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Linkage of tyrosine hydroxylase to four other markers on the short arm of chromosome 11

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ABSTRACT

Tyrosine hydroxylase is the rate-limiting enzyme in catecholamine synthesis; the gene has previously been cloned and localised to the short arm of chromosome 11. Because of the interest in tyrosine hydroxylase as a candidate gene for manic-depressive psychosis and other affective disorders, we carried out family studies to determine the linkage of tyrosine hydroxylase with insulin, β-globin, D11S12 and Harvey-ras 1, members of a linkage group which has previously been localised to 11p. Using DNA from the Centre d'Etude du Polymorphisme Humain (CEPH) and from two large British pedigrees, we show that tyrosine hydroxylase is closely linked to these four loci ($\hat{z}$=7.36, θ=0.04 for linkage to insulin) and suggest a gene order based on multipoint mapping.

INTRODUCTION

Tyrosine hydroxylase (TH) is an enzyme involved in amino acid and neurotransmitter metabolism and is the rate-limiting enzyme in catecholamine synthesis. The TH gene has been cloned by Mallet (1), assigned to chromosome 11 by analysis of human/mouse cell hybrids (2), and regionally localised to 11p15 by in situ hybridisation (3).

Catecholamine metabolism is thought to play a central role in the pathogenesis of manic-depressive psychosis and other affective disorders (4), and mutations in the TH gene may therefore be important in the aetiology of these disorders. Moreover, recent evidence in favour of close genetic linkage between the cellular oncogene Harvey-ras 1 (HRAS1), which has also been assigned to 11p, and an autosomal dominant form of manic-depressive psychosis (bipolar affective disorder) has stimulated further interest in TH as a candidate gene for this type of psychosis (5,6). In order to be able to examine the role of TH in this group of psychiatric disorders, we set out to identify polymorphic sequences within the TH locus. In addition, the genetic relationships between TH and other 11p markers were investigated so that multipoint analysis may be used to increase the information provided by linkage studies of pedigrees in which the disorders are segregating. The genes for HRAS1, β-globin and insulin have been localised to 11p15 (7) and a

gene order for these loci and for the arbitrary locus D11S12 has been established by a genetic linkage study of a large number of extended multigeneration families (8). Here we describe linkage studies to map the TH locus to the existing linkage group in the 11p15 region. A human cDNA clone of TH (Ty7), isolated and characterised by Mallet et al (1) was used to detect two restriction fragment length polymorphisms and these were used in linkage studies as part of the CEPH collaboration.

MATERIALS AND METHODS

Subjects: Samples from 20 families were made available by CEPH. EBV-transformed perennial cell lines have been established for members of large French, Venezuelan and Utah pedigrees by this Paris-based organisation. DNA extracted from these lines has been distributed to laboratories participating in the collaboration, along with software for data handling and linkage analysis. Data for other 11p markers were available only for certain Utah families (13 in all) which had been studied by White et al (8); these families were included in the present study. Two British pedigrees (9) (NDM-A, NDM-C) were also typed for the insulin (10) and HRAS1 (11) polymorphisms.
Methods: A panel of 19 random unrelated Northern Europeans was used to screen for restriction fragment length polymorphisms. DNA was digested with 19 restriction enzymes, electrophoresed through a 0.8% agarose gel and transferred to nylon filters (Hybond-N) by Southern blotting (12). The filters were hybridised with a 1.5 kilobase (kb) human cDNA tyrosine hydroxylase probe (Ty7) as described by Mallet (1), labelled with ^{32}P by nick-translation and washed at 65° for one hour in 0.1 X SSC.
Linkage analysis: Polymorphism data for the β-globin, D11S12, insulin and HRAS1 markers in 13 CEPH families were obtained from CEPH courtesy of Dr M. Leppert, (Howard Hughes Medical Insitute, Salt Lake City) and Dr H. Cann. Linkage studies in the CEPH families were based on marker data on all 5 loci and on 3 loci (insulin, HRAS1, TH) in NDM-A and NDM-C. Linkage analysis was carried out using the computer program package "LINKAGE" (13) on an IBM-AT computer.

RESULTS

Two restriction fragment length polymorphisms were detected using the TH cDNA probe. Three alleles were observed in EcoRI digests (A, B, C) and two in BglII digests (+,-). The sizes and frequencies of these alleles are given in Table 1. Complete linkage disequilibrium was, however, observed between the BglII + allele and the EcoRI A allele, and therefore the EcoRI

Table 1: Tyrosine Hydroxylase Polymorphisms

Enzyme	Alleles	Sizes (kb)	Frequencies
EcoRI	A	15.5	0.56
	B	15.0	0.03
	C	14.0	0.41
BglII	-	8.4	0.40
	+	6.9	0.60

Allele frequencies are those observed in 60 unrelated Northern Europeans.

Table 2: Lodscores for linkage between TH and 11p markers

	Recombination fractions (θ)						
	0.00	0.05	0.10	0.15	0.20	0.30	0.40
TH - β-globin	$-\infty$	2.91	2.87	2.70	2.46	1.82	1.01
TH - D11S12	$-\infty$	2.91	2.87	2.70	2.46	1.82	1.01
TH - Insulin	$-\infty$	7.35	6.91	6.31	5.63	4.02	2.23
TH - HRAS1	$-\infty$	4.51	4.19	3.73	3.23	2.16	1.12

polymorphism alone was used in linkage analysis. Four pedigrees were informative for TH: 1331, 1334, 1345 and NDM-A. Two-point lod scores for linkage between TH and 4 other polymorphic markers (HRAS1, β-globin, insulin, D11S12) are given for both sexes combined in Table 2. The maximum lod score for linkage between TH and insulin was $\hat{z}$=7.36, at a recombination fraction ($\hat{\theta}$) of 0.04.

The most likely gene order was inferred by inspection of crossovers. In pedigree 1334 there was a crossover between TH and the other 4 markers. In one individual of pedigree 1331, TH was linked to insulin and HRAS1 but not to β-globin or D11S12. The most likely gene order based on these observations is:

β-globin - D11S12 - insulin - HRAS1 - TH

The relative probability of this order compared with the second most likely order was obtained using the "LINKAGE" (13) program by combining the data on all 28 large three-generation pedigrees reported by White et al (8) with the data on the TH locus presented here. The suggested order is 16 times more likely than the second most likely order. A linkage map based on

the combined data is shown below. Distances between TH and the other loci are based on maximum likelihood estimates obtained from multipoint analysis, and are expressed in centiMorgans. Other distances are those estimated by White et al (8).

	2.9		9.1		3.2		3.8		
centromere	-	β-globin	-	D11S12	-	insulin	-	HRAS1	- TH - telomere

The least likely order, which is 60 times less likely than the order above, places TH between insulin and HRAS1.

DISCUSSION

As the density of markers on the genetic map of any region of the human genome rises, the effort required to establish the linkage relationships between the new marker and all the existing markers increases dramatically. Genetic data can however be accumulated more rapidly and efficiently if pedigree material is made available on a collaborative basis so that data on new polymorphisms can be combined with existing data contributed by other laboratories. This was the principle behind the foundation of CEPH: a Paris-based organisation headed by Professor Jean Dausset which distributes DNA from a large reference panel of families to its designated collaborators, who in turn submit data as typed polymorphic loci to CEPH for the use of other participants. This study shows the advantage of such a collaboration in that new data on the tyrosine hydroxylase locus was rapidly combined with a large amount of data on other polymorphic markers in the same pedigrees.

Gerhard et al (5,6) carried out linkage studies in a large Amish kindred segregating for an autosomal dominant form of manic-depressive psychosis, and found close linkage between the disorder and HRAS1 ($\hat{z}\simeq 4$, $\hat{\theta}=0$) and between the disorder and insulin ($\hat{z}\simeq 3$, $\hat{\theta}=0$). Joffe et al (14) have also reported evidence for linkage between β-globin and a similar affective disorder in a non-Amish kindred. In view of the central role of TH in catecholamine synthesis and the subchromosomal localisation of TH, insulin, HRAS1 and β-globin to the same region of chromosome 11, the genetic relationships of these four genes described here will be important in determining whether mutations in the TH gene might be responsible for manic-depressive psychosis. The two polymorphisms described here will be of use in further analysis of families with autosomal dominant manic-depressive psychosis. Moreover, the close linkage established between TH, HRAS1, D11S12 and insulin indicates that these markers may be used in combination to increase the information content of the

manic-depressive pedigrees and to refine the localisation of the disease mutation by multipoint mapping.

There is strong homology between the tyrosine hydroxylase gene and that for phenylalanine hydroxylase (PAH) which has been localised to chromosome 12 (15). The mapping of other pairs of homologous genes to chromosomes 11 and 12 has lead to speculation that these chromosomes result from an ancient tetraploidisation. However, there are differences in the subchromosomal localisation of the homologous genes suggesting that a rearrangement of gene order occurred at or after the tetraploidisation event. For example, PH has been localised to 12q22-q24.2 (16), whereas the homologous TH locus is on the short arm of chromosome 11. IGF-1 has been localised to 12q22-q24.1 (17) whereas IGF-2 lies at 11p15.5, 15kb 3' to the insulin gene (18). One explanation for the different comparative localisation of these homologous pairs is that a pericentric inversion event occured during the evolution of these chromosomes. In this model homologous pairs which continue to have comparable localisations on their respective chromosomes are assumed to lie outside the inverted region. The Kirsten ras-2 gene on the short arm of chromosome 12 bears an evolutionary relationship to HRAS1 and both of these genes reside on the short arms of their respective chromosomes (7). Our suggested gene order places the HRAS1 gene between TH and insulin/IGF-2. If a simple pericentric inversion event were responsible for the arrangement of the genes on these two chromosomes, one would expect the ras homologue on chromosome 12 to lie on the long arm between PAH and IGF-1. If the order suggested by our data is confirmed, this indicates that a simple pericentric inversion is unlikely to have been responsible for the differences in regional localisation of the homologous genes on chromosomes 11 and 12.

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