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RESEARCH LETTER

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A Novel Recurrent *LIS1* Splice Site Mutation in Classic Lissencephaly

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TO THE EDITOR:

Classic lissencephaly is a severe disorder of neocortical neuronal migration. The lissencephaly spectrum varies from complete or nearly diffuse agyria to subcortical band heterotopia. The most commonly mutated gene in patients with classic lissencephaly is *LIS1* (OMIM 601545) [Uyanik et al., 2007; Saillour et al., 2009]. More than 100 *LIS1* mutations have are known, with most being heterozygous large or small exonic deletions or duplications or truncating mutations, whereas missense mutations are less frequent [Sakamoto et al., 1998; Fogli et al., 1999; Cardoso et al., 2000, 2002;

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Sicca et al., 2003; Torres et al., 2004; Uyanik et al., 2007; Mei et al., 2008]. Ten splice site mutations in *LIS1* have been reported, but in most the effects of these mutations at the RNA level have not been demonstrated.

Here, we describe two unrelated patients with classic lissencephaly in whom a recurrent *de novo* mutation at the acceptor splice site of intron six was identified by targeted-gene sequencing. The first patient is the first boy of a non-consanguineous couple. He was born after an uneventful pregnancy, with a birth weight of 3240 g (19th centile), a birth length of 49 cm (12th centile), and an OFC of 33 cm (4th centile). Head control was achieved at 2 months but the ability of sitting independently and standing was delayed at 12 and 15 months, respectively. He walked at the age of 2 years. Seizures started at 4 years controlled with topiramate and valproate. At age of 8 years, his growth parameters were weight 50.1 kg (>97th centile), height 1.32 m (75th centile), and OFC 55 cm . He had normal visual interaction and used approximately 50 words. He walked unaided with bilateral genu flessum and varus deformity. Neurological examination was normal. Brain MRI showed posterior pachygyria sparing the fronto-temporal lobes (Fig. 1A–C). The second patient is the first boy of a non-consanguineous couple. He was born at full term, with a birth weight of 3670 g (57th centile). Intermittent right nystagmus and convergent strabismus were noted from the age of 2 months. He was brought to medical attention at 3.5 months because of repeated brief tonic clonic seizures. He was seizure-free from the age of 4 years. At last evaluation at the age of 5.5 years, his growth parameters were weight 21.3 kg (75th centile), length 113 cm (50th centile), and OFC 50 cm (10th centile). He had visual interaction and uttered articulate sounds with his parents. He could crawl and enjoyed walking a few steps. Neurological examination showed axial hypotonia and peripheral hypertonia. Brain MRI showed posterior pachygyria sparing the fronto-temporal lobes (Fig. 1D–F). In both patients, array CGH was normal. To identify the underlying cause of the classic lissencephaly in both patients, we performed targeted-gene sequencing on DNA extracted from a blood sample, using an NGS panel of 54 genes involved in cortical malformations. We identified a recurrent apparently *de novo* heterozygous mutation at the acceptor splice site of *LIS*1 (NM_000430) intron 7: c.569-10T > C (g.2575939T > C), not reported in public databases, nor in the ExAC database. All prediction tools (Alamut software using MaxENtSplice splice score (9.28 > 7.49), NNsplice (0.84 > 0.69), and ESE finder (6.31 > 5.88), except HSF predicted that this variant reduces the strength of the consensus acceptor splice site of intron 6. To assess the functional consequence of the mutation, an RNA study was performed after extraction and reverse transcription using fibroblast cell lines derived from skin biopsies of both patients.

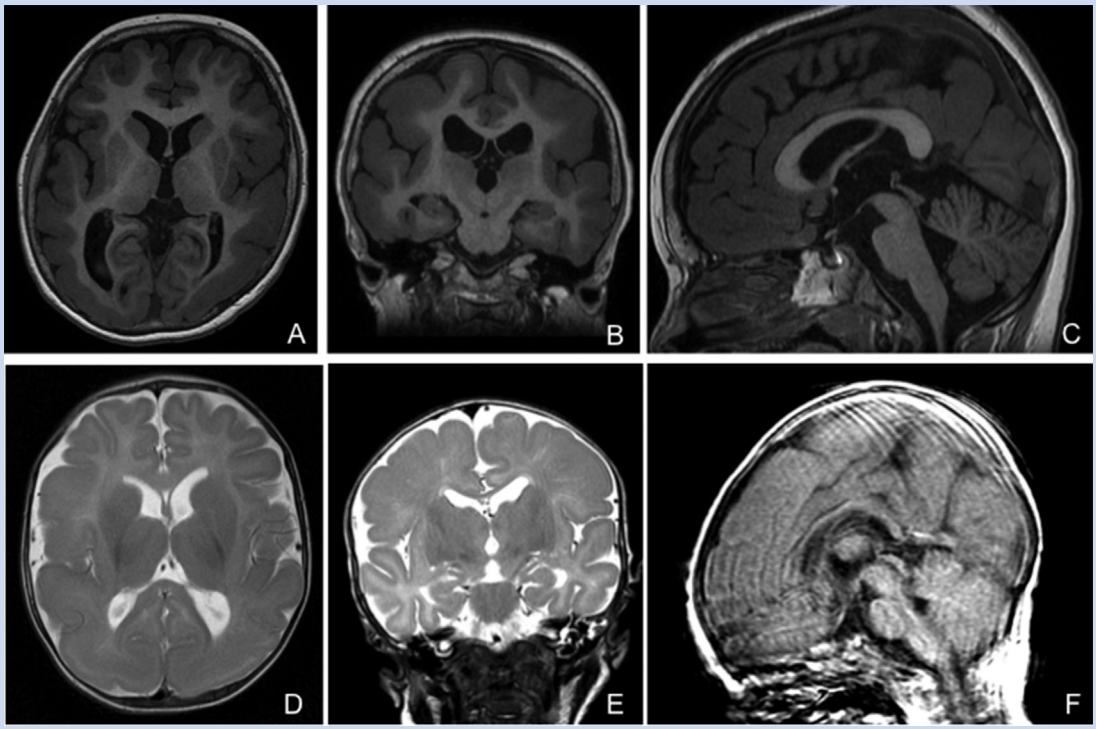

FIG. 1. Representative MRI images of patient 1 at the age of 5 years and 5 months (A–C), and patient 2 at the age of 4 months (D–F). Axial T1 weighted (A and B) and T2 (C and D) images at the level of the basal ganglia show pachygyria (thickened cortex) affecting the parietal and the occipital lobes (A and D), sparing the frontal lobes (A and C), and both temporal lobes in coronal section (B and E). Sagittal T1 weighted images (C and F) show normal corpus callosum, brain stem, and cerebellar vermis.

Compared to control samples for which a unique 600 bp RT-PCR fragment was observed, an additional shorter PCR product (497 bp) was detected from the patient samples, of which sequencing showed skipping of exon 7. Skipping induction was quantified and showed an approximately half decrease of wildtype mRNA abundance compared to control cells. Together, these results support the view that the mutation c.569-10T > C alters the consensus acceptor splice site of intron 6 Leading to exon seven skipping. Furthermore, the loss of exon 7 predicted (p.Gly190Alafs*3) (Supplemental Online Fig. S1).

The fraction of *cis* splicing mutations causing disease is estimated at 15% and mainly comprises mutations known to affect the canonical splice sites [Krawczak et al., 1992]. However, this proportion is likely to be underestimated and most *cis*-acting splicing mutations are likely undiagnosed. It has been proposed that 60% of mutations that cause disease do so by disrupting splicing [López-Bigas et al., 2005]. This wide range for the predicted frequency of splicing mutations reflects our incomplete knowledge of the splicing code and the fact that mRNAs from mutant alleles are rarely assayed for splicing abnormalities. In fact, one of the limitations of the systematic analysis of the impact of abnormal splicing as a cause of disease is the availability of RNA from disease relevant tissues [Wang and Cooper, 2007]. In classic lissencephaly related to *LIS1* mutations, more than 2/3 mutations are nonsense, frameshift mutations, or deletions. By contrast, splice mutations represent 10/137 mutations reported in HGMD, at least as uncommon as missense mutations. Although nonsense-mediated mRNA decay (NMD) is proposed as a key mechanism in the processing of the *LIS*1 transcript bearing premature stop codons [Uyanik et al., 2007], the documentation of this effect in human cells is scarce. Remarkably, the mutation c.569-10T > C described here was previously reported in six unrelated patients [Cardoso et al., 2002; Uyanik et al., 2007] ClinVar (RCV000020304.3) as pathogenic, and was referenced in dbSNP (rs113994202), without validation. Here, our data clearly demonstrate that the mutation alters the consensus acceptor splice site of intron 6 Leading to an alternatively spliced *LIS1* mRNA, and the skipping of exon 7 with the formation of a premature stop codon in exon 8 (p.Gly190Alafs*3).

Although previous reports have stressed the clustering of *LIS1* mutations in exon 6, only a few mutations are recurrent, reported in most cases twice (HGMD). The recurrence of this intronic mutation is of importance for clinical practice, although its predictive value for disease severity is not demonstrated. For instance, four out of the six patients previously reported with the c.569-10T > C mutation had severe neurological impairment being unable to sit or to interact, as is the case for the majority of patients with *LIS*1 Lissencephaly [Cardoso et al., 2002; Uyanik et al., 2007]. In contrast, both patients reported here, were less severely affected since they are both able to walk unaided, their seizures were controlled, and their MRI pattern showed pachygyria sparing the fronto-temporal lobes. These observations argue for the heterogeneity of the clinical consequences of the same mutation in *LIS1*, which has not been noted previously. Of note, we and others attempted to draw correlations with the type and the location of mutations [Pilz et al., 1998; Cardoso et al., 2002; Caspi et al., 2003; Uyanik et al., 2007; Saillour et al., 2009]. In all reports, the main limitation of such correlations is the reduced variability among patients with *LIS1* mutations who exhibit in most cases, mixed agyria–pachygyria, tetraplegia, and refractory epilepsy. The patients reported here further demonstrate that the phenotypic variability in patients with *LIS1* related lissencephaly may not be only explained by the type or the location of the mutation. In conclusion, our data demonstrate that the recurrent *LIS1* intronic mutation disrupt *LIS1* transcript splicing and is responsible for classic lissencephaly of variable severity. These observations further support that the type and the location of *LIS1* mutations is not predictive for the severity of the disorder.

ETHICS STATEMENT

The institutional review board of Necker Enfants Malades Hospital approved the use of human clinical materials and blood in this study. Written informed consent for genetic testing and medical photography were obtained from all subjects before participation.

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WEB RESOURCES

Human Gene Mutation Database http://www.hgmd.cf.ac.uk/ac/all.php

Exome Aggregation Consortium:

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