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A YAC contig encompassing the 11q14.3 breakpoint of a translocation associated with schizophrenia, and including the tyrosinase gene

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A balanced translocation t(1,11)(q42.1–q14.3) associated with schizophrenia in a Scottish family has been described (St Clair et al. 1990). It is assumed that a gene(s) responsible for the susceptibility to the disease is located in 1q42.1 or in 11q14.3 and is either broken by the translocation or has its expression altered by a position effect. A further possibility is that schizophrenia is caused by a mutation in a gene localized near the breakpoint and is, therefore, coinherited with the translocation. As a first stage towards positionally cloning the translocation breakpoints, a minilibrary of YACs belonging to the 1q41–q42 and 11q14–q22 regions was assembled (Petit et al. 1995) by screening a YAC library with microdissection clones from the area of interest (Muir et al. 1995). A contig spanning the region immediately proximal to the 11q14.3 breakpoint has been constructed and a YAC crossing the translocation breakpoint identified (Evans et al. 1995a). The aim of the present study was to integrate the genetic and physical maps of the 11q14.3 region and to extend the contig map on the centromeric side of the 11q14.3 breakpoint.

Microsatellite markers *D11S931,* known to map proximal (Evans et al. 1995a), and *D11S1342, D11S1358,* and *D11S1332,* known to map distal (Couillin et al. 1994 and our own data) to the breakpoint, were used to isolate YACs from the CEPH library (Chumakov et al. 1995); 15 YACs were identified (Fig. 1). STS content analysis of the YACs identified here, combined with the mapping of the microsatellite markers with respect to the 11q14.3 translocation breakpoint (Couillin et al. 1994; Evans et al. 1995a), allowed us to establish the following order of markers: CEN–*D11S931–D11S1342–D11S1358–D11S1332*–TEL.

All the YACs were then tested for the presence of the microdissection (MD) clones known to map closest to the breakpoint (Evans et al. 1995a). The results obtained allowed us to both localize the MDs with respect to the microsatellite markers and evaluate the extent of overlap between the YACs (Fig. 1). End fragments isolated from some of the YACs (Arveiler and Porteous 1991) helped to characterize the contig further. Some YACs were found to have small internal deletions (Fig. 1). This may be owing to the presence of repetitive sequences in the *D11S1358–D11S1132* interval that render the inserts unstable in *Saccharomyces cerevisiae.*

Two recently isolated cDNAs (Devlon et al. 1997), one corresponding to an α-tubulin pseudogene, the other one (C1) being of unknown function, were placed on the contig (Fig. 1).

Several YACs were shown to cross the translocation breakpoint since they contained both markers located on the proximal (*D11S931*) and distal (*D11S1342, D11S1358,* and *D11S1332*) sides of it. This was verified by fluorescent in situ hybridization (FISH) for most of the YACs. A representative example is provided in Fig. 2 for YAC 943C10: hybridization signals are displayed by both the derivative 1 and derivative 11 Chrs of a t(1,11) translocation carrier.

A 3-Mb contig spanning mainly the region immediately distal to the translocation breakpoint was established in a previous study (Evans et al. 1995a). One YAC (D0485) spanned the breakpoint. Based upon a number of markers analyzed in common, this contig and the one presented here could be integrated. YACs D0485, 14IA11, 37GE11, D11155, and A04135 from the previous study could thus be aligned with the new contig, as shown in Fig. 1 (lower part). A cosmid containing the right end of clone D0485 was identified. Two repeat-free subclones (D0485-R) were isolated and placed on the new contig.

None of the YACs of our contig contained the tyrosinase gene (*TYR*), which was known to map on the centromeric side of the 11q14.3 breakpoint (Evans et al. 1995b). Screening of the ICI (Anand et al. 1990) and CEPH YAC libraries by PCR and hybridization of high-density replica filters allowed us to identify seven clones containing the *TYR* gene: 4GD5, 12GE1, 17DE5, 24EG8, 32HA11, 974A4, and 982D2. The YACs were ordered by testing for the presence or absence of exons 1–5 of *TYR* and of end fragments isolated from some of the YACs (Fig. 1). Locus *D11S3987* (WI-9075) was found in the Massachusetts Institute of Technology and Genbank databases to be located immediately 3′ to exon 5 of *TYR,* and to be present in YAC 928D4. Overlap between YAC 928D4 and several YACs from the *D11S931–D11S1332* contig was demonstrated by hybridization of the left end of 928D4 and confirmed by comparing Alu-PCR fingerprints from 928D4 and 943C10 (data not shown). 928D4 did not, however, extend to the translocation breakpoint.

Since the *TYR* gene was located at the centromeric end of YAC 928D4 (940 kb) and the α-tubulin gene lay about 250 kb centromeric from the 11q14.3 breakpoint (Devon et al. 1997), we estimated that the distance between *TYR* and the 11q14.3 was at most 1.2 Mb.

The physical map presented in Fig. 1 combines the information resulting from the work presented here and from the contig previously published by Evans and associates (1995a). Novelties with regard to the previous map are that it contains YACs from three commonly used libraries (CEPH, ICI, and ICRF), that it integrates the genetic and physical maps by placing microsatellite markers of the region on the YACs, that it places a new set of markers (microdissection clones) on the CEPH Mega YACs, and that it extends towards the *TYR* gene. The extent of the overlaps between clones, and the size of the deletions found in some of the clones, were estimated by taking into account the size of the YACs and their STS content, such that the map fits all the data available.

The *TYR* gene was recently shown to be expressed in mouse and human brain (Tief et al. 1996; Xu et al. 1997). Although the function of tyrosinase in the brain remains unknown, it might be involved in the metabolism of catecholamines, and TYR was thus considered as a possible candidate for the predisposition to schizo-

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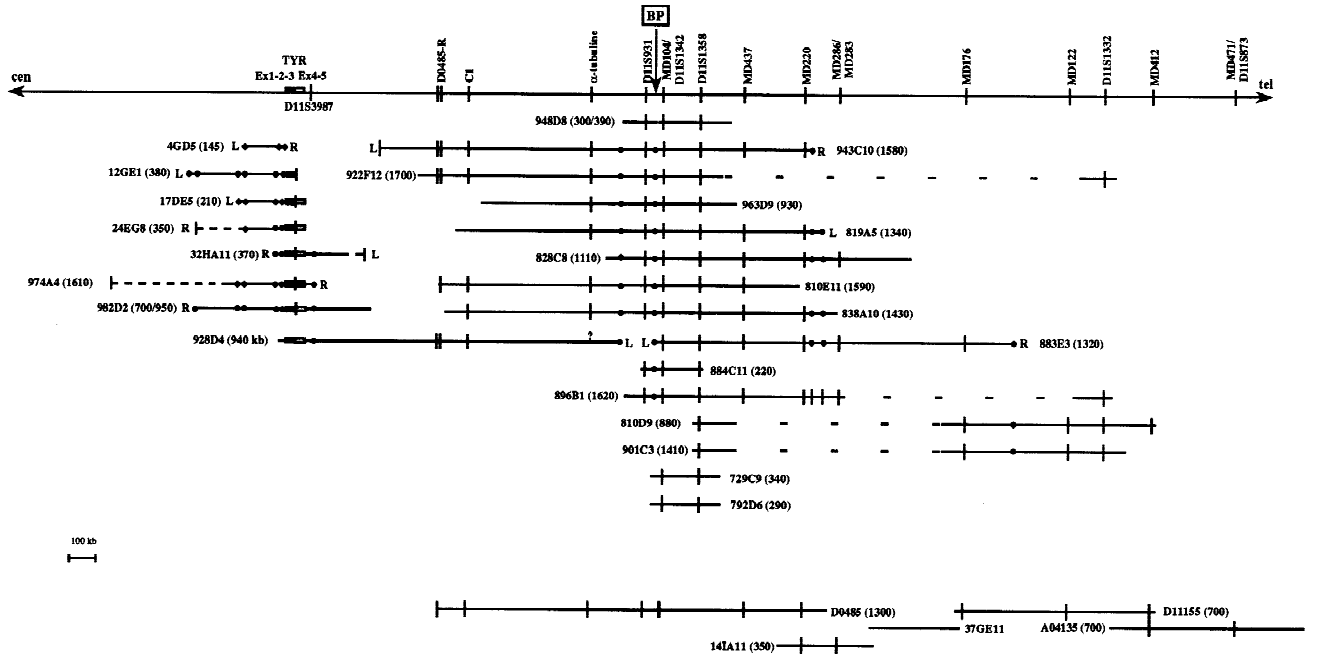

Fig. 1. YAC contig map of region 11q14.3. Markers that have been tested are represented in the upper part of the figure. Presence of a marker in a YAC is indicated by a vertical bar. YAC sizes in kb are indicated in parentheses. Right and left end fragments isolated from some of the YACs are indicated by R and L respectively, and their presence in YACs is indicated by a plain circle. Dashed lines indicate a probable chimerism, and dotted lines a deletion. YACs at the bottom of the figure are from Evans et al. (1995a).

phrenia. The five exons of the gene were amplified separately (see Fig. 3A for position of the primers) from a mouse hybrid cell line (MIS39.8) containing the derivative Chr 11 (Fletcher et al. 1993), as well as from one patient with the t(1,11) translocation, and sequenced. A single nucleotide change (T to C) was observed in exon 4 at nucleotide 2725 of the *TYR* gene sequence (Giebel et al. 1991) in a heterozygous state (Fig. 3B). It should be noted that the derivative Chr 11 present in the MIS39.8 somatic hybrid cell line harbors not only the *TYR* gene in 11q14.3, but also the *TYR* pseudogene that is located in 11p11 (Giebel et al. 1991). It remains unclear at the moment whether the nucleotide change occurred in the *TYR* gene or in its pseudogene, and whether it corresponds to a polymorphism. However, this nucleotide change will not have any effect on the protein sequence, since both the wild-type (AAT) and variant (AAC) sequences encode an Asn at codon 435.

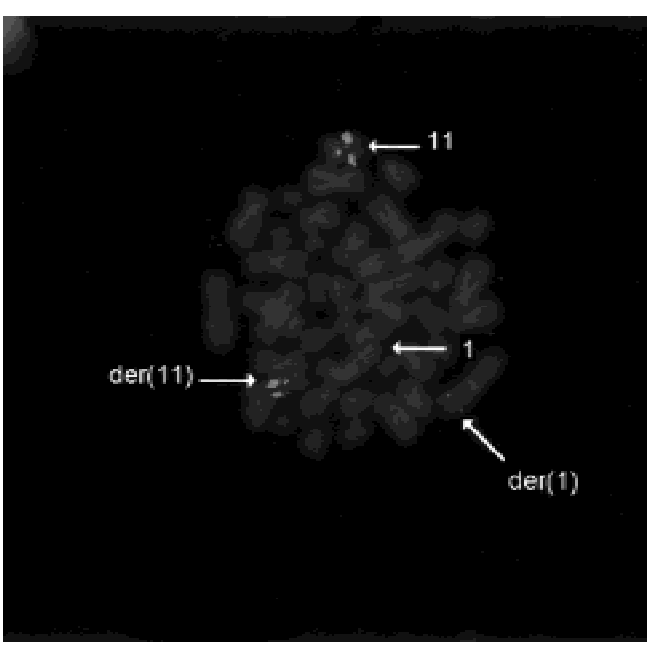

Fig. 2. FISH analysis of YAC 943C10 onto metaphase chromosomes from a t(1,11) translocation carrier. The probe was obtained by Alu-PCR, and the FISH was performed as described by Taine et al. (1998). Normal and derivative Chrs 1 and 11 are indicated.

The contig map presented here constitutes a valuable tool to establish a transcription map of cytogenetic band 11q14.3 and will help in identifying new genes involved in human diseases mapped to that region, such as Papillon-Lefevre syndrome (Fischer et al. 1997; Laass et al. 1997), and will hopefully lead to the identification of gene(s) implicated in the development of schizophrenia. Such a map is also useful to analyze the extent of regions of synteny between different species. Region 11q14–q21 is well known to be syntenic to part of region E1–E2 of mouse Chr 7. This region contains a locus for predisposition to atherosclerosis (ath3; Stewart Phillips et al. 1989) and the locus responsible for the taupe phenotype that is associated with generalized depigmentation of hair (Fielder 1952), the genes of which remain to be identified (data retrieved from the Mouse Genome Database, MGD, Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, Me., World Wide Web URL: http://www.informatics.jax.org/; August 1998). It is, therefore, possible that a gene responsible for susceptibility to atherosclerosis maps to 11q14.3 and is present in the region covered by our contig. Also Fzd4, a homolog of the *Drosophila melanogaster* frizzled gene (Wang et al. 1996), was localized immediately distal to *Tyr* on mouse Chr 7 and may represent a candidate for developmental defects mapped to that region in the mouse or to human 11q14–q21.

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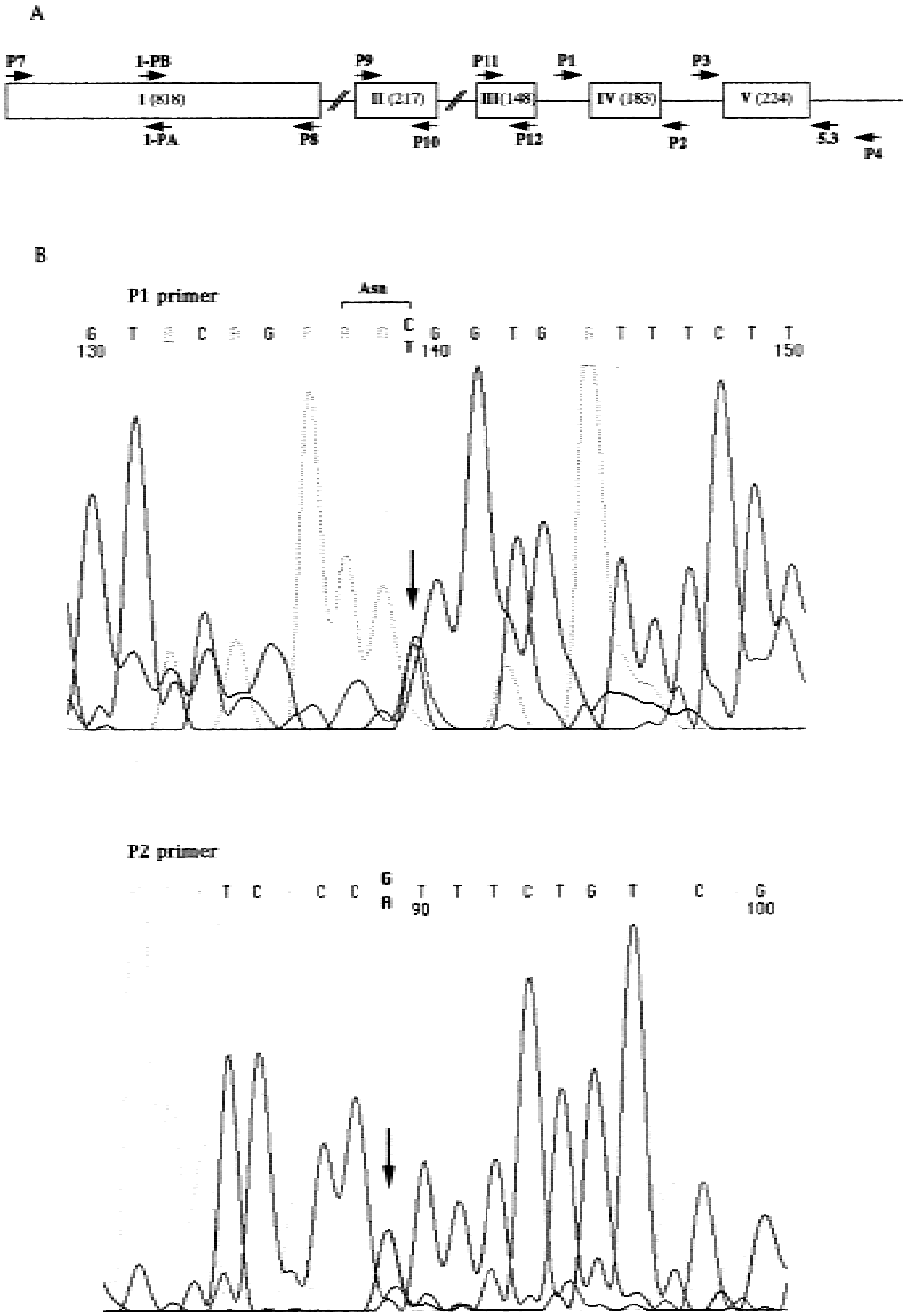

Fig. 3. Sequence analysis of the tyrosinase (*TYR*) gene coding region. **A)** Schematic representation of the *TYR* gene. Exons 1–5 are indicated as open boxes (sizes in bp are in parentheses). Primers P1–P12 were from Giebel et al. (1991). PCR was performed using Taq DNA polymerase from Promega Corporation (Madison, Wisc.) Optimal PCR parameters and cycling conditions for each pair of primers were: exon 1 (annealing 60°C; 1 mM $MgCl_2$; 5% formamide); exon 2 (annealing 50°C; 1 mM $MgCl_2$); exon 3 (annealing 55°C; 1 mM $MgCl_2$); exon 4 (annealing 50°C; 1 mM $MgCl_2$; 5% formamide); exon 5 (annealing 55°C; 1.5 mM $MgCl_2$). Internal primers 1-PA (5′TGGGGATGACATAG-TCTGAGC3)′ and 1-PB (5′CTTTAG-CAAAGCATACCATCAGC3′) were used for exon 1 sequencing. Exon 5 was sequenced with primers P3 and 5.3 (5′AGGCTTTTTGGCCCTACTCT3′). **B)** Sequence data obtained for the t(1,11)-associated exon 4 with the P1 and P2 primers. The arrows indicate the nucleotide change on both strands.

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