Title	Hemizygous deletion of the syntaxin 1A gene in individuals with Williams syndrome: Letters to the editor
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Letters to the Editor

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Hemizygous Deletion of the Syntaxin 1A Gene in Individuals with Williams Syndrome

To the Editor:

Williams syndrome (WS) is a microdeletion syndrome caused by the haploinsufficiency of genes at 7q11.23. The incidence of the disease is ~1/20,000–1/50,000 (Greenberg 1990), and it usually occurs in a sporadic manner, although rare cases of autosomal dominant transmission have been described (Morris et al. 1993; Sadler et al. 1993). WS is associated with a recognizable facies, cardiovascular disease, hypercalcemia, dental abnormalities, growth deficiency, generally mild mental retardation, and a distinct behavior profile (Pober and Dykens 1996). The behavioral profile is characterized by impaired cognition, hyperreactivity, sensory-integration dysfunction, delayed expressive and receptive language skills, and multiple developmental motor disabilities affecting balance, strength, coordination, and motor planning (Dilts et al. 1990). In addition, ~70% of WS individuals also suffer from attention-deficit and hyperactivity disorder (ADHD), and there is a high incidence of anxiety and simple phobias (Bellugi et al. 1990; Dilts et al. 1990; Pober and Dykens 1996).

Hemizygosity for the elastin gene, *ELN,* is observed in most WS individuals, resulting in supravalvular aortic stenosis (SVAS), which is a common cardiovascular lesion found in WS (Ewart et al. 1993; Nickerson et al. 1995). The gene *LIMK1* has been mapped near *ELN,* and it also is deleted in the vast majority of WS individuals (Frangiskakis et al. 1996; Osborne et al. 1996; Tassabehji et al. 1996). *LIMK1* has been proposed to have a role in proper visuospatial constructive cognition, since it and *ELN* are the only genes that have been found to be hemizygously deleted in affected individuals in a family with SVAS and impaired visuospatial cognition (but no other features of WS) (Frangiskakis et al. 1996).

Our chromosomal walking experiments using cosmid and P1-derived artificial chromosomes (PACs) have resulted in the assembly of a set of overlapping clones encompassing ~1,100 kb of DNA. At present, the contig extends 230 kb centromeric and 830 kb telomeric of *ELN.* The microsatellite marker *D7S1870,* which elsewhere had been shown to be deleted in 75%, but not all, of WS individuals (Gilbert-Dussardier et al. 1995), was found to reside in PAC clone 54h15, suggesting that the distal breakpoint of the WS commonly deleted region has been identified (fig. 1). The location of all of the known genes within the region are shown in figure 1; these include *ELN* (Ewart et al. 1993), *LIMK1* (Frangiskakis et al. 1996; Osborne et al. 1996), the replication factor C subunit 2 (*RFC2*) gene (Osborne et al. 1996; Peoples et al. 1996), a gene containing an RNA-binding motif (*WSCR1*) (Osborne et al. 1996), a gene with similarity to restin (*WSCR4*) (Osborne et al. 1996), the human *frizzled* homologue (*FZD3*) (Wang et al. 1997), and three transcription units (*WSCR2, WSCR3,* and *WSCR5*) (Osborne et al. 1996) predicting proteins of unknown function (fig. 1). Besides *ELN* and *LIMK1,* the other genes have no obvious connection with the WS phenotype. We have now identified the syntaxin 1A gene (*STX1A*) within the common WS deletion.

In order to characterize the centromeric end of the clone contig, DNA sequencing and FISH experiments have been completed. DNA sequencing of cosmid clone 16g10 (fig. 1) led to the identification of the neuronal-specific syntaxin gene (*STX1A*). Using the published cDNA sequence (GenBank L37792) as a template, our experiments confirmed that *STX1A* encoded a gene of 864 nucleotides, and sequencing of genomic DNA allowed seven exons, all <200 bp in length, and their boundaries to be determined (the size range of the introns was 93–3,200 bp) (fig. 1). The gene was found to

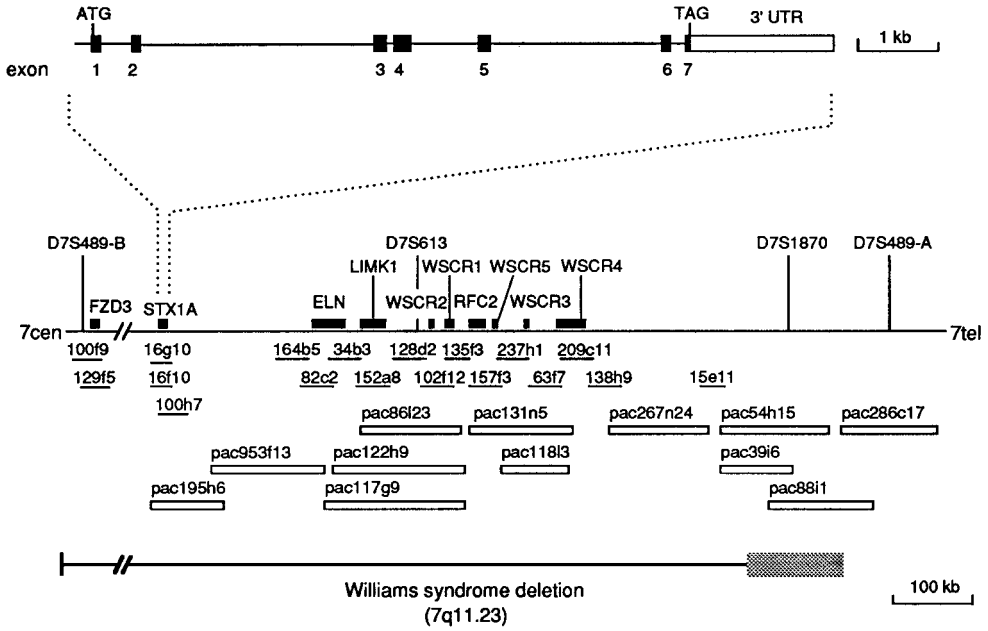

Figure 1 Map of WS deletion region at 7q11.23, showing position of *STX1A* in relation to genes and polymorphic markers (*D7S489, D7S613,* and *D7S1870*). *D7S489* is located in three places within the region (the three loci have been called "*D7S489-A,*" "*D7S489-B,*" and "*D7S489-C*"). The *D7S489-B* locus closest to *STX1A* is commonly deleted in WS individuals, whereas the most centromeric (*D7S489-C;* not shown) and most telomeric (*D7S489-A*) loci flank the commonly deleted interval. *FZD3,* which has been shown to be deleted in WS (Wang et al. 1997), is known to be linked to *D7S489-B* through cosmid clones 100f9 and 129f5, but these clones are not yet linked to the contig containing *STX1A*. The distal boundary of the deleted region varies and is shown here as a gray-shaded box at the bottom right. The PAC clones are from the Roswell Park Cancer Institute collection (kindly provided by Dr. P. de Jong), and the cosmid clones are from the Lawrence Livermore National Laboratory chromosome 7–specific library. Our decision to use these cloning systems was based on the observation that the genomic region shown here is extremely unstable when cloned in YACs. The genomic structure of *STX1A* is shown above (the orientation of the gene along the chromosome is unknown), with exons as blackened boxes, introns as lines, and the 3′ UTR as an unblackened box. No information on the 5′ UTR was available from the published cDNA sequence. Intron-exon boundaries were determined by genomic sequencing of a cosmid clone (cos16g10) containing the entire STX1A gene within a single 25-kb *Eco*RI restriction fragment (GenBank U87310–U87315).

span 9 kb. The gene structure is different from that of the *Drosophila* homologue, which is contained in a single exon (Schulze et al. 1995). In addition, we have confirmed that the recently isolated cDNA clone STX1C (Jagadish et al. 1997) is an alternatively spliced form of *STX1A*. It appears that this novel isoform is generated by the utilization of an alternative splice-donor site within intron 5, 91 nucleotides upstream of exon 6. FISH analysis with cosmid 16g10, which contains the entire sequence of *STX1A,* indicated that the gene was hemizygously deleted in all 20 typical WS individuals examined. These FISH data, in combination with the identification of clones in the contig containing *D7S1870,* indicate that the minimal size of the WS commonly deleted region is 950 kb.

Syntaxin 1A is an integral membrane protein found almost exclusively in neurons, and it is part of the preassembled vesicle-docking and vesicle-fusion machinery at the presynaptic plasma membrane (Bennett et al. 1992). It has been shown to bind other members of the presynaptic machinery, such as synaptobrevin (VAMP) and SNAP-25, and also N-type and P/Q-type Ca^{2+} channels, where it is believed to stabilize channel inactivation (Rettig et al. 1996; Sheng et al. 1996). To date, most experiments have been performed in the mouse or rat, but *STX1A* has also been isolated from human fetal brain cDNA libraries, suggesting that it is expressed in this tissue (Zhang et al. 1995; Jagadish et al. 1997). A series of allelic *stx1a* loss-of-function mutants has been generated in *Drosophila* (Schulze et al. 1995). Although complete-loss-of-function mutants had normal neuromuscular architecture, Ca^{2+}-dependent neurotransmitter release was abolished, resulting in both a lack of endogenous synaptic transmission and embryonic lethality. Mutants retaining 30% of syntaxin 1a protein showed no defects in neuronal number, size, or position, but thay had both an absence of endogenous synaptic transmission at the neuromuscular junction and an 80% decrease in evoked transmissions. The mutants also died before hatching but could be rescued by restoration of syntaxin 1a protein to control levels. These observations indicate that syntaxin 1a is essential for neurotransmitter release and suggest that hemizygosity for this gene might explain some aspects of WS.

The dramatic effects of reducing syntaxin 1a protein levels in *Drosophila* suggest that a 50% reduction in WS individuals could also evoke a phenotype. A clue to what that specific phenotype might resemble comes from studies of a naturally occurring mouse model of ADHD (named "coloboma"), which arises because of the semidominant deletion of several genes (Hess et al. 1992). Complementation experiments with one of these genes (named "*Snap*"), which encodes the Snap-25 protein, rescued the hyperactive phenotype in this ADHD mouse model (Hess et al. 1996). As mentioned above, SNAP-25 associates with the syntaxin 1A protein at the presynaptic membrane, which suggests that hemizygosity of *STX1A* could also give rise to hyperactivity and, possibly, other behavior profiles observed in WS patients. Further animal studies, as well as genetic analysis of individuals with psychiatric disorders (e.g., ADHD, Tourette syndrome, and obsessive-compulsive disorder), will help to clarify any contribution that *STX1A* might make to WS and other behavioral phenotypes.

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References

Bellugi U, Bihrle A, Jernigan T, Trauner D, Doherty S (1990) Neuropsychological, neurological and neuroanatomical profile of Williams syndrome. Am J Med Genet 6:115–125

Bennett MK, Calakos N, Scheller RH (1992) Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. Science 257:255–259

Dilts CV, Morris CA, Leonard CO (1990) Hypothesis for development of a behavioral phenotype in Williams syndrome. Am J Med Genet Suppl 6:126–131

Ewart AK, Morris CA, Atkinson D, Jin W, Sternes K, Spallone P, Stock AD, et al (1993) Hemizygosity at the elastin locus in a developmental disorder, Williams syndrome. Nat Genet 5:11–16

Frangiskakis JM, Ewart AK, Morris CA, Mervis CB, Bertrand J, Robinson BF, Klein BP, et al (1996) *LIM-kinase 1* hemizygosity implicated in impaired visuospatial constructive cognition. Cell 86:59–69

Gilbert-Dussardier B, Bonneau D, Gigarel N, Le Merrer M, Bonnet D, Philip N, Serville F, et al (1995) A novel microsatellite DNA marker at locus D7S1870 detects hemizygosity in 75% of patients with Williams syndrome. Am J Hum Genet 56:542–544

Greenberg F (1990) Williams syndrome professional symposium. Am J Med Genet Suppl 6:85–88

Hess EJ, Collins KA, Wilson MC (1996) Mouse model of hyperkinesis implicates SNAP-25 in behavioural regulation. J Neurosci 16:3104–3111

Hess EJ, Jinnah HA, Kozak CA, Wilson MC (1992) Spontaneous locomotor hyperactivity in a mouse mutant with a deletion including the *Snap* gene on chromosome 2. J Neurosci 12:2865–2874

Jagadish MN, Tellam JT, Macaulay SL, Gough KH, James DE, Ward CW (1997) Novel isoform of syntaxin 1 is expressed in mammalian cells. Biochem J 321:151–156

Morris CA, Thomas IT, Greenberg F (1993) Williams syndrome: autosomal dominant inheritance. Am J Med Genet 47:478–481

Nickerson E, Greenberg F, Keating MT, McCaskill C, Shaffer LG (1995) Deletions of the elastin gene at 7q11.23 occur in ~90% of patients with Williams syndrome. Am J Hum Genet 56:1156–1161

Osborne LR, Martindale D, Scherer SW, Shi X-M, Huizenga J, Heng HQ, Costa T, et al (1996) Identification of genes from a 500 kb region at 7q11.23 that is commonly deleted in Williams syndrome. Genomics 36:328–336

Peoples R, Perez-Jurado L, Wang Y-K, Kaplan P, Francke U (1996) The gene for replication factor C subunit 2 (RFC2) is within the 7q11.23 Williams syndrome deletion. Am J Hum Genet 58:1370–1373

Pober BR, Dykens EM (1996) Williams syndrome: an overview of medical, cognitive, and behavioral features. Child Adolesc Psychiatr Clin North Am 5:929–943

Rettig J, Sheng Z-H, Kim DK, Hodson CD, Snutch TP, Catterall WA (1996) Isoform-specific interaction of the α_{1A} subunits of brain Ca^{2+} channels with the presynaptic proteins syntaxin and SNAP-25. Proc Natl Acad Sci USA 93:7363–7368

Sadler LS, Robinson LK, Verdaasdonk KR, Gingell R (1993) The Williams syndrome: evidence for possible autosomal dominant inheritance. Am J Med Genet 47:486–470

Schulze KL, Broadie K, Perin MS, Bellen HJ (1995) Genetic and electrophysiological studies of *Drosophila* syntaxin-1A demonstrate its role in nonneuronal secretion and neurotransmission. Cell 80:311–320

Sheng Z-H, Rettig J, Cook T, Catterall WA (1996) Calcium-dependent interaction of N-type calcium channels with the synaptic core complex. Nature 379:451–454

Tassabehji M, Metcalfe K, Fergusson WD, Carette MJA, Dore JK, Donnai D, Read AP, et al (1996) LIM-kinase deleted in Williams syndrome. Nat Genet 13:272–273

Wang YK, Samos CH, Peoples R, Perez-Jurado LA, Nusse R, Francke U (1997) A novel human homologue of the *Drosophila frizzled* wnt receptor gene binds wingless protein and is in the Williams syndrome delrtion at 7q11.23. Hum Mol Genet 6:465–472

Zhang R, Maksymowych AB, Simpson LL (1995) Cloning and

sequence analysis of a cDNA encoding human syntaxin 1A, a polypeptide essential for exocytosis. Gene 159:293–294

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Further Evidence Suggesting the Presence of a Locus, on Human Chromosome 5q31-q33, Influencing the Intensity of Infection with *Schistosoma mansoni*

To the Editor:

Recently, Marquet et al. (1996) described a linkage study of the susceptibility to intestinal schistosomiasis, one of the most important worm infestations in humans. The individuals included in that study mostly showed a low-susceptibility phenotype, and a major gene (*SM1*) controlling the intensity of infection was found (Abel et al. 1991). Now, *SM1* has been localized to chromosome 5q31-q33 (Marquet et al. 1996). The study area was a hyperendemic schistosomiasis focus (infection with *Schistosoma mansoni*) in Brazil (Dessein et al. 1988).

We conducted a study in a newly emerged, epidemic focus of intestinal schistosomiasis, in northern Senegal, where the human population has been shown to be heavily infected with *S. mansoni,* as measured by the numbers of excreted worm eggs and the circulating antigen levels (Stelma et al. 1993). Recruitment, epidemiological study design, and parasitological methods have been described in detail elsewhere (Stelma et al. 1993). A total of 154 subjects were included. They belonged to 15 extended pedigrees, which contained 33 nuclear families. The distribution of logarithmically transformed egg counts ($\log_{10}$[egg count + 1]) is shown in figure 1. By use of the procedure described by Abel et al. (1991), the egg counts, after having been logarithmically transformed ($\log_{10}$[egg count + 1]), were adjusted for sex, age, and exposure, as estimated by water-contact measurements.

The resulting values differ, in distribution, from those calculated for the population in the Brazilian focus, in which a minority of individuals formed a distinct subgroup with relatively high egg counts (Abel et al. 1991). The Senegalese subjects, who have been exposed for no longer than 7 years (Stelma et al. 1993), present with a more balanced distribution of infection intensities, and no such subgroup is discernible. Complex segregation analysis of the nuclear families was performed, by use of complete selection as the mode of ascertainment and by use of the POINTER program (Lalouel and Morton 1981; Morton et al. 1983). The analysis revealed additional differences between the two populations. In Brazil, the familial distribution of phenotypes corresponded well to the Mendelian segregation of a codominant major gene (Abel et al. 1991). For the Senegalese sample, models of codominant (degree of dominance $d = .96$, displacement $t = 2.09$, and allele frequency $q = .47$), dominant ($d = 1$, $t = 2.04$, and $q = .46$), or recessive ($d = 0$, $t = 0.89$, and $q = .16$) modes of inheritance all were rejected ($P < .005$, in all cases); however, nontransmission of a major gene ($d = .55$, $t = 3.03$, and $q = .97$; transmission probability [τ] of $\tau 1 = \tau 2 = \tau 3 = .22$) was not rejected ($P > .14$). All the P values mentioned above are given for comparison with a general non-Mendelian model. A mixed Mendelian codominant model ($d = 1$, $t = 2.07$, $q = .45$, and heritability H in children = .03) does not have a significantly higher likelihood than a Mendelian codominant model without a multifactorial component ($d = .96$, $t = 2.09$, and $q = .47$); the corresponding P value for this comparison is .69. When the mixed Mendelian codominant model is compared with models allowing for non-Mendelian transmission probabilities, both the models assuming equal transmission probabilities and those allowing for free estimates of the transmission probabilities clearly have higher likelihoods ($P < .005$, in both cases) than the mixed Mendelian codominant model.

When a dominant mode of inheritance was assumed—which, as determined from the results of the segregation analysis, was the best-fitting model for our data—no significant LOD score was obtained by use of FASTLINK 2.0 (maximum LOD score of 0.322, with

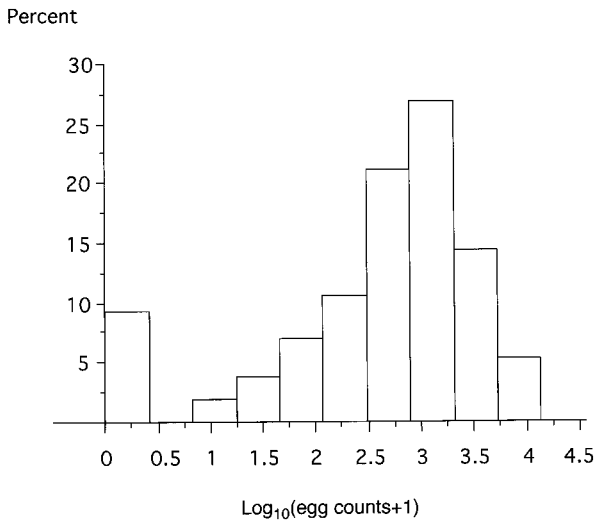

Figure 1 Distribution of egg counts, as $\log_{10}$(egg count + 1), among 154 Senegalese subjects recently exposed to *S. mansoni* transmission