No evidence of genetic heterogeneity in dominant optic atrophy

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Abstract

Autosomal dominant optic atrophy (OPA, MIM 165500) is an eye disease causing a variable reduction of visual acuity with an insidious onset in the first six years of life. It is associated with a central scotoma and an acquired blue-yellow dyschromatopsia. A gene for dominant optic atrophy (OPA1) has recently been mapped to chromosome 3q in three large Danish pedigrees. Here, we confirm the mapping of OPA1 to chromosome 3q28-qter by showing close linkage of the disease locus to three recently reported microsatellite DNA markers in the interval defined by loci D3S1314 and D3S1265 in four French families (Zmax = 5.13 at θ = 0 for probe AFM 308yf1 at locus D3S1601). Multipoint analysis supports the mapping of the disease gene to the genetic interval defined by loci D3S1314 and D3S1265. The present study provides three new markers closely linked to the disease gene for future genetic studies in OPA.

Materials and methods

Eighteen affected subjects and 14 healthy relatives belonging to four unrelated families of

![Figure 1 Pedigrees of families with OPA.](http://jmg.bmj.com/)

Autosomal dominant optic atrophy (OPA, MIM 165500) and mitochondrialy inherited Leber’s hereditary optic neuropathy (LHON) represent the commonest forms of inherited, uncomplicated optic atrophy. Each disease has an incidence of 1/50,000. OPA was originally described by Kjer in Danish pedigrees and later by Smith, Kline and Glaser, Hoyt, and Elliott. The disease usually starts in the first six years of life with a progressive reduction of visual acuity. The visual prognosis is relatively good with stable or slowly progressive visual loss but impairment can vary considerably among affected relatives. An acquired blue-yellow dyschromatopsia with a central, para-central, or centrocecal scotoma and an inversion of the peripheral field, more confined to blue than to red test objects, are present in most cases. Appearance of the optic nerve ranges from mild temporal pallor to complete atrophy.

Recently, a gene responsible for OPA has been mapped to the distal long arm of chromosome 3 (OPA1, 3q28-qter) by linkage analysis in three large Danish families. Here, we confirm the localisation of OPA1 to chromosome 3q in four unrelated French families and provide evidence for genetic homogeneity of this condition.

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Pairwise lod scores between OPAl and five polymorphic DNA markers on chromosome 3q

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Figure 2. Support for location of OPAl with respect to five markers on chromosome 3q. Likelihood estimates are given in log base 10. Distances between marker loci are shown in cM along the abscissa.

French origin (fig 1) were ascertained from Hôpital des Enfants Malades, Paris (families 1 and 4), Hôpital de Vannes (family 2), and Hôpital de Caen (family 3). Ophthalmological examinations were obtained for each person over 6 years of age and inclusion criteria in the study were: (1) reduced visual acuity (1/10 to 5/10), (2) onset in the first decade, (3) a rapidly progressive course, (4) an acquired blue-yellow dyschromatopsia, (5) a characteristic appearance of the optic nerve on fundus examination. At risk subjects over 10 years were considered as unaffected when their visual acuity was normal with a normal colour vision test and a normal appearance of the fundus. The status of subjects under 10 years of age was considered uncertain when their ophthalmological examination was normal. Those who took part in the linkage study are indicated by an asterisk in fig 1.

For each person, a 20 ml EDTA blood sample was collected and DNA was prepared from lymphocyte pellets by SDS lysis, proteinase K digestion, phenol/chloroform extraction, ethanol precipitation, and Tris-EDTA resuspension. The hypervariable microsatellites developed by Weissenbach et al. and Gypay et al. were used for genotyping. Genomic DNA (50 ng) was submitted to PCR amplification using 1 unit Taq polymerase in a buffer containing 50 pmol of each primer, 6.25 nmol of each deoxynucleotide, 50 mmol/l KCl, 10 mmol/l Tris HCl, pH 8, 1.5 mmol/l MgCl2, 0.1% gelatin in a final volume of 50 μl. Taq polymerase was added after the first step of denaturation (10 minutes, 95°C) followed by 30 cycles of denaturation (94°C, 40 seconds), annealing (55°C, 30 seconds), and elongation (72°C, 40 seconds), and a last step of elongation (10 minutes, 72°C). An aliquot of the amplified DNA was mixed with the loading buffer. The samples were denatured for 10 minutes at 94°C and loaded on a 6% polyacrylamide denaturing gel. After blotting, nylon membranes were fixed in 0.4 mol/l NaOH and hybridised for two hours with (CA)n[32P] labelled probes. Blots were washed once in 2 x SSC, 0.1% SDS for 10 minutes at room temperature and autoradiographed.

OPA was tested assuming that the disease is transmitted as an autosomal dominant trait (gene frequency f = 1/50 000) with complete penetrance. Linkage analysis was performed using the MLINK and LINKMAP options of the 5.1 version of the LINKAGE package1011 using a SUN computer station.

Results

Linkage analyses using microsatellite DNA markers of chromosome 3 showed a maximum pairwise lod score for marker AFM 308yr1 at the D3S1601 locus (Zmax = 5.13 at 0 = 0, table). The location score method was used to estimate the position of the OPAl gene. In this procedure, the map of the marker loci is fixed and the position of the disease locus is varied throughout the map. The order 3pter-D3S1314-(0.02)-D3S2747-(0.03)-D3S1601-(0.02)-D3S2748-(0.06)-D3S1265-3qter (with recombination estimates in parentheses) has been established by analysis of Généthon markers in CEPH reference families. The maximum likelihood estimate of OPAl was obtained in the interval defined by loci D3S1314 and D3S1265 (location score in log base 10 = 5.19, fig 2). A recombination event at locus D3S1265 was observed in one affected member of family 1 and in all loci D3S1314 in two affected subjects in family 3 (data not shown). No linkage disequilibrium between OPAl and one particular allele was observed with the markers tested.

Discussion

We report the mapping of a gene for dominant optic atrophy to the long arm of chromosome 3 in four pedigrees of French ancestry. This study gives support to the recent localisation of OPAl to chromosome 3q28-qter in Danish families.7 Eiberg et al7 showed that a disease gene is located in the 10 cM genetic interval defined by loci D3S1314 and D3S1265. The present study gives support to the localisation of the disease gene in this interval and provides no evidence of genetic heterogeneity of OPAl, as all families hitherto tested were consistent with linkage to chromosome 3q.

In the last few years, several protein coding
genes have been mapped to 3q28-qter, including tracheobronchial mucin 4 (MUC4),\(^2\) melanoma associated antigen p97 (MIF2),\(^3\) and A2HS-glycoprotein (AHSG).\(^4\) None of them can be regarded as candidate genes for OPA1. Continuing studies will help to narrow the genetic interval encompassing the disease gene and hopefully to identify the disease causing gene.

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