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Dominant optic atrophy (OPA1) mapped to chromosome 3q region. I. Linkage analysis

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Dominant optic atrophy, type Kjer (McKusick no. 165500) is an autosomal dominant eye disease. The disease is characterized by moderate to severe visual impairment with an insidious onset during the first decade of life, blue-yellow dyschromatopsia and centrocecal scotoma of varying density. We examined three extended Danish pedigrees using highly informative short tandem repeat polymorphisms and found linkage of the disease gene (OPA1) to a $(CA)_n$ dinucleotide repeat polymorphism at locus D3S1314 (Z_{max} = 10.34 at $\theta_{M=F}$ = 0.075). Using two additional chromosome 3 markers we were able to map the OPA1 gene in the region between D3S1314 and D3S1265 (3q28 – qter).

INTRODUCTION

Autosomal dominant infantile optic atrophy, type Kjer (IOA) is the most common type among the hereditary optic atrophies (1,2), with a disease frequency in the range of 1:50.000 (3). IOA is transmitted as a dominant Mendelian trait with nearly complete penetrance (0.98) (4), and with a highly variable expression ranging from subclinical cases to legally blind persons. This disorder, identical with juvenile optic atrophy (MIM # 165500, gene symbol OPA1) (5), is characterized by an insidious onset of optic atrophy in early childhood with moderate to severe decrease of visual acuity, blue-yellow dyschromatopsia and centrocecal scotoma of varying density (6–8). Many affected members may be unaware of having the disease or of its hereditary aspects. The clinical picture has been described in several studies during the past years (1–3,6–12). Histopathological studies (13,14), have shown that dominant optic atrophy is a primary degeneration of the ganglion cell layer in the retina, accompanied by ascending optic atrophy. Linkage studies between OPA1 and the blood group systems ABO, MNS, RH, FY, HP, and KEL (15) showed no evidence of linkage. In the study of Kivlin *et al.* (4) a positive lod score (Z = 2.0 at θ = 0.18) to the Kidd blood group locus on chromosome 18 was obtained in a single family of 121 persons.

Other hereditary optic atrophies, however, have been described. The best known among these is Leber's hereditary optic neopathy (LHON), with which dominant optic atrophy has previously been confused. In LHON the characteristic symptom is an acute stage resembling optic papillitis with severe visual loss typically occurring in young men. LHON is transmitted exclusively through females due to a mutation in mitochondrial DNA. Rare types of hereditary optic atrophies have also been described in connection with neurological diseases, such as hearing loss and ataxia or diabetes mellitus and diabetes insipidus.

In this paper we present the linkage data from a study of three large Danish families, indicating that the gene OPA1 is located in the telomeric part of the long arm of chromosome 3.

The clinical aspects of the present study are to be published in a separate paper (Kjer *et al.* in prep.).

RESULTS

After excluding approximately 80% of the human autosomes by typing of 140 polymorphic loci on pedigree IOAKA168 (Fig. 1), an allele of the microsatellite marker D3S1314 (16) was found to segregate conspicuously together with the disease phenotype. By two-point linkage analysis employing the computer program LINKAGE (17) a significant maximum lod score was obtained Z = 4.91 (θ_M = 0.073, θ_F = 0.089).

Families IOAKS184 and IOALL161 were then typed for D3S1314. These pedigrees both gave positive lod scores to D3S1314 (Z = 3.08 in family IOAKS184 and Z = 2.95 in family IOALL161), i.e. the results support the contention that the disease locus is the same in these three families. All three families were typed for the flanking markers D3S1265 and D3S1262. The following combined lod scores were obtained, Z = 10.34 (θ_M = 0.075, θ_F = 0.074) to D3S1314; Z = 4.67 (θ_M = 0.149, θ_F = 0.205) to D3S1262 and Z = 10.15 (θ_M = 0.23, θ_F = 0.0) to D3S1265 (Table 1). All markers are located on chromosome 3q, in the telomeric area. The order and sex average recombination fractions between the marker loci, as estimated from data on a subset of CEPH families are: D3S1262-0.11-D3S1314-0.11-D3S1265-qter (16). The most likely order of OPA1 in this linkage groups was found, by four-point analysis using LINKMAP to be: D3S1262-D3S1314-OPA1-D3S1265 (Fig. 2). The order of markers was supported over the next most likely order with odds 1.23×10^5:1. The maximum lod scores and distances between the microsatellite markers in this material were calculated to: D3S1314-D3S1265 (Z = 5.13 at $\theta_{(M=F)}$ = 0.147), D3S1314-D3S1262 (Z = 9.72 at $\theta_{(M=F)}$ = 0.091) and D3S1262-D3S1265 (Z = 1.59 at $\theta_{(M=F)}$ = 0.253).

The disease in these three families is associated with entirely different haplotypes, but the distances between the disease locus and the markers are too large for drawing conclusions with regard to possible different mutational origins.

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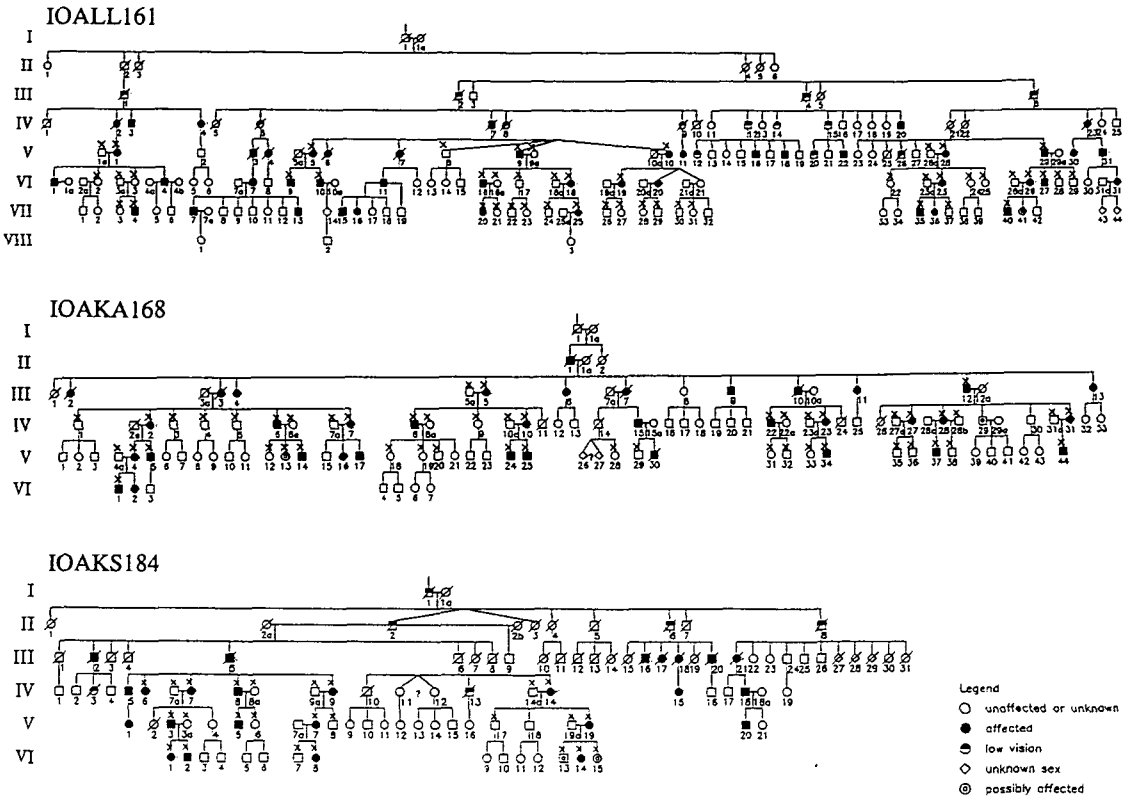

Figure 1. Three multigeneration families IOALL161 (6), IOAKA168 (7), and IOAKS184 (7) from the Danish Family Register of Hereditary Eye Diseases. Persons included in the linkage study are marked with an X.

Penetrance

It is a general experience that non-affected persons, despite careful examination for minor visual defects, may carry the mutation. A direct count of 'skipped generations' in the three pedigrees, disclosed one female (IOALL161 VI-3), of a total of 36 living affected persons, i.e. a penetrance of 0.97. The person (IOALL161 VI-3) was analysed for DNA markers and was found carrying the haplotype flanking the disease gene.

DISCUSSION

We have established the most likely location of the OPA1 gene within the interval between the chromosome 3q microsatellite markers D3S1314 and D3S1265. Provisional suggestion of linkage was earlier found to the Kidd blood group locus on chromosome 18 (4) with a lod score of Z = 2.18. This might indicate genetic heterogeneity, but a random accidental derivation may be more likely. The Kidd locus gave negative lod scores in our three families. Penetrance values for IOA were determinated to 0.98 (4) in a family with 121 members. Direct count of 'skipped generations' in our families gave a similar penetrance of 0.97 (35 of 36).

The present locus assignment for OPA1 provides the first significant biological marker for this disease. The most likely physical location of OPA1 in the area of chromosome 3q should be determined by its linkage to physically mapped markers. The somatostatin gene (SST) (18), which maps to 3q28, gave a maximum lod score Z = 0.61 at $\theta_M = 0.123$, $\theta_F = 0.275$ (Table 1) while D3S1265 that maps very close to the telomere in region 3q29 (16) gave a high positive lod score Z = 10.15 at $\theta_M = 0.23$, $\theta_F = 0.00$. A LINKMAP analysis mapped OPA1 between D3S1314 and D3S1265 (Fig. 2) indicating that the disease is most likely located in 3q28−q29 region. SST was excluded as a candidate gene for OPA1 by its negative lod score to OPA1 (Z = −9.94 at θ =0.0).

A few more genes have been mapped in the region (3q28−3qter) of D3S1314, namely AHSG (α-2HS-glycoprotein, a serum protein) (19), MUC4 (tracheobronchial mucin 4)(20) and MFI2 (melanoma associated antigen p97)(21). None of these genes are considered as obvious candidate genes for OPA1. To identify the gene for OPA1, we first plan to type with new primer pairs in the region between D3S1314 and D3S1265 to narrow down the region of OPA1, second to screening a retina cDNA library using YAC and cosmid contigs from this particular region.

MATERIAL AND METHODS

Materials

Three multigeneration families (Fig. 1) previously published: family IOALL161 as pedigree I and IV (6), and families IOAKA168 and IOAKS184 as pedigree A and S (7) respectively have now been updated in the Danish Family Register of Hereditary Eye Diseases at the National Eye Clinic for the Visually Impaired.

Blood samples from 123 individuals, older than 6 years of age were collected for linkage studies. The material comprised 53 affected and 67 non-affected persons. Three persons could not be classified as either affected or unaffected. Serum, erythrocytes and DNA were isolated from all blood samples (22).

The clinical aspects of the present study are to be published in a separate paper (Kjer *et al.* in prep.).

Table 1. Linkage relations of dominant optic atrophy and chromosome 3q markers

	Lod (Z) scores at various recombination fractions of ($\theta_M = \theta_F$) in all families							
Penetrance = 98%	0.00	0.01	0.05	0.10	0.20	0.30	Z_{max}	at θ (M;F)
OPA1 (D3S1314):								
IOAKA168	1.70	3.92	4.80	4.87	4.21	2.76	4.91	(0.073;0.089)
IOAKS184	3.08	3.02	2.77	2.44	1.78	1.12	3.08	(0.000;0.000)
IOALL161	−1.22	1.34	2.62	2.91	2.61	1.85	2.95	(0.138;0.081)
Total	3.56	8.28	10.18	10.23	8.60	6.02	10.34	(0.075;0.074)
OPA1 (D3S1262):								
IOAKA168	−8.14	3.40	4.91	5.19	4.61	3.40	5.22	(0.073;0.114)
IOAKS184	−10.73	−2.50	−0.65	0.08	0.52	0.49	0.67	(0.129;0.298)
IOALL161	−11.60	−4.69	−2.49	−1.46	−0.46	−0.19	0.00	(0.500;0.500)
Total	−29.89	−3.80	1.78	3.81	4.58	3.70	4.67	(0.149;0.205)
OPA1 (D3S1265):								
IOAKA168	−3.59	3.78	4.66	4.59	3.75	2.57	5.43	(0.150;0.000)
IOAKS184	−1.86	1.46	1.90	1.87	1.49	0.97	2.26	(0.138;0.000)
IOALL161	−6.98	−0.83	0.81	1.36	1.46	1.09	2.75	(0.339;0.000)
Total	−12.43	4.41	7.37	7.82	6.71	0.63	10.15	(0.223;0.000)
OPA1-SST:								
Total	−9.94	−2.46	−0.51	0.16	0.54	0.52	0.61	(0.123;0.275)

Linkage analysis

A total of 35 classical markers was first tested and analysed for linkage. Later 140 polymerase chain reaction (PCR) pairs were tested until a highly significant positive lod score was obtained. The scores $L(\theta_M = \theta_F)$ on chromosome 3q markers, as shown in Table 1 and the gene order was assessed by manual haplotype drawing and ordering, and by the program package LINKAGE (5.2) (17). A penetrance of 0.98 was chosen for our initial linkage studies.

DNA amplification

The radioactive PCR was performed in a volume of 10 μl in microtitreplates (Hybaid). This comprises 50 ng of templated DNA, 1−3 μM of non-labelled and ^{32}P end-labelled primer, 0.1 mM of each dNTP, 1.5 mM $MgCl_2$, and 0.15 U of *Taq* DNA polymerase in buffer (Promega). The amplification was run in a programmable heating block (Omnigene, Hybaid) for 27 cycles comprising of 94°C for 30 s, 55°C for 0.30 s and 72°C for 30 s. The amplified fragments were separated by electrophoresis for 1−3 h at (100 W) in 6% denaturing polyacrylamide sequencing gel (25×42×0.4 cm). Gels were fixed for 10 min (10% acetic acid), washed for 20 min, dried and exposed to X-ray film (XAR-5 Kodak) overnight.

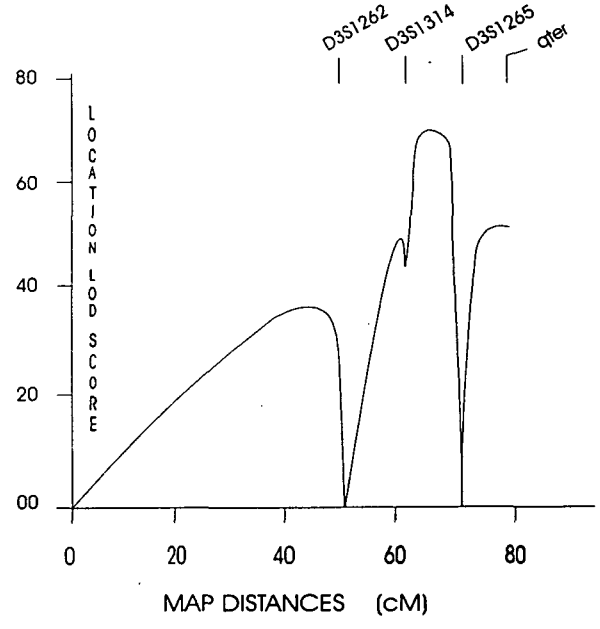

Figure 2. Multipoint linkage data for the OPA1 locus between chromosome 3q markers from the Généthon collection (16).

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