kb (NM_000425.3). *L1CAM* encodes for the L1 cell adhesion molecule (L1-CAM), a protein of 1257 amino acids, member of the immunoglobulin (Ig) superfamily of neural cell adhesion molecules. L1-CAM is a transmembrane glycoprotein containing several domains that include six immunoglobulins, five fibronectin type III domains in the extracellular part, a single-pass transmembrane segment and a very short cytoplasmic domain.

L1 is predominantly expressed in the developing nervous system and plays a key role in cell–cell adhesion and interaction, neuronal migration, neurite growth and fasciculation, myelination, synaptic plasticity and hippocampal long-term potentiation [1].

To date more than 240 different mutations have been reported in the *L1CAM* gene (*L1CAM* Mutation Database) [2]. These mutations are associated to the large phenotypic spectrum of L1 syndrome that includes: X-linked hydrocephalus due to stenosis of the aqueduct of Sylvius (HSAS, MIM#307000), MASA syndrome (mental retardation, aphasia, shuffling gait, and adducted thumbs, MIM#303350), X-linked complicated Spastic Paraplegia Type I (SPG1, MIM#303350) and X-linked partial agenesis of the corpus callosum (MIM#304100) [3,4]. Moreover, the known *L1CAM* mutations are scattered over the whole coding region, therefore the entire gene has to be sequenced in order to achieve molecular diagnosis.

2. Materials and methods

2.1. Patients

Sixteen index cases were screened for mutations in the *L1CAM* gene. Each of them showed at least one of the phenotypic features typical of L1 syndrome according to the classification of Finckh [4], as described in Table 1. Among them, case 7421 was already described [5], the remaining have not been previously reported.

2.2. Molecular analysis

Genomic DNA was extracted from peripheral leukocytes according to standard protocols from patients after having obtained written informed consent. Mutation screening of the *L1CAM* gene was performed by standard PCR amplification and direct sequencing of all coding exons and exon–intron boundaries (primer sequences and amplification conditions are available upon request). Relatives of mutated patients were included in the genetic analysis in order to identify female carriers.

In order to exclude the possibility that the detected variants were rare polymorphisms, we tested their presence in 300 X-chromosomes

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doi:10.1016/j.jns.2010.03.030

Table 1
Summary of clinical and anamnestic data of selected patients.

Patients	Family history	Adducted thumbs[a]	Hydrocephalus[a]	ACC[a]	Spastic paraplegia[a]	Mental retardation[a]	Other clinical features	Disease onset	Actual age	No. of typical features
7421		X	X	X	X	X		pediatric	12	5
8083			X	X		X	Hirschsprung syndrome	Perinatal	1	3
9107		X	X	X	X	X		Perinatal	15	5
9125				X	X	Mild		18 years	25	3
9156	X	X	X		X	X		Perinatal	24	4
9178		X	X	X		X		Perinatal	27	4
9227		X	X	X	X	X			3	5
9408	X	Adducted forefinger	X		X	X			10	4
9604		X			X	X		Pediatric	16	3
9608		X	X	X	X	X	Bartter syndrome		12	5
9618			X						3	1
9658			X			X			17	2
9659		X	X	X	NA	NA		Perinatal	3 months	3
9664		X	X	X	X	X	Club feet		11	5
9745			X		Mild	Mild		Perinatal	6	3
9754	X		X	X	X	X		Pediatric	54	4

ACC = agenesis of the corpus callosum.
[a] Typical phenotypic features accordingly to the classification of Fickh [4].

(150 ethnically-matched, healthy females). Mutation nomenclature is based on the *L1CAM* reference sequences available at the NCBI (cDNA: NM_000425.3 and protein: NP_000416.1).

3. Results

A total of 7 different *L1CAM* mutations were found in 16 index cases (Table 2). Five of these were novel, one had already been described by our group elsewhere [5] and one had been previously reported by Saugier-Veber [6]. The novel mutations identified were absent in 300 control chromosomes. All mutations identified were located in the coding region: 3 were frameshift and 4 were missense.

L1CAM mutations were identified in 44% of patients. However, the detection rate increased to 100% when considering only the familial cases.

Once a mutation was identified, females that were at risk of being carriers within the patient's family were considered for molecular testing. This was done in 5 families (index cases 7421, 9107, 9156, 9659 and 9754) in which we identified 13 healthy heterozygous carriers out of 19 investigated subjects (Fig. 1). Moreover in families in which other affected males were available, the molecular testing confirmed the segregation of the mutations supporting their pathogenicity.

4. Discussion

In this study, direct sequencing of the *L1CAM* coding region in 16 patients with clinical features related to L1 syndrome, allowed us to identify seven mutations, five of which had never been reported before (Table 2).

Patient 9227 carried a missense mutation (p.Trp9Arg) involving the signal peptide of the L1 protein. A different mutation affecting the same codon (p.Trp9Ser) has been previously reported in a 6 year-old patient with prenatal-onset hydrocephalus, mental retardation, adducted thumbs and spastic displegia [7,8]. Clinical features and phenotype severity were similar to those of our patient. As suggested by functional studies, the tryptophan residue in position 9 seems to play a key role in controlling protein sorting. Its substitution severely impairs translocation through the endoplasmic reticulum, resulting in low levels of L1 molecule on the cell membrane [9].

A second novel missense mutation (p.Tyr310Cys) was identified in a 54 year-old man (case 9754) with hydrocephalus, mild mental retardation, shuffling gait and agenesis of corpus callosum. Within the same family, five other subjects currently aged between 30 and 73 were reported to have similar clinical findings. The mild phenotype observed in this family seems in agreement with the hypothesis that mutations of residues at the surface of L1-CAM extracellular domains (like p. Tyr310Cys) are more likely associated to milder phenotypes than those involving amino acids in the core [10].

Three novel frameshift mutations were also identified (Ile225-LeufsX11, Asn825LysfsX63 and Ile804AsnfsX14). All these variations produce stop codons yielding L1 proteins truncated at different domains in the extracellular portion. It has been suggested that this kind of mutation is more likely associated to severe hydrocephalus, severe mental retardation and early mortality (<1 year) than missense mutations [11]. Clinical features of our patients are in agreement with this except for the early mortality aspect. Cases 9107 and 9156 have now reached 15 and 24 years of age but they both have undergone ventricular shunt implant within their first months of life.

In this study, we have identified *L1CAM* mutation in 44% of tested patients. This detection rate was similar to those reported by other studies [4,12]. Finckh has found that the probability of detecting *L1CAM* mutation in L1-patients is directly related to the number of L1-typical features, the absence of atypical signs and the presence of other affected subjects in the pedigree [4]. Recently, Vos et al. confirmed this observation reporting that the probability of finding *L1CAM* mutation increases up to 66% in patients with three or more clinical features [13]. By adopting similar criteria, our mutation rate increased to 58% (7/12) underlining the importance of careful clinical assessment before

Table 2
L1CAM mutations indentified in our sample.

Patients	Family history	Type of mutations	DNA	Mutations locus	Protein	Domain	No. of patients	No. of carriers	References
7421		Missense	c.2308G>A	Exon 18	p.Asp770Asn	Fn2	1	3	[5]
9107		1 bp deletion	c.670delC	Exon 6	p.Ile225LeufsX11	Ig2	1	3	novel
9156	Yes	1 bp insertion	c.2410insA	Exon 18	p.Ile804AsnfsX14	Fn2	1		novel
9227		Missense	c.25T>A	Exon 1	p.Trp9Arg	E1	1		novel
9408	Yes	Missense	c.1417C>T	Exon 12	p.Arg473Cys	Ig5	2		[6]
9659		5 bp deletion	c.2470_2474del	Exon 19	p.Asn825LysfsX63	Fn3	1	3	novel
9754	Yes	Missense	c.929A>G	Exon 8	p.Tyr310Cys	Ig3	4	4	novel

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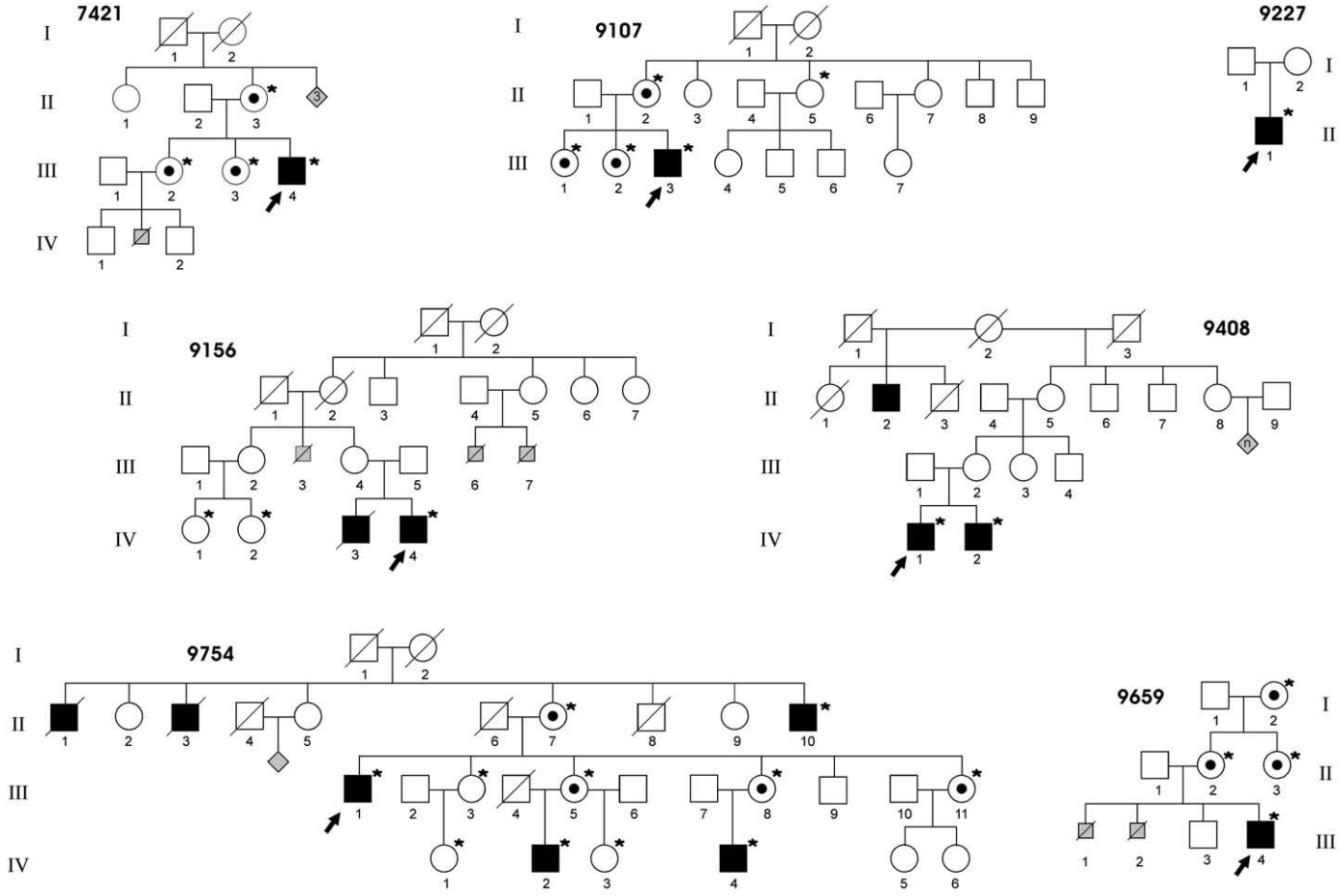

Fig. 1. Family trees of *L1CAM* mutated patients. Black arrows: probands; asterisk: subjects available for genetic analysis.

planning molecular analysis. Likewise, family history is confirmed to be highly predictive of the involvement of the *L1CAM* gene. Indeed, we found mutations in all cases with documented familiarity.

In conclusion, our results are in agreement with those of previous reports, even though the number of patients investigated here was relatively small. Moreover, our findings confirm that both familial and sporadic cases with more than three L1-typical phenotypic features provide a promising detection rate for *L1CAM* mutation (over 50%) and strongly encourage *L1CAM* genetic testing.

More than half of our patients did not show mutations in the *L1CAM* gene. With the exception of three subjects with additional atypical features, the clinical presentation in such cases did not grossly differ from mutated patients. Although the presence of non-coding *L1CAM* mutations could not be excluded, the proportion of non-mutated patients reinforces the previous notion of genetic heterogeneity of these forms.

Since there are no predictive bio-markers for L1-syndrome, molecular analysis of the *L1CAM* gene is the only diagnostic tool for the differential diagnosis of affected individuals and for the identification of healthy female carriers. However, it must be considered that about 7% of *L1CAM* mutations are *de novo* or due to maternal germ cell mosaicism [13]. In the present study we identified *L1CAM* mutations in four apparently sporadic cases (7421, 9107, 9659 and 9227). The analysis of probands' relatives evidenced several female carriers in three families (7421, 9107 and 9659; for pedigree 9227 no relatives' DNA was available). This allowed to exclude *de novo* mutation events in three out of four apparently sporadic cases. These findings underline the need to extend the molecular analysis to the relatives in order to provide an effective and reliable genetic counseling to these families.

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Please cite this article as: Bertolin C, et al, Novel mutations in the *L1CAM* gene support the complexity of L1 syndrome, J Neurol Sci (2010), doi:10.1016/j.jns.2010.03.030