Refinement of the Genomic Structure of *STX1A* and Mutation Analysis in Nondeletion Williams Syndrome Patients

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Abstract

Williams syndrome (WS) is a contiguous gene deletion disorder in which the commonly deleted region contains at least 17 genes. One of these genes, Syntaxin 1A (*STX1A*), codes for a protein that is highly expressed in the nervous system and is essential for the docking of synaptic vesicles with the presynaptic plasma membrane. In this study, we refine the complete genomic structure of the human *STX1A* gene by direct sequencing and primer walking of bacterial artificial chromosome (BAC) clones and show that *STX1A* contains at least 10 exons and 9 introns. The length of exons range from 27 bp to 138 bp and all splice sites conform to the GT-AG rule. Investigation of the *STX1A* gene sequence in five WS patients without detectable deletions did not identify any point mutations. Although the regulatory elements that control *STX1A* transcription were not examined, these results do not support a role for *STX1A* in the WS phenotype.

Keywords

Williams syndrome; *STX1A*; genomic structure; point mutation

INTRODUCTION

Williams syndrome (WS) is a neurodevelopmental disorder characterized by mental disability with unique cognitive and personality profiles, distinctive facial features, vascular and connective tissue abnormalities, and infantile hypercalcemia [Francke, 1999; Osborne, 1999]. WS is a contiguous gene deletion disorder involving at least 17 genes within a commonly deleted region on chromosome 7q11.23. Hemizygosity for elastin has been shown to be responsible for several features of WS, such as supravalvular aortic stenosis and possibly the connective tissue abnormalities [Ewart et al., 1993; Li et al., 1997; Tassabehji et

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al., 1997; Tassabehji et al., 1998], but no other gene has been definitely implicated in the WS phenotype.

Syntaxin (STX1A) was originally described as a neuronal-specific protein that co-immunoprecipitated with the synaptic vesicle membrane protein synaptotagmin [Bennett et al., 1992; Yoshida et al., 1992]. Described as an integral membrane protein, STX1A was localized to the plasma membrane of axons and presynaptic terminals in the nervous system [Inoue et al., 1992; Koh et al., 1993; Sollner et al., 1993]. STX1A is found almost exclusively in neurons, where it is a component of the pre-assembled vesicle docking and vesicle fusion machinery which is essential for neurotransmitter release [Bennett et al., 1993]. We had previously mapped human *STX1A* to within the common WS deletion region [Osborne et al., 1997] and determined that it contained at least 7 exons [Osborne et al., 1997, GenBank Accession numbers U87310–U87314], unlike the *Drosophila* Syntaxin 1a, which has only a single exon [Schulze et al., 1995]. Because *STX1A* is a neuronally expressed gene that is located within the common WS deletion region [Nakayama et al., 1997], we hypothesized that STX1A may have a role in certain behavioral characteristics observed in individuals with WS [Osborne et al., 1997].

The identification of individuals with features of WS and mutations in a specific gene from the common WS deletion region would firmly implicate that gene in the pathophysiology of WS. To facilitate the evaluation of the role of *STX1A* in WS, we have refined its genomic structure and used this information to develop oligonucleotide primers suitable for mutation analysis. We have then proceeded to analyze the *STX1A* gene in several individuals who have clinical WS but do not harbor the common deletion.

MATERIALS AND METHODS

Genomic Screening

In order to determine the complete genomic structure of *STX1A*, a human genomic bacterial artificial chromosome (BAC) library (Research Genetics) was screened by PCR using primers designed from the published *STX1A* exon 5 DNA sequence (Table I, newly designated exon 8 primer set). Two positive human BAC clones, 137N23 and 137N19, were isolated. Fluorescence in situ hybridization (FISH) was performed to confirm that the clones mapped to 7q11.23 (data not shown). To confirm the exon-intron boundaries of the *STX1A* gene, PCR primers were designed using the previously published sequence as a starting point. Products from both BACs and total human genomic DNA were amplified according to a published protocol [Wu et al., 1998], sized by agarose gel electrophoresis, purified by GeneClean Kit (Bio 101), and sequenced directly in the Baylor College of Medicine sequencing facility.

WS Patients

In order to attempt to implicate *STX1A* in the features of WS, we chose five unrelated individuals with clinical features of WS and no identifiable deletion in the WS critical region for *STX1A* mutation screening. All individuals met the clinical criteria for WS by history and physical findings, but had normal karyotypes. Four of the individuals had been reported

previously [Nickerson et al., 1995; Wu et al., 1998] and the other was newly enrolled for the present study (Fig. 1). This individual was a 14-year-old Hispanic male with several clinical features suggestive of WS, including developmental delay, mild hyperactivity and extreme sociability. His head circumference, height, and weight are at the 5th, 35th, and 25th centile, respectively, and he has characteristic facial features, with prominent periorbital tissue, anteverted nares, prominent lips with a long philtrum, and a large-appearing mouth. A geneticist gave him the clinical diagnosis of WS when he was a toddler but he was not seen in the Texas Children's Genetics Clinic until age 12 years. A cardiac echocardiogram, serum calcium, a chromosome analysis, and a FISH study with a probe containing the elastin gene were normal.

Prior to entering the study, molecular analyses of the WS region was performed on all individuals, using 10 polymorphic markers within the common deletion region and FISH analyses with a *ELN-5'* probe, *LIMK1* probe [Wu et al., 1998] and a *STX1A* probe [Osborne et al., 1997]. No deletions were detected in any of the five study subjects.

Point Mutation Analysis

Each *STX1A* exon, including the exon-intron boundaries, was amplified from genomic DNA from the five WS individuals and from unrelated controls. The products were purified and sequenced as described above.

RESULTS AND DISCUSSION

Complete Genomic Structure

Based on the published sequence, amplification products of the expected size were found for exons 2, 5, 6, and 7, but larger products were obtained for exons 1, 3, and 4. Using primer walking in the BAC clones to define the boundaries of exons 1 (STX1A-OF AAG GAC CGA ACC CAG GAG; STX1A-OR CTC CTG GGT TCG GTC CTT), 3 (STX1A-gap3 CTA TCC ACC TTC CCA CAT CC) and 4 (STX1A-gap4 CAC CAC ACA GCG TCA CAG), we found that each could be separated into two distinct exons. Primers were subsequently designed for the amplification of the newly defined exons and are shown in Table I. Ten exons, ranging in size from 27 to 138 bp, and the complete genomic sequence, spanning exons 4 to 7, have been elucidated. Exon-intron boundaries for exons 1, 2, 4, 5, 6, and 7 have been newly defined with GenBank accession numbers: AF297001, AF297002, and AF297003, and the boundaries for exons 3, 8, 9, and 10 were described previously [accession number: U87311, U87314, U87315, Osborne et al., 1997]. Table II summarizes the splice junctions and surrounding sequence of the newly defined *STX1A* exons. All splice sites conform to the GT-AG rule [Green, 1991]. The 864 bp coding sequence of this newly constructed genomic region agrees with that originally published by Zhang et al. [1995] (GenBank L37792) and with that previously published by us [Osborne et al., 1997]. Newly deposited sequence in the high throughput database (accession number NT_023557) agrees with our sequence. However, there is a gap in this deposited sequence around exon 1. Our study has completed the genomic structure for *STX1A*.

Point Mutation Analysis

This analysis uncovered two common variants in the exon 3 coding region that do not change the amino acid sequence (Table III). The two polymorphic nucleotides were 150C/T and 204T/C (Table III). Aside from these polymorphisms, no other sequence variations were identified in any of the individuals tested, either control or WS. Thus, no point mutations were found in these five nondeleted WS patients.

CONCLUSIONS

Our previous studies showed that the majority of WS carry a similar sized deletion spanning an identical number of genes [Wu et al., 1998]. This renders the characterization of genes that contribute to specific features difficult. A small number of individuals with smaller deletions of the region have been identified, including two children with apparently classical WS [Botta et al., 1999]. These two patients, plus the results presented here, suggest that *STX1A* does not play a major role in the cognitive and behavioral features of WS. Although these results do not support a role of *STX1A* in the WS phenotype, analysis of the promoter region or continued investigation of other non-deletion patients may help to elucidate any potential role of *STX1A* in WS. Additionally, it is possible that these nondeletion patients represent phenocopies of a different etiology. Therefore, the possible effects of hemizygosity of *STX1A* on the WS phenotype still need to be examined further.

Acknowledgments

The authors thank Dr. L. Potocki (Baylor College of Medicine) for supplying Figure 1 and the Patients for their Participation in this study.

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Fig. 1.
Patient 5. Note periorbital fullness.

TABLE I

PCR Primers for Mutation Analysis of the Human *STX1A* Gene (5' to 3')

Exon	Forward	Reverse	Annealing (°C)
1	GGCGCGTTGGCGCTGTCG	GTCGGCATGGGGGGCTCAC	68
2	AGGTGGGTCTGTACATACGTCC	CCTCAAAACGGTTCATTCGTTG	60
3	GCAGCCCTAATTGGGCC	CATGAAGGCTGCAGAGGTCC	56
4	CTTCCCAGAGCCTCCAC	GCTGCTGAGTGAATGGGC	60
5	GTGGATAGATGCTGGGTG	ACCGACCCAGAGACTCAG	60
6	GCTCCTCCGCTGACCTGA	CTCGGGCCCCTAACCTAAG	57
7	CAATGCTGCTGCTGAAT	CCACCTTTGCCGCTGACA	57
8	TCTGCCCTGCCCTTTCAGCAAGC	GAGGTAGTGCAGCCCTG	58
9	CGAGCCCCAACCCAACCGTGAG	CGATGCCCAGGATCACACAGCA	56
10	CCCCGAATGTCAGTCTCAGC	GCTGCCTCCCTCTGCCTCTTCC	56

TABLE II

STX1A Splice Junctions and Surrounding Sequence*

Splice acceptor	Flanking exon sequence (5' → 3')	Splice donor
ATG AAG GAC	ATG AAG GAC	CTC CGC ACG
GCC AAG GAC	GCC AAG GAC	TTT GAG CAG
GTTG GAG GAG	GTTG GAG GAG	CCC GAT GAG A
AG ACG AAG	AG ACG AAG	AAG TTA AAG A
GC ATC GAG	GC ATC GAG	ACA CAG
CAC TCC ACG	CAC TCC ACG	CTG GAG ATC A
CC GGC AGG	CC GGC AGG	GCC TCT GGG
ATC ATC ATG	ATC ATC ATG	GAG AGC CAG
GGA GAG ATG	GGA GAG ATG	GCG CGC CGG
AAG AAA ATC	AAG AAA ATC	ATC TTC GCC

*. Bold indicates splice sites.

TABLE III

Polymorphisms Observed in *STX1A*

Position	Nucleotide	Control chromosomes	Non-deletion WS chromosomes	Deletion WS chromosomes
150	C	27	10	22
150	T	5	0	4
204	C	17	4	11
204	T	15	6	15

Controls, n = 16; non-deletion WS individuals, n = 5; WS individuals, n = 26.