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NTNG1 Mutations are a Rare Cause of Rett Syndrome

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Abstract

A translocation that disrupted the Netrin G1 gene (*NTNG1*) was recently reported in a patient with the early seizure variant of Rett syndrome (RTT). The netrin G1 protein (NTNG1) has an important role in the developing central nervous system, particularly in axonal guidance, signalling and NMDA receptor function and was a good candidate gene for RTT. We recruited 115 patients with RTT (females: 25 classic and 84 atypical; 6 males) but no mutation in the *MECP2* gene. For those 52 patients with epileptic seizure onset in the first six months of life, *CDKL5* mutations were also excluded. We aimed to determine whether mutations in *NTNG1* accounted for a significant subset of patients with RTT, particularly those with the early onset seizure variant and other atypical presentations. We sequenced the nine coding exons of *NTNG1* and identified four sequence variants, none of which were likely to be pathogenic. Mutations in the *NTNG1* gene appear to be a rare cause of RTT but NTNG1 function demands further investigation in relation to the central nervous system pathophysiology of the disorder.

Keywords

Rett syndrome; Netrin G1; Autism; NMDA receptor

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Introduction

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder that predominantly affects females (OMIM#312750). A series of clinical criteria which characterize the disorder have been developed [Hagberg et al., 1983; Trevathan and Naidu, 1988] and recently modified [Hagberg et al., 2002]. Patients with all of these criteria are generally diagnosed with classic RTT. Mutations in the *MECP2* gene (methyl CpG binding protein 2 gene, OMIM#300005) account for most cases of classic RTT [Amir et al., 1999]. Even in those without an identifiable *MECP2* mutation, the features may be related to dysfunction of the MeCP2 protein [Renieri et al., 2003]. The *MECP2* mutation detection rate is much lower in patients with atypical RTT, suggesting that this group is both clinically and genetically more heterogeneous [Charman et al., 2005]. The early onset seizure variant of RTT is associated with an atypical presentation in which early seizures mask the onset of the disorder [Hanefeld., 1985; Goutieres and Aicardi., 1986] and in which MECP2 mutations are uncommon [Charman et al., 2005]. Mutations in the X-linked *CDKL5* gene (cyclin-dependent kinase-like 5, OMIM#300203) were found in some patients with this RTT variant [Weaving et al., 2004; Tao et al., 2004; Scala et al., 2005; Evans et al., 2005; Mari et al., 2005].

A recently published report described a patient with atypical RTT who presented with early onset of epileptic seizures (not infantile spasms) and a *de novo* translocation: 46,XX,t(1;7)(p13.3;q31.33) which disrupted the Netrin G1 gene (*NTNG1*, OMIM#608818), located on chromosome 1 [Borg et al., 2005]. When evaluated at 10 years of age by one of the authors (HA), she had many features of RTT but still had poor eye contact and no interest in people.

NTNG1 spans 340 kilobases and has recently been shown to contain ten exons, nine of which are coding [Aoki-Suzuki et al., 2005]. The membrane bound product of this gene netrin G1 (NTNG1) is involved in axonal guidance and signaling and NMDA receptor functioning [Lin et al., 2003; Aoki-Suzuki et al., 2005]. Its important role in the developing central nervous system made it a good candidate gene for RTT.

We recruited patients with both classic and atypical RTT but no mutation in *MECP2* to determine whether mutations in *NTNG1* accounted for a significant proportion of patients with these clinical phenotypes.

Materials and Methods

Patient recruitment

Patients with suspected RTT (total 115), but in whom a *MECP2* mutation had not been found were identified with consent from within the UK (85 cases) and from the Australian Rett Syndrome Database (30 cases) [Colvin et al., 2003] (see Table I). Of 109 female patients, 25 had classic RTT and 84 atypical RTT, of which 46 had seizure onset in the first 6 months of life. The remaining six patients were male. In the 102 patients without infantile spasms, exon 1 mutations and large genomic rearrangements of *MECP2* had also been excluded [Laccone et al., 2004; Ravn et al., 2005; Evans et al., 2005]. No *MECP2* mutations have been reported so far in patients with infantile spasms, so this additional analysis was not likely to yield any further mutations in the other 13 patients. Mutations in *CDKL5* were excluded in the subset of 52 patients with seizure onset in the first six months of life or infantile spasms, either by sequence analysis or DHPLC [Weaving et al., 2004; Evans et al., 2005].

Ethical approval for this research study was granted by MREC (Wales): reference number 02/9/33.

Molecular analysis

The coding exons, 2-10, of NTNG1 were screened by sequence analysis in all 115 patients in the study group. The sequence of the exons of *NTNG1* was obtained by alignment of the mRNA sequences NM_014917 and AY764265 with the genomic sequence of chromosome 1 (www.ensembl.org). PCR primers and conditions are shown in the online supplementary material (supplementary table I). PCR was performed in a 20μl volume containing 1× PCR buffer (supplied with Taq), 25mMgCl2, 200μM dNTPs, 250μM each primer, 0.5U AmpliTaq Gold (Applied Biosystems) and 20ng genomic DNA. Sequencing reactions were performed using a Big Dye kit v1.1 (Applied Biosystems) according to the manufacturer's instructions. We screened a panel of unrelated healthy female controls for sequence variants identified within the study group.

Results

No pathogenic mutations were identified in *NTNG1* in 109 female and six male patients with suspected RTT. In total, four sequence variations were identified in the study group, all of which were unlikely to be pathogenic (see Table II). Three were intron sequence variations which did not involve any sequences known to interact with the splicing machinery. One was a silent polymorphism within a coding region. Examination of this sequence using splice site prediction programs did not suggest that this sequence variation leads to the generation of an exonic splicing enhancer site (http://www.fruitfly.org/seq_tools/splice.html, ESE Finder Release 2.0: http://rulai.cshl.edu/tools/ESE/ [Cartegni et al., 2003] and http://www.genet.sickkids.on.ca/~ali/splicesitefinder.html).

Discussion

We have investigated a large group of patients with a clinical diagnosis of RTT for mutations in *NTNG1*. We did not identify any likely pathogenic mutations and only found four sequence variants. We did not identify the synonymous SNP (A282A) nor the intronic variant IVS7+60T>G in the control panel. This was not surprising given the low frequency of these variants within the study group. Although we did not find any pathogenic *NTNG1* mutations in our study group, it is possible that large genomic rearrangements such as exonic deletions, which would not be identified by sequencing, may represent the common mutations in *MECP2* mutation negative RTT patients. While this may explain our negative results, it remains likely that *NTNG1* mutations are a rare cause of RTT.

Four further exons (exons 6-9) of *NTNG1* were identified after the publication of the translocation case [Aoki-Suzuki et al., 2005]. By alignment of the flanking sequences described in the published translocation case with the sequence of *NTNG1*, we have re-defined the location of the chromosome 1 breakpoint to intron 8 (IVS8+570) of *NTNG1* [Borg et al., 2005]. *NTNG1* contains 10 exons and there are at least ten different Ntng1 mRNA transcripts in mice, nine of which include the coding part of exon 10 [Aoki-Suzuki et al., 2005]. It has already been shown that the translocation patient has at least one functional NTNG1 isoform: AB023193 (see online supplementary Figure 1) [Borg et al., 2005]. This isoform is not membrane bound and little is known about its expression pattern. For the remaining nine isoforms, which all contain exon 10, loss of the functional C-terminal domain would lead to loss of the glycosyl phosphatidylinositol lipid (GPI) anchor encoded by this exon [Meerabux et al., 2005]. Effective removal of the GPI anchor in tissue culture severely disrupts neurite outgrowth of thalamocortical neurons [Nakashiba et al., 2002]. Even if the truncated transcripts were translated, it is unlikely that they would retain critical functions in a non-membrane bound state.

Further investigation of the specific regional brain expression of the isoforms of NTNG1 may be helpful in understanding both the translocation patient's phenotype and the overlap with RTT. NTNG1 is expressed in the brain, particularly strongly in the thalamus [Yin et al., 2002], and is important for normal NMDA receptor function [Nishimura et al., 2004]. Isoforms G1a, c, d, e and l are expressed in human fetal brain, and of these G1c and d are the most highly expressed [Meerabux et al., 2005]. Glc binds to the NTNG1 ligand in tissue culture, promoting outgrowth of thalamic neurons [Lin et al., 2003]. Of the remaining five isoforms not expressed in fetal brain, at least four are expressed in human adult brain [Meerabux et al., 2005]. This differential expression demonstrates that NTNG1 is developmentally regulated in humans. It is interesting that there is also strong expression of G1c in the kidney, and that this does not bind to the one known NTNG1 ligand [Meerabux et al., 2005]. It was hypothesized that *NTNG1* mutations may also be found in patients with renal vascular disease [Meerabux et al., 2005]. However, the translocation patient did not have any apparent renal abnormalities nor have they been reported, so far, in mouse knockouts.

Normal function of both the dopaminergic pathways and glutaminergic pathways are required for normal NMDA receptor function and for normal neurogenesis. In patients with RTT it is clear that these and other neurotransmitter systems are impaired and that neuronal maturation and synaptogenesis is abnormal [Johnston et al., 2003; Johnston et al., 2005]. It has been shown that CSF glutamate levels are increased [Hamberger et al., 1992] and while NMDA receptor numbers are initially increased they later significantly decrease in number [Blue et al., 1999; Johnston et al., 2001]. Glutamate deficiency, NMDA receptor blockade and *Ntng1* knockouts in rodents produce a phenotype that overlaps with that of RTT and the MECP2 knockout mice [Hauber., 1998; Hohmann et al., 1998; Mohn et al., 1999; Ohtake et al., 2000; Moretti et al., 2005; Aoki-Suzuki et al., 2005]. Partial NMDA receptor blockade in mice results in stereotypes, abnormal motor activity, social withdrawal as well as sensory and cognitive deficits [Mohn et al., 1999]. The translocation patient, whom we have independently investigated, presented with these features: all of which are also found in patients with RTT. However, social withdrawal is typically a temporary state in RTT but this appeared to be permanent in the translocation patient. The overlap in phenotype of the translocation patient and those with RTT may reflect converging end pathways resulting in disruption of the NMDA system. Further research is needed to investigate the potential role of NTNG1 in those with RTT, atypical autism, mental retardation and epilepsy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table I

The study group. EOS: epilepsy onset before 6 months of age. IS: infantile spasms. Other: all other RTT patients in each category.

RTT type	EOS	IS	Other	Total
Classic RTT female	na	na	25	25
Atypical RTT female	35	11	38	84
male suspected RTT	4	2	0	6
GRAND TOTAL	39	13	63	115

Table II

Sequence variants identified in *NTNG1*. (For details of *NTNG1* reference sequence see methodology section).

Type of variant	Sequence change	Frequency in study group	Frequency in controls (30 control chromosomes)	Reference
Silent	c.846A>G (A282A)	0.9%	0%	this study
Intronic	IVS5-43A>G	20%	20%	Aoki-Suzuki et al. 2005
Intronic	IVS5-6G>A	9%	10%	this study
Intronic	IVS7+60T>G	0.9%	0%	this study