Idiopathic torsion dystonia (ITD) is a group of movement disorders which is usually inherited in an autosomal dominant manner with reduced penetrance. Most patients with ITD present with focal dystonia at adult age. However, thus far, this common subform remained unmapped chromosomally. In contrast, a rare early onset, more generalized form of ITD has been mapped to chromosome 9q34. Our linkage study in a large pedigree with seven definitely affected, six possibly affected and 16 phenotypically unaffected family members assigns an ITD gene for the common focal form with a maximal lod score of 3.17 to the region telomeric of D18S1153 on chromosome 18p.

INTRODUCTION

Idiopathic torsion dystonia (ITD) is a clinically and genetically heterogeneous group of movement disorders, characterized by sustained dystonic muscle contractions causing involuntary twisting movements and/or postures, where causes like cerebral lesions (especially of the basal ganglia), drugs or other neurological disorders have not been found (1). Various clinical subtypes are classified by the distributions of symptoms resulting in focal, segmental, multifocal or generalized dystonia. Furthermore, ITD has been divided into early onset dystonia, typically with onset of symptoms in the limbs and a tendency to generalize, and adult onset dystonia which usually remains focal and is localized in the upper part of the body. Variants of ITD are defined by additional characteristics like myoclonic events, paroxysmal occurrence, or DOPA responsiveness (2).

The etiology of ITD is unknown. For some variants of ITD, the localization or identification of underlying genes has been achieved: For example, the autosomal dominant DOPA-responsive dystonia (DRD) was linked to 1q43 (3) and is due to different point mutations of the GTP cyclohydrolase I gene (4). The autosomal recessive form of DOPA-responsive dystonia is due to point mutations of the tyrosine hydroxylase gene (5). A gene locus for a subtype of paroxysmal dystonia/chooreathetosis on chromosome 1p was reported by Auburger et al. (6). No linkage assignment has been reached so far for paroxysmal kinesiogenic dystonia and for myoclonic dystonia responsive to alcohol, although some families were reported in which these disorders showed a clearly autosomal dominant inheritance (7,8).

The only known gene for classic ITD, DYT1, is localized on 9q34 (9,10) and the trait is inherited in an autosomal dominant manner with reduced penetrance. It is responsible for early onset generalized dystonia in Ashkenazi Jews and seemed to be involved in earlier onset dystonia in several non-Jewish families (11). However, outside of the Jewish population, this form of ITD has a prevalence of only 3.4 per 100 000 (12).

The most common ITD form with adult onset and focal distribution was estimated to have a 10 times higher prevalence of 30 per 100 000 (12), and several families have been identified showing apparent autosomal dominant inheritance (13–17). However, a chromosomal linkage assignment could not be reached thus far, possibly due to the low penetrance or further locus heterogeneity.

In this study, we investigated a large family of German origin with seven definitely affected and six possibly affected members plus three obligate carriers, and demonstrated significant linkage to chromosome 18p.

RESULTS

Clinical investigation

The family was chosen because of the high density of dystonic individuals, and for this motif three neurologists agreed to examine clinically a total of 31 family members over the age of 20 years. The investigation identified seven definitely affected and six possibly affected individuals and three deceased individuals who are obligate transmitters, including the founder of this family. Autosomal dominant inheritance was assumed based on affected individuals in successive generations and also on the basis of roughly equal sex distribution and two male-to-male transmissions involving the obligate gene carrier II.1. Six of seven patients with definite dystonia suffer from torticollis, the remaining one from typical spasmodic dysphonia.
responding to therapy with botulinum toxin. Two of these patients with torticollis have slight additional dystonic symptoms: one suffers from Meige’s disease, the other one from dystonic writer’s cramp. Five of six patients with possible dystonia suffer from less obvious forms of torticollis (e.g. hypertrophic muscles and/or higher shoulder position on the affected side but only minimal head rotation), the sixth patient (III.11) only from postural hand tremor. Postural hand tremor was also observed in three definitely and three possibly affected persons. The dystonic symptoms had remained focal in all cases over 9 years of disease duration on average. Age of onset ranged between 28 and 70 years, with a mean of 58 years in generation II, of 37 years in generation III and of 43 years in total, but was difficult to establish precisely in the older generation due to lack of introspection.

**DISCUSSION**

We report the first linkage assignment of an ITD gene with the common purely focal and adult onset presentation. The result is based on the significance of a lod score of 3.17 being generated, and on the fact that all seven definitely affected individuals and all three obligate transmitters share the same chromosome 18p haplotype. The presence of one possibly affected individual in this pedigree who does not share the presumptive disease haplotype underlines the difficulties in unequivocal diagnoses of this clinical entity. On the other hand, the presence of eight family members with the disease haplotype (D18S1153-18pter) in spite of no clinical affection underlines a reduced degree of penetrance, as has been observed previously in the early onset, generalized ITD form on chromosome 9q34 and in DOPA-responsive dystonia (19). Both diagnostic difficulties explain why focal ITD gene loci remained unmapped so far by linkage studies. The results of the haplotype analysis in this family localized this gene for focal idiopathic dystonia to a region of ∼30 cM between D18S1153 and the telomere of 18p, possibly within 16 cM between D18S843 and D18S52.

Associations between our candidate region on chromosome 18 and dystonia syndromes have been reported previously in two cases: recently, a patient with deletion of chromosome 18p was reported who presented with a dystonic syndrome and accessory visual and pyramidal signs (20). Also on chromosome 18, the gene for autosomal recessive juvenile dystonic lipidosis (M. Niemann–Pick type C) has been localized (21). An overlap of both candidate regions cannot be excluded at present. The biochemical background in this syndrome is a defect in the esterification of cholesterol (22).

Within our ITD candidate region, two genes have been mapped which are involved in neurologic functions or structures: laminin is a non-collagenous macromolecule, is part of basement membranes and consists of the three subunit polypeptides A, B1 and B2. Neurite outgrowth was found to be dependent on the availability of carbohydrate residues of this glycoprotein to neurons (23). The gene of laminin chain A (LAMA1) maps to 18p11.31 (24). On the other hand, ADCAV1 is a human gene coding for a neuropeptide (pituitary adenylate cyclase-activating polypeptide) which remarkably stimulates adenylate cyclase in rat anterior pituitary cell cultures, and which has been localized to 18p11 by chromosomal in situ hybridization (25).

It remains to be shown whether this ITD gene can be identified through an improved sublocalization. Further narrowing of the candidate ITD region on chromosome 18p could be achieved by linkage disequilibrium analysis in nuclear families from the same geographical region, although the existence of founder effects has so far remained speculative. Such analyses will also clarify to which extent other gene loci are responsible for the clinical variability of ITD.

**MATERIALS AND METHODS**

**Patients**

All family members were carefully investigated by three neurologists at different times. Persons were considered as definitely affected only when all investigators had reached the diagnosis of ITD independently. Possible affection was diagnosed when only one or two investigators had noted ITD symptoms, usually reflecting a milder affection. The index patient

**Linkage and haplotype analysis**

A genome-wide linkage search was performed with Généthon multiplexing technology (18) using 206 microsatellites of which 118 were informative in this family. Linkage to the DYT1 locus on 9q34 could be excluded by three highly informative microsatellites, D9S290, D9S179 and D9S164, generating overlapping exclusion regions of 10, 2 and 10 centiMorgans (cM), respectively. Only one marker with a lod score of >2.0 was detected in the genome search (D18S62). In consequence, 34 microsatellites on the short arm and the pericentromeric region of chromosome 18 were analyzed on two-point linkage analysis. The order and the distance of the markers were taken from the microsatellites on the short arm and the pericentromeric region of D18S62 (cM), respectively. Only one marker with a lod score of >2.0 was detected in the genome search (D18S62). In consequence, 34 microsatellites on the short arm and the pericentromeric region of chromosome 18 were analyzed on two-point linkage analysis. The order and the distance of the markers were taken from the CHLC sex-averaged recombination minimization maps. 

A change in the phenotypic definition of individual III.11 (with only postural hand tremor) to ‘unaffected’ resulted in a core dystonia (20). Within our ITD candidate region, two genes have been mapped which are involved in neurologic functions or structures: laminin is a non-collagenous macromolecule, is part of basement membranes and consists of the three subunit polypeptides A, B1 and B2. Neurite outgrowth was found to be dependent on the availability of carbohydrate residues of this glycoprotein to neurons (23). The gene of laminin chain A (LAMA1) maps to 18p11.31 (24). On the other hand, ADCAV1 is a human gene coding for a neuropeptide (pituitary adenylate cyclase-activating polypeptide) which remarkably stimulates adenylate cyclase in rat anterior pituitary cell cultures, and which has been localized to 18p11 by chromosomal in situ hybridization (25).

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Figure 1. German pedigree with purely focal, adult onset ITD in seven definitely affected individuals, three obligate gene carriers and six possibly affected individuals. For the sake of anonymity, the sex was not always given. Definite dystonia is indicated by black symbols, possible dystonia in generation III by symbols with stripes. Four individuals in generation IV were typed for the sake of haplotype construction and have unknown affection status at ages <20 years. Individuals I.2, II.1 and II.3 are obligate transmitters under the assumption of autosomal dominant inheritance. Individual haplotypes are shown, with paternal alleles written on the left. The designation 00 indicates that the marker was not run or did not work well in that individual. The distance in cM between the markers is shown on the right. The ancestral disease haplotype and its different parts in descendants are indicated by bars. Eight unaffected individuals at risk and one individual with unknown affection status contained parts of the disease haplotype. Reconstructed haplotypes are indicated by dotted frames.
III.6 was admitted to the Neurological Department of Düsseldorf University and evaluated by physical examination, CCT, electroencephalography, hemogram including a blood smear for the exclusion of acanthocytosis and including copper metabolism. An extensive report of the clinical study and the individual symptoms in each affected member has been submitted independently (Leube et al., in preparation). Blood samples were collected from 60 family members, 45 of whom were >20 years of age.

**DNA analysis**

DNA was extracted using standard protocols involving the Scot-Lab II· procedure and then stored at +4°C. Microsatellite genotyping was carried out by the protocol of Genéthon (18) or alternatively as follows: PCR was performed in a total volume of 5 μl using 40 ng of genomic DNA, 10 pmol of each primer, 200 μM dGTP, dCTP, dTTP, 2.5 μM A TP and 0.50 μCi [α-35S]dATP, 10 mM Tris, 1.5 mM MgCl2, 50 mM KCl, 0.01% gelatine and 0.1 U Super-Taq (Stehelin). Thirty-five PCR cycles were performed with 1 min at 94°C, 55°C and 72°C. Separation of amplified fragments occurred on a 6.5% denaturing polyacrylamide gel in 30x60 cm chambers (S2, BRL) for 3–4 h at 70 W. Gels were fixed in 5% ethanol, 5% acetic acid for 30 min, dried and then exposed on Kodak Biomax films for one night.

**Linkage analysis**

All family members, whose blood samples were available, were included in the genome-wide microsatellite genotyping in order to allow haplotype construction and the reconstruction of deceased gene carrier haplotypes with a maximum certainty. Four members of generation IV were included in linkage analysis for the sake of haplotype construction, although the affection status was unknown due to an age of <20 years.

Haplotypes were established under the assumption of minimal recombination. The linkage analysis was conducted using the MLINK option in the LINKAGE software package (26) assuming an equal male–female recombination rate, autosomal dominant inheritance, a gene frequency set at 3:10 000 and its downcoding into a nine allele system, as shown in Figure 1. The specific disease haplotype 1 was set at a population allele frequency of 0.01, and all other alleles representing cumulative haplotypes were set at equal frequencies. This core haplotype was treated as one locus in two-point linkage analysis with disease inheritance.

**REFERENCES**


