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SHORT REPORT

Refined mapping of the human serotonin transporter (*SLC6A4*) gene within 17q11 adjacent to the *CPD* and *NF1* genes

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The *SLC6A4* gene encodes the serotonin transporter, the target of an important class of antidepressant drugs (serotonin selective reuptake inhibitors). Polymorphisms in the *SLC6A4* gene have been reported to be associated with susceptibility to depression and other psychiatric disorders. We have constructed a 1 Mb YAC and PAC contig which harbours both the *SLC6A4* and the carboxypeptidase D (*CPD*) genes. The order of loci within the contig was cen–D17S975–D17S1549–24R–D17S1294–*SLC6A4*–28L–(*CPD*, D17S2009, D17S2004)–D17S2120–ter. Both genes were deleted in one of 17 neurofibromatosis type 1 (NF1) patients carrying submicroscopic *NF1* contiguous gene deletions. *European Journal of Human Genetics* (2000) **8**, 75–78.

Keywords: serotonin transporter; *CPD*; *NF1*; YAC contig

Introduction

The actions of the neurotransmitter serotonin (5-HT) are terminated by reuptake via a Na^+-dependent serotonin transporter (SERT) encoded by the *SLC6A4* gene. The SERT is the target for an important class of antidepressant drugs (the serotonin selective reuptake inhibitors) and also of certain drugs of abuse including 3,4-Methylenedioxymethamphetamine (MDMA or 'ecstasy'). Polymorphisms in the *SLC6A4* gene have been reported to be associated with depression and other psychiatric disorders and to influence personality traits (reviewed by Lesch[1]). To facilitate studies of the molecular basis of these associations, we have constructed a map of a 1 Mb region encompassing the human *SLC6A4* and *CPD* genes. We have evaluated the contribution of these genes to the phenotype observed in patients with *NF1* contiguous gene deletions.

Materials and methods

Construction and analyses of physical contig

Positive clones were identified by hybridisation of [α-^{32}P]dCTP-labelled SLC6A4 cDNA to the RPC-1 PAC library and YAC libraries from ICRF (35D8, 132C6 and 49A9) and CEPH (704F1, 782E2 and 765D1). Yeast DNA was prepared by combined methods of Schedl *et al*[2] and Bellis *et al*.[3] Pulsed-field gel electrophoresis was performed in 0.5 × TBE buffer at 6 V/cm for 24 h at 14°C with 60 s switch time and gels were blotted overnight onto Appligene Positive Membrane. Filters were sequentially hybridized with: (i) a 854 bp Pst I fragment of *SLC6A4* cDNA (bp 785–1639, GenBank accession no. L05568); (ii) a 2.3 kb Eco RI–Pvu II fragment of pBR322; (iii) 28L, a 3.2 kb Not I – Eco RI fragment of PAC 50G6, about 40 kb 5' of the *SLC6A4* gene adjacent to the T7 promoter sequence from the vector pCYPAC2N; (iv) 24R, a 8.4 kb Not I – Hind III fragment of PAC 50G6, about 15 kb 3' of the *SLC6A4* coding sequence adjacent to the SP6 promoter sequence of the vector. YACs were sized by hybridisation to pBR322 plasmid DNA, using the endogenous yeast chromosomes as size references. PCR was performed for 30 or 35 cycles with primers listed in Table 1.

Patient and somatic cell hybrid lines

Patient UWA106-3 is hemizygous for a microdeletion of about 1–1.5 Mb that spans the entire *NF1* gene.[4] The human/rodent somatic hybrid cell lines UWA106-3-#36 and UWA106-3-#41 harbour, respectively, the deleted and non-deleted chromosomes 17 from this patient.[4] Fluorescence *in*

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situ hybridisation (FISH) was carried out with biotin-11-dATP-labelled 50G6 PAC DNA.[5]

Results

Characterisation of the *SLC6A4* gene and PAC clones

Long-range PCR of human genomic DNA with six pairs of primers indicated that the *SLC6A4* gene spanned approximately 40 kb (Table 1). One PAC (50G6) contained the entire coding sequence of the *SLC6A4* gene. Southern blot analysis of NotI digested 50G6 DNA revealed a 40 kb fragment that hybridised to end clone 28L, a 55 kb fragment that hybridised to end clone 24R, and a 16 kb fragment from the PAC vector (data not shown). We concluded that 50G6 contains the entire *SLC6A4* gene flanked by about 40 kb of 5' sequence and about 15 kb of 3' sequence.

Construction of *SLC6A4* contig

The contig depicted in Figure 1 was assembled by analysis of 6 YACs and the PAC 50G6 for the presence of 6 STS markers and for the PAC end clones 24R and 28L. Five of the six YACs harboured the entire *SLC6A4* gene, whereas 704F1 lacked the 5' untranslated region and first exon. These data oriented the 3' end of the *SLC6A4* gene towards the chromosome 17 centromere. D17S1294 mapped < 15 kb centromeric to the 3' end of the *SLC6A4* gene, since it was present in 704F1 but not in 49A9. All clones carried the 10 repeat allele of *SLC6A4* intron 2 polymorphism;[6] YAC clones from ICRF library carried the short allele of the promoter polymorphism,[7] while those from the CEPH library carried the long allele.

Carboxypeptidase D gene maps telomeric to *SLC6A4*

A 163 kb genomic sequence from human chromosome 17 (GenBank AC006050) encodes the entire *CPD* gene, with D17S2004 and D17S2009 within the predicted 3' untranslated region. The presence of the *CPD* gene in YACs 35D8, 765D1 and 49A9 was confirmed using both forward primers of D17S2009 and D17S2004 and with two pairs of primers (cpd6740F/cpd6858R, and cpd1065F/cpd1768R: Table 1) from *CPD* cDNA. We concluded that the *CPD* gene lies telomeric to the *SLC6A4* gene within the contig.

Hemizygotic deletion of the *SLC6A4* and *CPD* genes in a NF1 patient

Our YAC contig does not contain the *NF1* gene, since none of the YAC clones yielded products with PCR primer pairs from exons 1, 27a and 49.2 of the gene.[8] However, because the deletion in UWA106-3 included loci predicted to map nearer the centromere than *SLC6A4* , we subjected metaphase chromosomes prepared from immortalised lymphoblasts of this patient to FISH using 50G6 as the probe. Only one hybridisation signal was observed, confirming the hemizygosity of the SLC6A4 locus (Figure 2a). PCR of DNA from rodent/human somatic hybrid cell lines carrying either the deleted or non-deleted chromosomes 17 of UWA106-3 indicated that the deletion encompassed the *CPD* and *SLC6A4* genes and D17S2120 but not D17S1294 or D17S1549 (Figure 2b). Therefore, the centromeric breakpoint of the deletion in this patient lies in a < 15 kb region between exon 14 of the *SLC6A4* gene and D17S1294 (arrow head, Figure 1b). Neither the *SLC6A4* nor the *CPD* gene was deleted in 16 additional *NF1* microdeletion patients[4] examined.

Discussion

We report here the construction of a 1 Mb contig encompassing the *SLC6A4* and *CPD* genes and eight marker loci. Our results confirm the localisation of *SLC6A4*[9,10] and *CPD*[11] to chromosome 17q11.2–17q12. The *SLC6A4* and *CPD* genes were deleted in 1/17 *NF1* microdeletion patients tested, but our data suggest that the majority of such patients will not be haploinsufficient for SERT and *CPD*. The identification of eight marker loci flanking the *SLC6A4* and *CPD* genes will facilitate future studies of their role in susceptibility to developmental and psychiatric disorders. This contig will also provide a centromeric anchor for chromosomal walking towards the *NF1* gene, which may lead to the discovery of other genes contiguous to *NF1*. Functional analysis of these

Table 1 PCR primers for *SLC6A4* and *CPD* genes

Forward primers (5′–3′)		*Reverse primers (5′–3′)*		*Product*	*Size*
31013	CACCTAACCCCTAATGTCCCTACT	31014	GGACTGAGCTGGACAACCAC	SLC6A4 5-HTTLPR	458/502 bp
1AP(F)	GCGTCTAGGTGGCACCAGAATC	1AP(R)	TCGCGCTTGTGTTCCCAGCTAC	SLC6A4 Exon 1a	545 bp
43084	CCTGCGAGGAGGCGAGGAGG	43085	AACTCCTCTCGGTGACTAATCG	SLC6A4 Exon 1–Intron 1a	10 kb
44771	CTAGTGACTGACATTGCCTGG	44772	TGTCCAGTCTATCTGCACATG	SLC6A4 Exon 1b	824 bp
43088	GCCTGGCGTTGCCGCTCTGAATGC	43089	TAGCAGCAGCAGTGAGCAGTTACC	SLC6A4 Intron 1a–Exon 2	3.5 kb
26373	ACTAACCAGCAGGATGGAGACG	26374	TAGAGTGCCGTGTGTCATCTCC	SLC6A4 Exon 2	199 bp
18564	GTCAGTATCACAGGCTGCGAG	18565	TGTTCCTAGTCTTACGCCAGTG	SLC6A4 Intron 2 VNTR	249/266/299 bp
E2F	ACTAACCAGCAGGATGGAGACG	2B	TTAGACCGGTGGATCTGCAG	SLC6A4 Exons 2–5	5 kb
54073	TGGCAAGGTGAGGAAGGCTCTGG	54074	CCACCTCAGACACATCTTCATTCC	SLC6A4 Exons 5–8	4 kb
5A	ATGAAGATGTGTCTGAGGTGG	5B	ACAGCGACTGCTTCGATCAG	SLC6A4 Exons 8–11	3.8 kb
6A	TACGTGGTGAAGCTGCTGGA	P3	GAGGAGGAGGTTGTGGAGAAGCC	SLC6A4 Exons 11–14	>12 kb
26375	AGTTCTGATGAGGCACGC	26376	TTCATCACCTCCATCCACATCC	SLC6A4 Exon 14	223 bp
60703	ATCACATTAGAACTGTCTTGTTGC	60704	AGGTATTCTATGAGGTTCAACAGC	CPD 1065F + CPD 1768R	2.7 kb
60705	TTATGTAGTTCAGTAAGATGTGCC	60706	GCAAGTATTCTTCAACTGGATAGG	CPD 6740F + CPD 6858R	118 bp

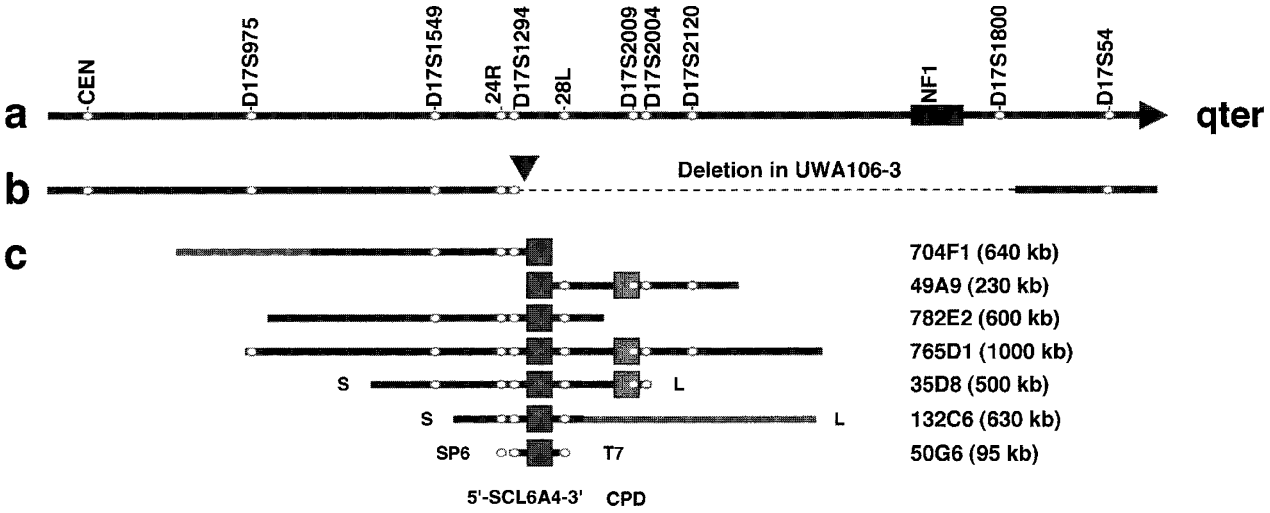

Figure 1 Mapping of the SLC6A4 and CPD genes to 17q11 in a YAC/PAC contig. **a** Physical map of the region between 17cen and D17S54; **b** the extent of the deletion (– – –) in the patient UWA106-3; **c** positions of the SLC6A4 (■) and CPD ▨ genes, STS markers and other probes (circles) in 6 YACs and one PAC (50G6) clone. Putative regions of chimaerism in YACs 704F1 and 132C6 are shaded. The orientations of the long (L) and short (S) YAC vector arms are indicated where known. Orientation of the insert in PAC 50G6 is shown relative to the SP6 and T7 promoters of the vector.

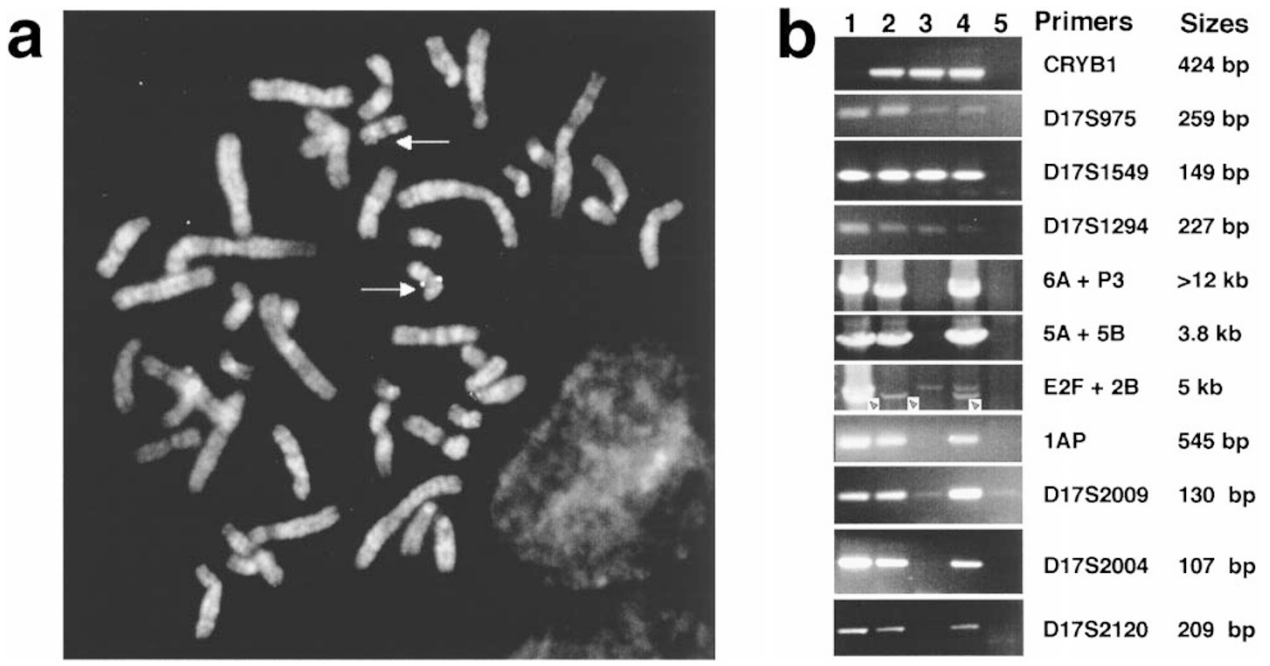

Figure 2 **a** Hemizygosity of the SLC6A4 in patient UWA106-3. Metaphase chromosome preparations from lymphoblasts of UWA106-3 were hybridised with 50G6 DNA. The two chromosomes 17 are arrowed: only one of them hybridised to the SLC6A4 probe; **b** Localisation by PCR of the centromeric breakpoint of the deletion in patient UWA106-3. DNA templates were as follows: (lane 1) YAC 765D1; (lane 2) genomic DNA from patient UWA106-3; (lane 3) cell line UWA106-3-#36 carrying the deleted chromosome 17; (lane 4) cell line UWA106-3-#41 carrying the non-deleted chromosome 17; (line 5) the Chinese hamster RJK cell line. The primers (see Table 1 and the Genome Database [http://gdbwww.gdb.org]) and sizes of PCR products are indicated. The specific PCR product of the E2F/2B primer pair is arrowed.

genes should play a role in the understanding of neurofibromatosis as well as other developmental/psychiatric disorders.

Acknowledgements

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