

Disruption of a Synaptotagmin (*SYT14*) Associated With Neurodevelopmental Abnormalities

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We report cytogenetic and molecular studies of a de novo, apparently balanced t(1;3)(q32.1;q25.1) identified in a 12-year-old female (designated DGAP128) with cerebral atrophy, macrocephaly seizures, and developmental delay. A combination of fluorescence in situ hybridization (FISH) and Southern blot analysis demonstrated disruption of a synaptotagmin gene (*SYT14*) at the 1q32 breakpoint. Expression of *SYT14* in human brain was confirmed using Northern analysis. Because members of the synaptotagmin family of proteins function as sensors that link changes in calcium levels with a variety of biological processes, including neuro-

transmission and hormone-responsiveness, *SYT14* is an intriguing candidate gene for the abnormal development in this child. This is the first known constitutional rearrangement of *SYT14*, and further systematic genetic analysis and clinical studies of DGAP128 may offer unique insights into the role of *SYT14* in neurodevelopment. © 2007 Wiley-Liss, Inc.

Key words: reciprocal translocation; neurodevelopment; seizures; synaptotagmin

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INTRODUCTION

The cytogenetic and molecular study of rare individuals with apparently balanced chromosomal rearrangements has been a successful approach to identify genes causing an abnormal phenotype [Krantz et al., 2004; Abelson et al., 2005]. Recently, identification of constitutional rearrangements causing gene disruption or dysregulation also has provided insight into potentially novel pathways for known genes [Ligon et al., 2005].

DGAP128 is a 12-year-old female who carries a de novo, apparently balanced rearrangement, initially reported as t(1;3)(q32.1;q25.1). Her clinical evaluation was significant for macrocephaly, cerebral atrophy, seizures, and developmental delay. Cytogenetic and molecular approaches were used to map the translocation breakpoints in DGAP128, and subsequently to uncover disruption of a known gene, *SYT14*, which we propose contributes to the abnormal phenotype.

CLINICAL BACKGROUND

DGAP128 was born at 39 weeks gestation by normal spontaneous vaginal delivery, weighing 3,005 g and without any neonatal complications. Her mother had two prior spontaneous pregnancy losses and one healthy son. Her maternal grandmother and great aunts had a history of learning difficulties and childhood seizures, the latter of which resolved. Her mother's occipital-frontal circumference (OFC) was enlarged at 58 cm (+2 SD), and this woman has mild bilateral 2–3 syndactyly of

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the toes. Her father is 182.9 cm tall, weighs 99.8 kg, and has an OFC within normal range.

Previously, both height and weight parameters for DGAP met or exceeded the 95th centile, though on most recent examination she was 160.2 cm (90th centile) and her weight was 56.7 kg (90th centile). She had an OFC of 55.5 cm (> +2 SD). She was not dysmorphic. A single telangiectatic lesion measuring 3 × 1.5 cm was identified over her right flank. There was mild bilateral 2–3 syndactyly of the toes, identical to that of her mother. Deep tendon reflexes were slightly brisk. The physical examination was otherwise unremarkable.

The following investigations were all normal: plasma amino acids, urine organic acids, liver enzymes, cholesterol, 7-dehydrocholesterol, uric acid, and molecular analysis for fragile X syndrome. Her bone age was appropriate for her chronological age. A head MRI showed diffuse atrophy, especially of the frontal and parietal lobes with possible mild cerebellar and vermian atrophy. Ophthalmologic assessment revealed mild convergence insufficiency type of exotropia requiring no intervention. WISC III scores were 58, 57, and 64 for full scale, verbal, and performance IQ, respectively. Her weak areas noted by her school-teacher were in logic, analysis, planning, sequencing, and abstract thinking.

METHODS

Fluorescence In Situ Hybridization Analysis

Breakpoint mapping studies were initiated to map precisely the chromosomal rearrangement using fluorescence in situ hybridization (FISH). For this purpose, a peripheral blood specimen was received after informed consent was obtained in accordance with institutional policies and procedures for human research at Partners HealthCare System. Cell transformation was performed at the Massachusetts General Hospital Center for Human Genetic Research tissue culture resource using standard protocols. Transformed lymphoblastoid cells were then cultured to generate sufficient material for GTG-banding and FISH-mapping using standard protocols (see below).

Metaphase chromosome spreads were prepared using conventional cytogenetic protocols [Ney et al., 1993]. FISH was performed with directly labeled cosmid or BAC probes. BAC or fosmid clones were selected using the University of California Santa Cruz Genome Browser (<http://genome.ucsc.edu/>; May 2004 freeze used for probe selection, March 2006 freeze used to confirm results) and were hybridized simultaneously in pairs (SpectrumGreen and SpectrumRed/Orange; Abbott Molecular/Vysis, Inc., Des Plaines, IL). At least 10 metaphases were studied per hybridization.

Southern Blot Analysis

Southern blotting was performed using standard methods. RepeatMasker (<http://www.repeatmasker.org>) was used to identify single copy sequences for probe design. Genomic DNA from DGAP128 and from a phenotypically and karyotypically normal control was digested with several restriction enzymes (*StuI*, *BglII*, *BlnI*, *EcoRI*, *HpaI*, *NcoI*, *NsiI*, *SspBI*; Webcutter: <http://rna.lundberg.gu.se/cutter2/>) and hybridized to a 700 bp probe amplified from within the sequence of BAC RP11-168F20. The following primers were used to generate the probe: (F) 5'-TCTTCCCGATTTAGGGCATT-3' and (R): 5'-CAAATGCAACTACAGCACATCA-3' (Primer design: http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi; <http://www.ncbi.nlm.nih.gov/BLAST/>). Probe labeling was performed using the MegaPrime labeling kit (Amersham/GE Healthcare, Piscataway, NJ), following the manufacturer's instructions.

Northern Blot Analysis

A commercially prepared Northern blot (Clontech, Mountain View, CA) of polyadenylated RNA isolated from multiple regions of human adult brain (amygdala, caudate nucleus, corpus callosum, hippocampus, thalamus, and whole brain), was hybridized with a probe representing a 683 bp region of the 3'UTR of *SYT14*. Labeling, hybridization, and blot washes were performed using standard methods, as described above. The blot was normalized with β -actin as a control (data not shown).

RESULTS

Translocation Breakpoint Mapping

FISH was performed with BAC and fosmid clones (Table I, also see Supplementary Material) mapping in the vicinity of the 1q32 breakpoint. Ultimately, hybridization of BAC RP11-168F20 showed a signal split between the derivative chromosomes 1 and 3 (Fig. 1), indicating that the 1q32 breakpoint maps within this clone. RP11-168F20 spans synaptotagmin 14 (*SYT14*), which is transcribed in a centromere to telomere orientation and has two alternative promoters that can produce five transcripts via alternative splicing [Craxton, 2004]. A relatively weaker hybridization to the der(1) is consistent with the majority of *SYT14* being translocated to the der(3) (Fig. 1). The 29 kb FISH-defined 1q32 breakpoint interval was refined by Southern blot analysis (Fig. 2A) to a 2,880 bp interval within intron 3 of *SYT14* (Fig. 2B).

Similarly, the 3q25 breakpoint was studied by FISH (data not shown) and the critical interval narrowed to ~383 kb, flanked proximally by RP11-372M20 and distally by RP11-664C7, and including no known genes or conserved non-genic (CNG)

TABLE I. BAC and Fosmid Clones Used to Map DGAP128 Breakpoints

Breakpoint	Clone	Accession number	Signal position	
1q32	RP11-148K15	AQ374947	Centromeric to break	
	RP11-571I7	AL137789	Centromeric to break	
	RP11-45F21	AL355533	Centromeric to break	
	RP11-167J2	AQ421322	Centromeric to break	
	RP11-168F20	AL355335	CROSSES break	
	RP11-216F1	BZ749095	Telomeric to break	
	RP11-91C15	AZ518690	Telomeric to break	
	RP11-123O6	BZ749030	Telomeric to break	
	RP11-91I7	AL691441	Telomeric to break	
	G248P85272B11	—	Telomeric to break	
	G248P88070E8	—	Telomeric to break	
	3q25	RP11-362A9	AC026347	Centromeric to break
		RP11-89L22	AQ286615	Centromeric to break
RP11-372M20		AC107423	Centromeric to break	
RP11-664C7		AQ433955	Telomeric to break	
RP11-451C20		AQ583106	Telomeric to break	
RP11-270G15		AC106724	Telomeric to break	

Representative BAC and fosmid clones used to map the translocation breakpoints in DGAP128. Accession numbers are indicated, where available, as well as the relative map location of each clone with respect to a given breakpoint. Note that BAC RP11-168F20 crosses the 1q32 breakpoint.

sequences; Southern blot analysis was not performed for this breakpoint. Following FISH-mapping of DGAP128, the GTG-banded breakpoints were revised and the rearrangement was designated as t(1;3)(q32.2;q25.2)dn.

SYT14 Expression

Based upon previous mouse studies it has been suggested that *SYT14*, unlike other synaptotagmins, is expressed mainly in non-neural tissues [Fukuda, 2003]. Consequently, we examined the expression pattern of *SYT14* in human brain using a commercially prepared Northern blot and a specific cDNA probe amplified from within the 3'UTR of *SYT14*. A ~2.9 kb full-length transcript (NM_153262) was identified, as well as a smaller (~2.5 kb) related transcript derived from a synaptotagmin pseudogene (not shown), in the corpus callosum, amygdala, caudate nucleus, hippocampus, and thalamus (Fig. 3), supporting the view that *SYT14* may be responsible for the phenotype observed in DGAP128.

DISCUSSION

The incidence of apparently balanced reciprocal translocations is reported to be approximately 1/500–1/2,000 live births, with a risk for congenital anomalies twice that of an unselected population of newborns [Warburton, 1991]. This increased risk is generally attributed to disruption or dysregulation of genes mapping at or near the individual breakpoints or, in some cases, to submicroscopic deletions or duplications that occur in the vicinity of the breakpoints [Kumar et al., 1998; Gribble et al., 2005]. We report here on the results of molecular cytogenetic analysis of DGAP128, a 12-year-old

female with multiple neurological abnormalities including cerebral atrophy, absence seizures, and developmental delay. Breakpoint-mapping of the t(1;3)(q32.2;q25.2) demonstrated direct disruption of *SYT14*.

SYT14 belongs to a family of at least 15 related mammalian genes [Andrews and Chakrabarti, 2005], some of which are conserved in *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Arabidopsis thaliana* [Craxton, 2004]. Synaptotagmins include a transmembrane domain, linker domain, and a cytoplasmic region comprised of tandem C2A and C2B domains [Rickman et al., 2004]. As a group, synaptotagmins can undergo both homo- and hetero-oligomerization, either in a calcium dependent or independent manner [Perin et al., 1991; Littleton et al., 1994; Bai et al., 2000; Sudhof, 2002]. Calcium-dependent phospholipid binding specifically involves the C2 domains, both of which show homology to the regulatory domain of protein kinase C and, in the case of *SYT1*, affect calcium-dependent activation [Perin et al., 1990; Fernandez-Chacon et al., 2001].

Fukuda [2003] reviewed synaptotagmin function in processes as diverse as peptide hormone secretion [Gao et al., 2000], fertilization [Roggero et al., 2005], and neuronal communication [Geppert et al., 1994; Chapman, 2002; Sudhof, 2002]. While some synaptotagmins show ubiquitous expression, vertebrate synaptotagmins are expressed mainly in neurons and neuroendocrine cells [Sudhof, 2002]. Expression of synaptotagmins in such a diverse range of organisms is consistent with evolution of a role that may extend beyond neurotransmission [Craxton, 2004].

The best-described member of this family, *SYT1*, encodes a synaptic vesicle membrane protein that responds to calcium influx and mediates the rapid

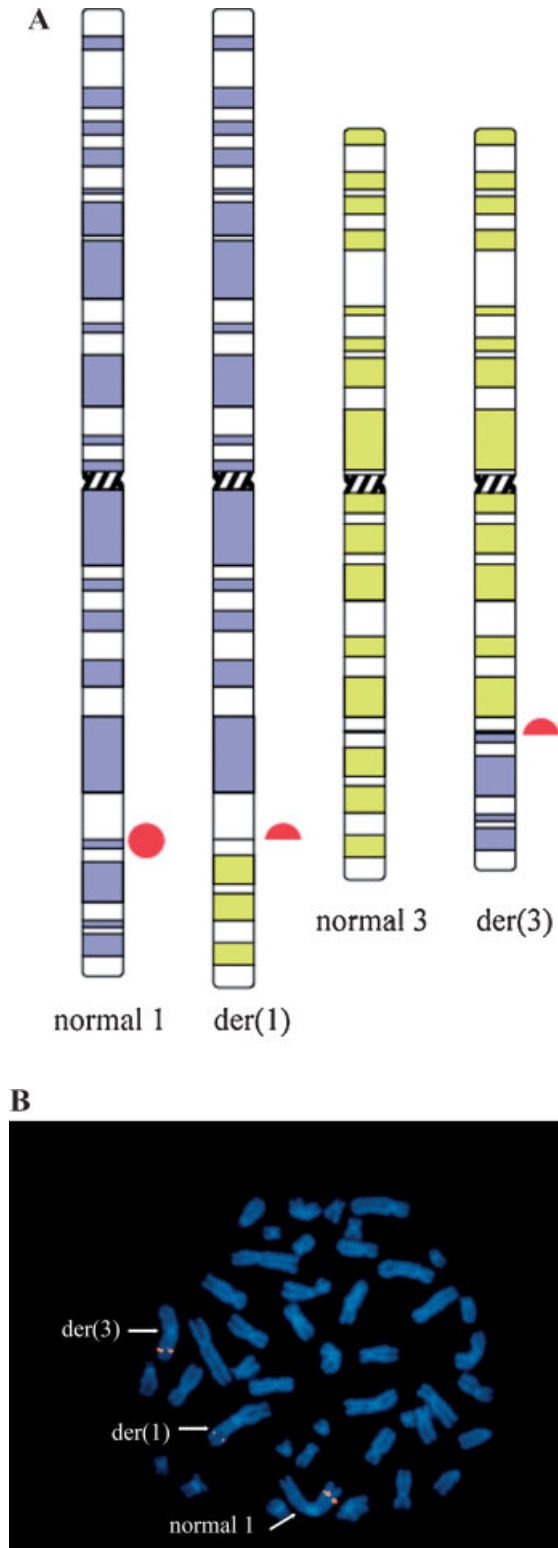


Fig. 1. Metaphase FISH with RP11-168F20 showing hybridization to the normal chromosome 1, der(1) and der(3).

release phase of presynaptic vesicle exocytosis, thus serving a critical function in neurotransmission [Perin et al., 1990; Geppert et al., 1994]. An essential step in neuronal communication is release of neurotransmitters from presynaptic vesicles, a process mediated by specific synaptotagmins and associated proteins [Davis et al., 1999; Fernandez-Chacon et al., 2001; Bai et al., 2004].

Study of murine and rat Syt proteins has provided additional insight into synaptotagmin function and plasticity. For certain synaptotagmins (e.g., *Syt1* and *Syt7*), alternative splicing has been demonstrated [Perin et al., 1990; Sugita et al., 2001]. *Syt7* encodes a thyroid hormone-responsive protein that may play a role in thyroid-regulated development of the central nervous system [Thompson, 1996]. Interestingly, *Syt4* has been implicated in hippocampal-based memory mediated by calcium-dependent glutamate release [Zhang et al., 2004]. Though *Syt4*^{-/-} mice lack any gross phenotypic abnormalities, hippocampal-dependent learning and memory, as well as fine motor coordination, appear to be compromised.

Murine Syt14 can undergo calcium-independent oligomerization and phospholipid association, and structural analyses suggest a role in membrane transport that is conserved across several phyla [Fukuda, 2003]. Additional diversity might be achieved through differential *SYT14* splicing [Craxton, 2004]. Because brain tissue was unavailable from DGAP128, it was not feasible to assess *SYT14* expression in this individual. However, a Northern blot analysis of normal human brain demonstrated expression of a 2.9 kb *SYT14* mRNA (Refseq: NM_153262) in adult amygdala, caudate nucleus, corpus callosum, hippocampus, and thalamus. Interestingly, microarray data (U133A and GNF1H chips, UCSC Genome Browser) indicate *SYT14* expression in the atrioventricular node, trachea, salivary gland, ovary and fetal liver, suggesting a function for *SYT14* that may not be limited to brain [Fukuda, 2003; Su et al., 2004].

Given the critical role of certain synaptotagmins in neurotransmission and the demonstrated expression of *SYT14* in human brain, the possibility that disruption of one *SYT14* allele contributes to the neurodevelopmental abnormalities in DGAP128 is intriguing. Potential mechanisms by which the translocation might contribute to the DGAP128 phenotype include haploinsufficiency or unmasking of a recessive allele. Furthermore, the mother's large OFC suggests that macrocephaly in DGAP128 likely reflects a familial trait that is unrelated to the chromosomal translocation. Those maternal relatives with seizures reportedly did not have absence seizures, as documented for DGAP128, and so would not exclude a role for disruption of a neurodevelopmentally critical gene by the t(1;3) in pathogenesis of the DGAP128 seizure phenotype. Currently

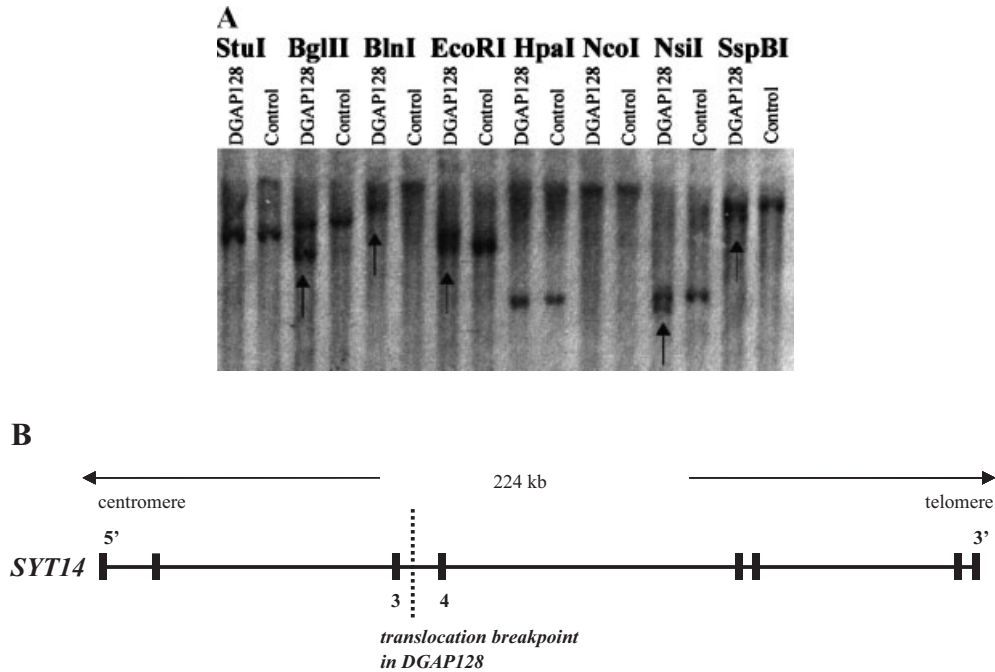


Fig. 2. Molecular characterization of the 1q32 breakpoint. **A:** Southern blot analysis of DGAP128. A probe generated from RP11-168F20 identified aberrant bands (black arrows) in DGAP128 and indicate that the breakpoint occurred within the respective restriction fragments. **B:** Schematic diagram of *SYT14*. The 8 exons span a genomic region of 224 kb (black vertical bars). The 1q32 breakpoint maps within intron 3 (dotted line).

there are no reports of individuals with 1q32 deletions known to include *SYT14*, with similar or overlapping phenotypes. Similarly, though a murine knockout model for *SYT14* does not currently exist, it will be an important step in further assessing the role of *SYT14* in this neurodevelopmental phenotype. Finally, the lack of any known genes disrupted by the 3q25.2 breakpoint does not exclude possible disruption of a regulatory element or of position effect. As such, our data provide an impetus for further studies exploring the role of *SYT14* in neurodevelopment and for seeking potential disruption of this locus in other individuals with similar or overlapping phenotypes.

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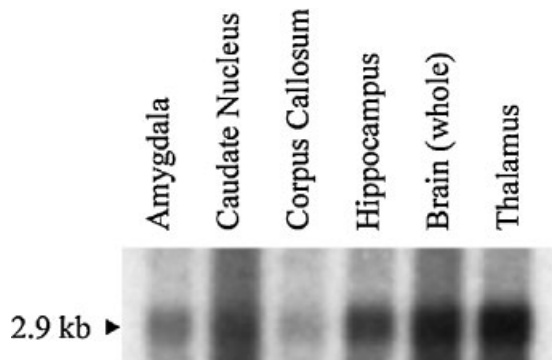


Fig. 3. *SYT14* expression in human adult brain. A multiple tissue northern blot, hybridized with a probe derived from the 3’UTR of *SYT14*, identifies a 2.9 kb transcript (NM_153262) expressed in several regions of the brain.

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