

ORIGINAL INVESTIGATION

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Linkage mapping of serotonin transporter protein gene SLC6A4 on chromosome 17

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Abstract Abnormalities in monoamine metabolism, including serotonin metabolism, have been implicated in the pathophysiology of affective disorders, schizophrenia, suicide, and other psychiatric disorders. Serotonin transporter protein (SERT) allows neurons to retrieve serotonin that has been released into a synapse. SERT is a site of action for several drugs with CNS effects, including both therapeutic agents (e.g., antidepressants) and drugs of abuse (e.g., cocaine). This gene had previously been physically mapped to chromosome 17. We used a PCR product corresponding to the 3' untranslated region of the gene as a probe to identify restriction fragment length polymorphism (RFLP), which we then used to establish that the SLC6A4, genetic locus for SERT, is near 17q12 and probably flanked by D17S58 and D17S73 (a location consistent with observed crossovers). These data should be useful for linkage studies of neuropsychiatric disorders.

Introduction

The serotonin transporter protein is the cellular reuptake site for serotonin (5HT) and a site of action for many tricyclic antidepressant medications (such as imipramine) and certain drugs of abuse. In the brain, after 5HT is released into the synapse it is taken up into the presynaptic neuron at the serotonin transporter protein; this terminates the synaptic actions of 5HT and recycles it into the neurotransmitter pool. Abnormalities in serotonin transporter protein density have been observed in schizophrenia

(Joyce et al. 1993). Neurotransmitter reuptake sites (including also the norepinephrine transporter protein and the dopamine transporter protein) are logical candidate genes for susceptibility to psychiatric illness. We have previously (Gelernter et al. 1993) mapped the norepinephrine transporter protein to chromosome 16q21. We describe here linkage mapping of the serotonin transporter protein gene (gene symbol SLC6A4, for "solute carrier family 6 (neurotransporter, serotonin), member 4"), which was cloned in 1991 (Blakely et al. 1991; Hoffman et al. 1991) and previously assigned to chromosome 17, most likely to band 17q11.2, by in situ hybridization (Ramamoorthy et al. 1993). Our linkage results confirm the initial mapping of SLC6A4 and place it in the linkage map of proximal 17q.

Materials and methods

We first identified a polymorphism at the SLC6A4 locus. We amplified bases 1947–2509 of the 3' untranslated region (sequence from Ramamoorthy et al. 1993) using the polymerase chain reaction (PCR). We were unable to identify a single-strand conformation polymorphism (SSCP) in this PCR product, so we used it as a probe to screen for a restriction fragment length polymorphism (RFLP); we identified a *Pst*I RFLP (heterozygosity, 0.43) (Gelernter and Freimer 1994). We then typed the polymorphism, using standard RFLP methods, on a series of extended reference pedigrees (two segregating for Tourette's syndrome, known as TS Canadian, Branch "C" and TS Oregon; one segregating bipolar affective disorder, OOA110; and one segregating ocular albinism; altogether 249 individuals were genotyped for SLC6A4. These kindreds are described in Gelernter et al. 1990.)

Pairwise linkage analyses were carried out using LIPED (Ott 1976). These results were used to identify markers likely to be useful for multipoint analysis and to define a realistic sex recombination ratio.

For multipoint analysis, we used a newly parallelized version of Linkmap (original program, Lathrop et al. 1985; new version, Carriero and Gelernter 1995. The parallelization was accomplished using C-Linda (Gelernter 1985).

Results

Pairwise linkage analysis provided strong evidence for linkage to markers located on proximal chromosome 17q

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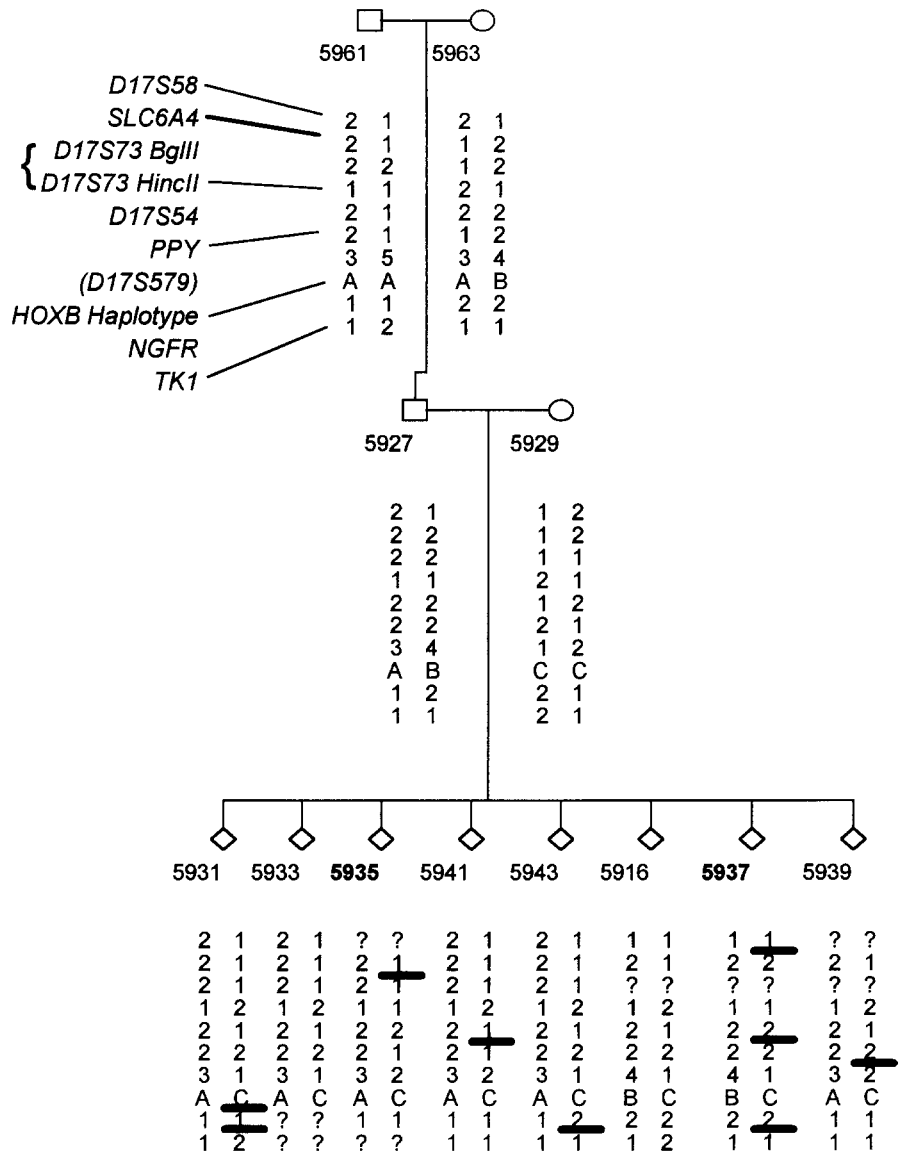
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Table 1 Pairwise lod scores by LIPED between SLC6A4 and several other chromosome-17 markers from four non-CEPH extended pedigrees

| Marker (location) ^a | Probe | Enzyme | Z _{max} | Θ _{max} (M, F) | Z _{max} (M = F) | Θ _{max} (M = F) |
|--------------------------------|--------|--------------------|------------------|-------------------------|--------------------------|--------------------------|
| D17S58 (17p11.2-cen)* | EW301 | TaqI | 11.4 | 0,0.10 | 10.5 | 0.05 |
| D17Z1 (cen)** | 17H5 | EcoRI | 5.2 | 0.05,0.10 | 5.1 | 0.10 |
| D17S73 (1711.2-q12)** | EW207 | (Hap) ^b | 10.7 | 0,0.05 | 10.5 | 0.05 |
| D17S98 (17q12-q21.1)** | EW122 | MspI | 16.4 | 0,0 | 16.4 | 0 |
| D17S47 (17q12-q24)*** | LEW110 | BglII | 3.3 | 0.10,0.30 | 3.0 | 0.20 |
| NGFR (17q21-q22)* | PE51 | HincII | 9.6 | 0,0.20 | 8.7 | 0.05 |
| EPB3 (17q21-qter)* | p242 | PstI | 7.2 | 0,0.20 | 6.5 | 0.05 |

^a Marker locations are from:
 * Fain (1992); ** Fain et al. (1991); *** Barker et al. (1989)
^b Haplotype: BglII and HindIII systems

Fig. 1 Chromosome-17 marker genotypes in a subset of OOA110 (see text). Thick lines indicate approximate points of crossovers. SLC6A4 segregates with proximal markers in a maternal crossover gamete transmitted to 5953 and with distal markers in a maternal crossover gamete transmitted to 5937. This provides evidence for localization of SLC6A4 between D17S58 and D17S73. The position shown for D17S79 is that which was most consistent with these data



(Table 1). These analyses also suggested that the female:male recombination ratio exceeds 2:1 for this region. Of the markers studied, D17S58, D17Z1, and D17S73 are chromosome-17 reference markers (Solomon et al. 1993).

Crossovers observed in OOA110 place SLC6A4 distal to D17S58 (individual 5937) and proximal to D17S73 (individual 5935; Fig. 1).

We used multipoint analyses for finer localization of the SLC6A4 locus (Fig. 2). A five-point analysis moving SLC6A4 across a fixed map (markers D17S58, D17Z1, D17S73, and NGFR) gave the best resolution. (The > 220 individual computations needed for this problem were completed in 1582 using the newly parallelized version of Link-map running on 10 IBM RS6000/340s and 6 IBM RS6000/560s, whereas the original version could not compute

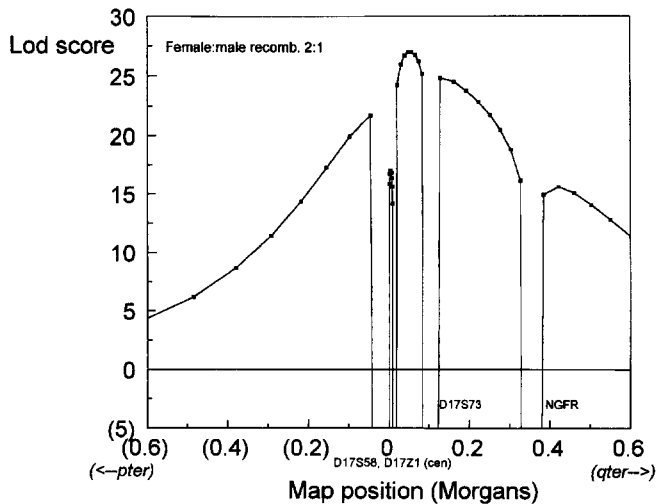


Fig. 2 Serotonin transporter protein locus (SLC6A4) moved across a fixed map of chromosome-17 markers. Female: male recombination is 2:1; male map position given in Morgans; Haldane map function

even a single point in 90 min of CPU time on a VAX 7610. A single computation, or point, refers to a lod score for one pedigree at one map position). The maximum lod score of > 27 occurred in the interval between D17Z1 and D17S73. The interval containing the next highest lod score was D17S73-NGFR, with a maximum lod score of 24.9. Placement of SLC6A4 flanked by D17S73 and D17Z1 is favored over all of the other intervals (except D17S73-NGFR) by > 3 lod units of support. An observed crossover (described above) is most consistent with placement of *HTT* in the D17Z1-D17S73 interval, rather than the D17S73-NGFR interval. These observations, together with that of a lod score of 16.4 with D17S98, located at 17q12-q22.1 (Fain et al. 1991) at 0 cM, supports localization of SLC6A4 at 17q12, the only point of overlap between the D17Z1-D17S73 interval and D17S98.

Discussion

We have confirmed the genetic mapping of SLC6A4 to proximal 17q, using linkage analysis. The most likely location according to our analysis is at 17q12, flanked by D17Z1 and D17S73, but possibly distal to that interval.

Alterations in various neurotransmitter levels are found in many neuropsychiatric disorders. Mutations in the neurotransmitter transporter proteins could predispose to psychiatric illness by either increasing or decreasing synaptic availability of a particular neurotransmitter. A mutation at SLC6A4 specifically could result in either excessive or insufficient amounts of serotonin (5HT) in the synaptic cleft. 5HT system dysfunction is implicated in schizophrenia (van Kammen and Gelernter 1987), affective disorders, violence and impulsivity, suicide, and Tourette's syndrome. Tricyclic antidepressants, which are used to treat some of these disorders (especially affective disorders), also bind SERT. SLC6A4 is therefore a candi-

date gene for causation of the several psychiatric illnesses that may involve abnormalities in serotonergic neurotransmission or may respond to tricyclic antidepressant medications. We have excluded genetic linkage between SLC6A4 and bipolar affective disorder in one large kindred, Old Order Amish pedigree 110, both indirectly based on our earlier exclusion of the entire region using several RFLPs (Pakstis et al. 1991a) and directly using this SLC6A4 polymorphism (Gelernter et al., unpublished data). Since this region of 17q has also already been excluded for linkage with TS (Pakstis et al. 1991b) using several closely linked markers, SLC6A4 can also safely be considered to be excluded as the major causative locus in those specific families for TS.

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