Genetic refinement of dominant optic atrophy (OPA1) locus to within a 2 cM interval of chromosome 3q

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Abstract

Autosomal dominant optic atrophy (OPA, MIM 165500) is an eye disease characterised by variable optic atrophy and reduction in visual acuity. It has an insidious onset in the first decade of life and is clinically highly heterogeneous. It is associated with a centrocecal scotoma of varying size and density and an acquired blue-yellow dyschromatopsia. Recent studies of three large Danish pedigrees have mapped a gene for dominant optic atrophy (OPA1) to a 10 cM region on chromosome 3q, between markers D3S1314 and D3S1265 (3q28-qter). Genetic linkage analysis in five British pedigrees confirms mapping to chromosome 3q28-qter. Haplotype analysis of a seven generation pedigree positions the disease causing gene between loci D3S3590 and D3S1305, corresponding to a genetic distance of 2 cM. This represents a significant linkage refinement and should facilitate positional cloning of the disease gene.
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Keywords: dominant optic atrophy; genetic refinement; linkage.

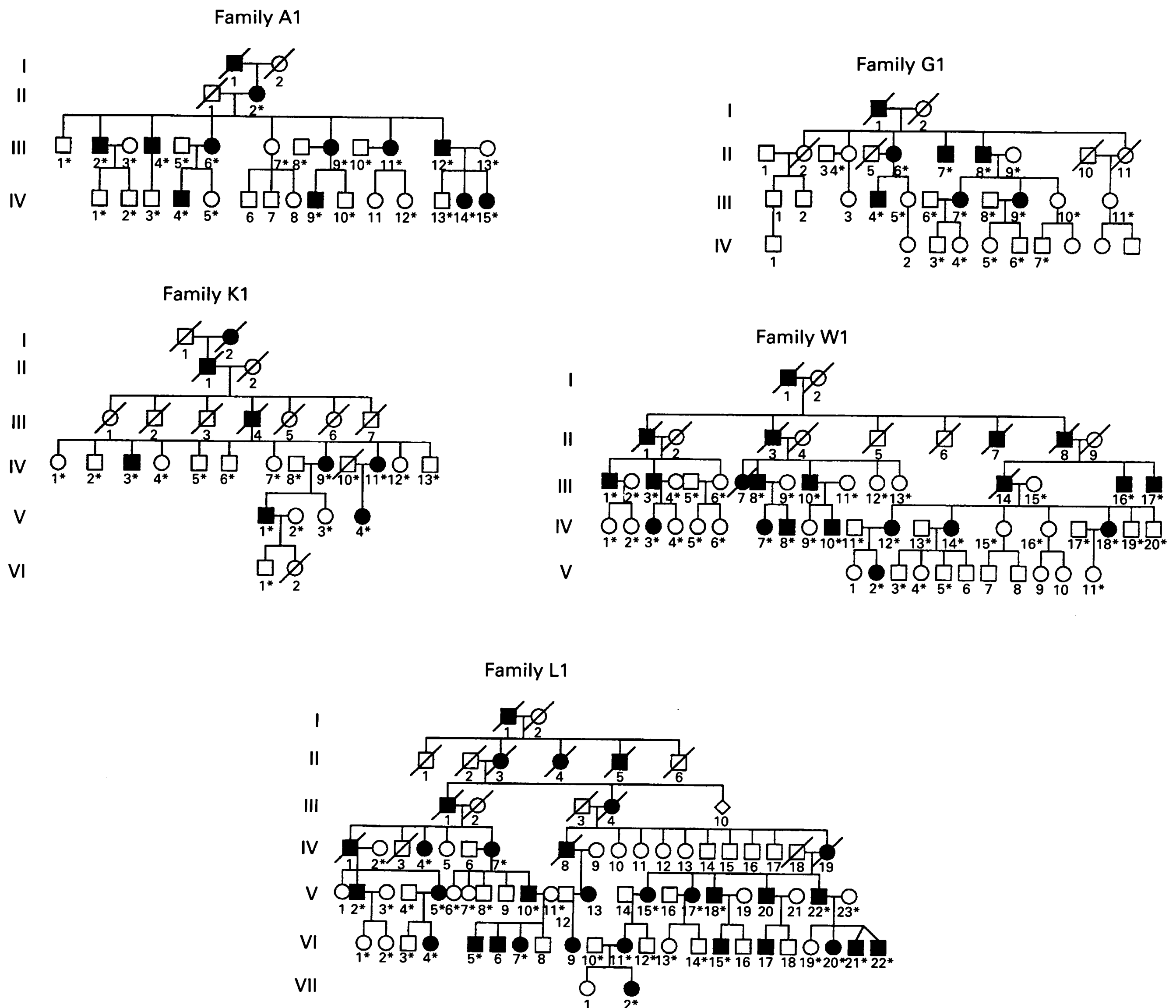

Figure 1 Five British pedigrees of families with OPA.

Dominant optic atrophy (OPA, Kjer type) is the commonest hereditary optic neuropathy with an estimated incidence of 1:50 000[1] and a prevalence of 1:10 000.[2] By comparison, Leber's hereditary optic neuropathy is estimated to affect 1:50 000.[3] The clinical features of OPA have been described in several studies in the past decades.[4-8]

OPA is characterised by an insidious onset of visual loss in the first decade of life. There is a variable reduction of visual acuity ranging from very mild to severely affected. Affected people may show the classical picture of an acquired blue-yellow dyschromatopsia, although mixed colour vision defects are common. There is usually a central, paracentral, or centrocecal scotoma on visual field testing. The optic nerve appearance ranges from mild temporal pallor through to complete optic atrophy. The disease is usually symmetrical and only slowly progressive. However, there is considerable clinical heterogeneity both within and between families.

Histopathological studies suggest that OPA is primarily a ganglion cell degeneration.[9] OPA has recently been mapped in three large Danish pedigrees to the distal long arm of chromosome 3 (OPA1, 3q28-qter)[10] in the 9-10 cM genetic interval defined by loci D3S1314 and D3S1265. We have performed linkage studies on five British pedigrees that give support to the localisation of the disease gene in this interval and, in addition, substantially refine the disease gene containing interval.

Patients and methods

PHENOTYPING AND GENOTYPING

Fifty three affected subjects and seventy eight unaffected relatives from five unrelated English families (fig 1) identified from the Moorfields Genetic Clinic Database were studied.

Subjects underwent a full clinical examination, including visual acuity, slit lamp examination, standard pseudoisochromatic plates, Mollon Reffin "Minimalist" test for colour vision deficiencies,[11] colour contrast sensitivity, Humphrey 30-2 visual field assessment, and fundal photography. EDTA blood (20 ml) was obtained for linkage and haplotype analysis. Subjects were assigned as affected if they were over the age of 6 years and had onset of mildly to severely decreased vision in the first decade, dyschromatopsia, a centrocecal scotoma, and optic nerve pallor. People included in the linkage study are marked by an asterisk in fig 1. No unaffected subject had an abnormal visual acuity, visual field on Humphrey perimetry, or abnormal colour vision. Affected status was only assigned if the above criteria were met.

Of particular interest are subjects V.5, VI.3, and VI.4. Subject V.5 was a 59 year old female with visual acuity of 6/60 in both eyes, with a history of poor vision since the age of 4 years. She had an extensive centrocecal scotoma on Humphrey 30-2 perimetry and virtual achromatopsia on colour testing with a modified 100 Hue test and pseudoisochromatic plates. Her daughter, VI.4, was an 18 year old with visual acuity of 6/36 in both eyes and a large centrocecal scotoma on testing. She had a profound colour vision abnormality, with no one axis predominating. Subject VI.3 was a 22 year old male with visual acuity of 6/6 in both eyes and no refractive error. He had no scotoma on perimetry and normal colour vision.

Genomic DNA was extracted using a Nucleon II DNA extraction kit (Scotlab Bioscience). PCR amplification[12] using *Taq* polymerase from 100 ng DNA template was performed using primers labelled with radioactive ^{32}P. PCR conditions were 30 cycles at 94°C

Table 1 Summed two point lod scores (Z) for five markers on chromosome 3q, flanking the optic atrophy locus (OPA1), in five British pedigrees with dominant optic atrophy

	Recombination fraction								
Marker	0.00	0.01	0.05	0.1	0.2	0.3	0.4	Zmax	θmax
Pedigree A1									
D3S1314	4.52	4.45	4.16	3.78	2.96	2.04	0.98	4.52	0.00
D3S1601	3.66	3.60	3.36	3.05	2.36	1.60	0.75	3.66	0.00
D3S1265	−∞	0.37	5.14	5.99	5.34	3.61	1.54	1.05	0.11
D3S1311	−∞	2.15	2.60	2.57	2.16	1.53	0.75	2.62	0.07
D3S1272	−∞	2.15	2.60	2.57	2.16	1.53	0.76	2.62	0.07
Pedigree G1									
D3S1314	2.41	2.36	2.19	1.95	1.44	0.89	0.36	2.41	0.00
D3S1601	1.51	1.48	1.37	1.22	0.88	0.51	0.16	1.51	0.00
D3S1265	1.56	1.56	1.53	1.43	1.13	0.76	0.35	1.56	0.04
D3S1311	1.92	1.89	1.75	1.58	1.19	0.78	0.36	1.92	0.00
D3S1272	1.71	1.67	1.54	1.35	0.96	0.55	0.17	1.71	0.00
Pedigree K1									
D3S1314	0.27	0.27	0.38	0.94	0.77	0.47	0.18	0.94	0.17
D3S1601	2.71	2.61	2.46	2.21	1.65	1.02	0.38	2.71	0.00
D3S1265	0.83	0.81	0.74	0.65	0.47	0.29	0.12	0.83	0.00
D3S1311	0.30	0.28	0.23	0.19	0.16	0.15	0.07	0.03	0.00
D3S1272	−∞	0.95	1.44	1.47	1.19	0.75	0.27	1.48	0.08
Pedigree L1									
D3S1314	−∞	5.50	5.72	5.39	4.35	3.03	1.54	5.75	0.04
D3S1601	−∞	3.91	4.16	3.90	3.02	1.97	0.87	4.17	0.04
D3S1265	−∞	3.13	3.46	3.29	2.58	1.69	0.72	3.46	0.05
D3S1311	−∞	1.51	3.16	3.46	3.14	2.30	1.19	3.45	0.11
D3S1272	−∞	0.97	2.02	2.17	1.85	1.26	0.54	2.17	0.10
Pedigree W1									
D3S1314	−∞	0.17	1.35	1.65	1.57	1.17	0.63	1.85	0.13
D3S1601	−∞	−2.14	0.03	0.78	1.11	0.92	0.50	1.81	0.20
D3S1265	−∞	−4.71	−1.52	−0.42	0.23	0.22	0.08	0.26	0.24
D3S1311	−∞	−3.31	−1.81	−1.22	−0.55	−0.19	−0.03	0.19	0.47
D3S1272	−∞	−4.26	−1.68	−0.74	−0.07	0.10	0.09	0.12	0.34

Table 2 Two point lod scores (Z) for linkage between the dominant optic atrophy locus (OPA1) and chromosome 3q markers in family L1

	Recombination fraction								
Marker	*0.00*	*0.01*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*Zmax*	*θmax*
D3S1314	−∞	5.50	5.72	5.39	4.35	3.03	1.54	5.75	0.04
D3S1601	−∞	3.91	4.16	3.90	3.02	1.97	0.87	4.17	0.04
D3S3669	−∞	1.83	2.23	2.15	1.66	1.05	0.45	2.23	0.06
D3S3590	−∞	−0.19	0.35	0.47	0.41	0.27	0.12	0.47	0.12
D3S3562	2.79	2.72	2.48	2.17	1.59	1.02	0.47	2.79	0.00
D3S2748	0.03	0.03	0.03	0.03	0.01	0.01	0.00	0.03	0.00
D3S3642	0.91	0.88	0.79	0.69	0.50	0.33	0.17	0.91	0.00
D3S1305	−∞	0.00	1.12	1.37	1.24	0.85	0.32	1.38	0.12
D3S1265	−∞	3.13	3.46	3.29	2.58	1.69	0.72	3.46	0.05
D3S3707	−∞	3.26	3.69	3.60	2.98	2.11	1.07	3.70	0.06
D3S1311	−∞	1.51	3.16	3.46	3.14	2.30	1.19	3.49	0.11
D3S1272	−∞	0.97	2.02	2.17	1.85	1.26	0.54	2.17	0.10

D3S2748 and D3S3642 are uninformative in this family.

for one minute, 55°C for one minute, and 72°C for one minute. The products were separated by denaturing polyacrylamide gel electrophoresis and visualised by autoradiography.

Five polymorphic microsatellite DNA markers[13 14] on chromosome 3q known to flank the region bounding the OPA1 gene were used to obtain linkage data in all five British families.

In the seven generation family L1, 33 family members were studied (family L1, fig 1): 18 affected members, nine unaffected members, and six spouses. Twelve microsatellite markers were used to genotype subjects in family L1.

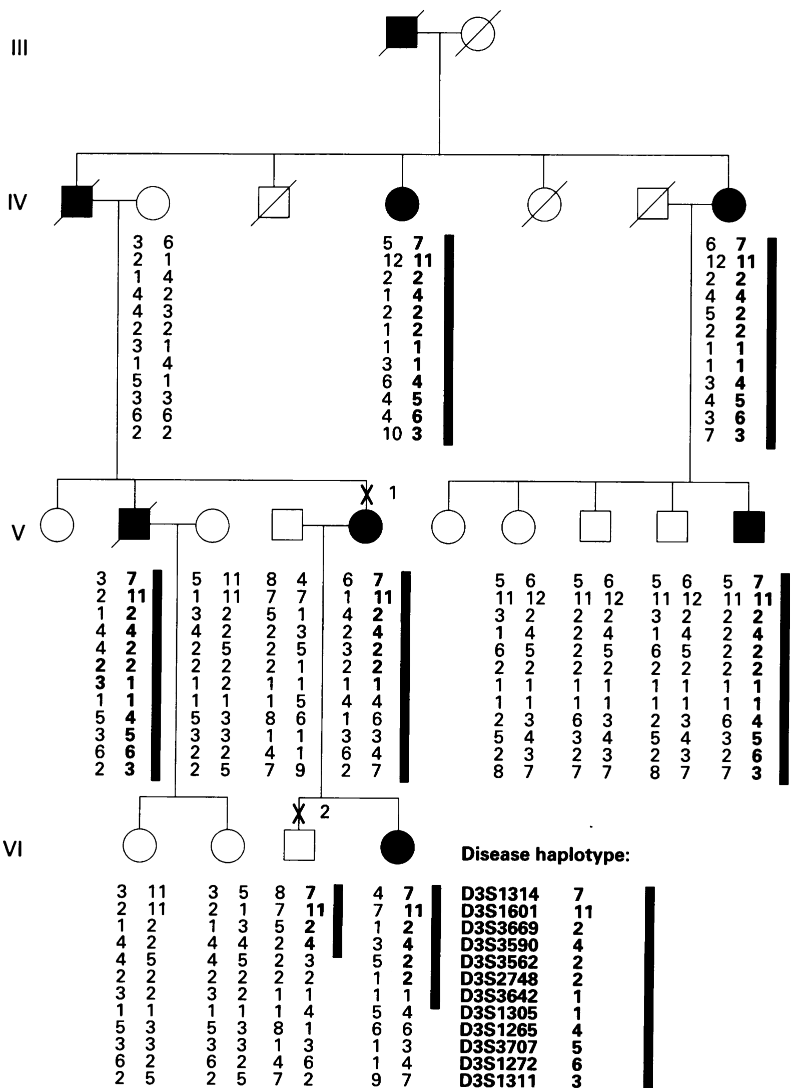

Figure 2 Abridged autosomal dominant optic atrophy pedigree L1 illustrating the segregation of 12 3q loci. Haplotype linked to disease is indicated in bold type. Solid symbols indicate affected members of the family. X1 denotes critical recombination event in V.5. X2 denotes critical recombination event in VI.3.

LINKAGE ANALYSIS

Data were analysed using LINKSYS (version 3.1)[15] and two point lod scores were obtained using MLINK (version 5.10) of the LINKAGE package.[16] Maximum lod scores were obtained using ILINK. It was assumed that the disease is transmitted as an autosomal dominant trait with a gene frequency of f =1/50 000 and full penetrance.

Results

TWO POINT ANALYSIS

Maximum two point lod scores for the five unrelated British pedigree are shown in table 1. The families studied show evidence of linkage to the region 3q. Additionally, pedigree W1 has been tested for markers centromeric to D3S1314, with no evidence for linkage. For example, with D3S1262, which is 12 cM centromeric to D3S1314, the lod score is -3.07 at a θ value of 0.10.

Two point lod scores for family L1 with 12 microsatellite markers mapping to the OPA1 region are presented in table 2. A maximum two point lod score of 5.75 (θ=0.04) with marker D3S1314 confirmed tight linkage between OPA1 and the 3q28 region in this family.

Table 3 HOMOG data for five British pedigrees, by marker on chromosome 3q, showing values of Max. lnL, alpha and theta

	Estimates of:		
Hypothesis	*Max. lnL*	*Alpha*	*Theta*
H2: linkage, heterogeneity			
D3S1314	32.17	1.00	0.05
D3S1601	26.20	1.00	0.05
D3S1265	13.79	1.00	0.10
D3S1311	15.84	0.80	0.10
D3S1272	15.71	0.95	0.10
H1: linkage, homogeneity			
D3S1314	32.17	(1)	0.05
D3S1601	26.20	(1)	0.05
D3S1265	13.79	(1)	0.10
D3S1311	15.15	(1)	0.10
D3S1272	15.70	(1)	0.10
H0: no linkage			
D3S1314	(0)	(0)	(0.5)
D3S1601	(0)	(0)	(0.5)
D3S1265	(0)	(0)	(0.5)
D3S1311	(0)	(0)	(0.5)
D3S1272	(0)	(0)	(0.5)

Table 4 HOMOG data for five British pedigrees, for five markers on chromosome 3q, showing chi-squared and likelihood ratios for heterogeneity, homogeneity, and linkage

Components of chi-square source	*df*	*Chi-square*	*L ratio*
H2 *v* H1 heterogeneity			
D3S1314	1	0.00	1.00
D3S1601	1	0.00	1.00
D3S1265	1	0.00	1.00
D3S1311	1	1.38	1.99
D3S1272	1	0.01	1.00
H1 *v* H0 linkage			
D3S1314	1	64.33	9.33×10^{13}
D3S1601	1	52.41	2.39×10^{11}
D3S1265	1	27.59	9.77×10^{5}
D3S1311	1	30.30	3.80×10^{6}
D3S1272	1	31.41	6.61×10^{6}
H2 *v* H0 total			
D3S1314	2	64.33	9.33×10^{13}
D3S1601	2	52.41	2.39×10^{11}
D3S1265	2	27.59	9.77×10^{5}
D3S1311	2	31.68	7.59×10^{6}
D3S1272	2	31.41	6.62×10^{6}

HOMOG ANALYSIS

An analysis of the likelihood of the presence or absence of genetic heterogeneity within the group of five British families was performed, using the HOMOG data analysis programme. The results show that there is no statistical evidence for the presence of genetic heterogeneity within the families studied (tables 3 and 4).

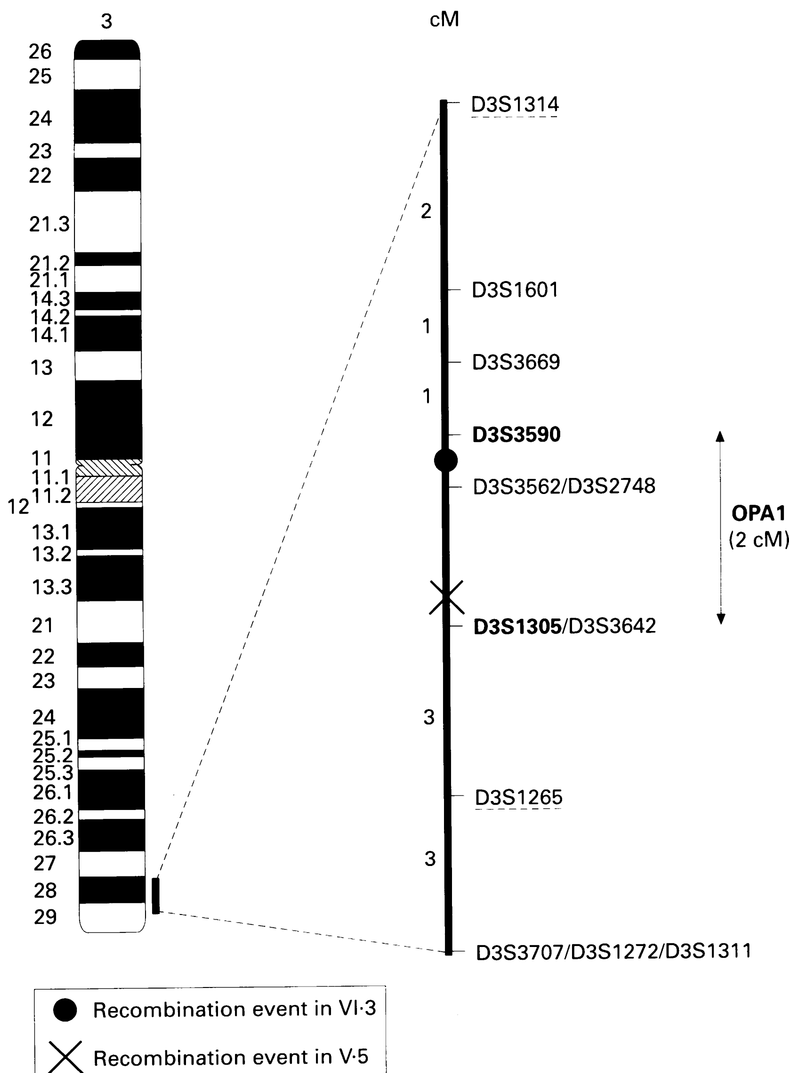

Figure 3 Schematic diagram of chromosome 3 indicating the physical localisation of the OPA1 locus. The marker order is as shown in the Genethon map, 1996.[14]

HAPLOTYPE ANALYSIS

Haplotype analysis across the disease gene containing region in the families studied did not suggest a founder effect.

Two crucial recombination events in family L1 were identified (fig 2). Haplotype analysis for affected subject V.5 identified a crossover event centromeric to the D3S1305 locus, and the recombinant chromosome was inherited by affected subject VI.4. This localises OPA1 centromeric to D3S1305 (fig 3). Unaffected subject VI.3 shows a crossover at locus D3S3590 (fig 2), indicating that OPA1 lies telomeric to this marker. Thus the disease interval lies between D3S3590 and D3S1305, a genetic interval of 2 cM (fig 3).

Discussion

Eiberg *et al*[10] showed that a disease gene for dominant optic atrophy is located in the 10 cM interval defined by loci D3S1314 and D3S1265. We report the genetic refinement of the locus for dominant optic atrophy to within a 2 cM interval. This represents a significant improvement in the genetic refinement. The confirmation of linkage of dominant optic atrophy to the 3q region in five unrelated British pedigrees gives support to the lack of genetic heterogeneity found by other authors.[17] This suggests that the highly variable clinical phenotype described may be because of the effect of different mutations within the same gene, as seen in sectorial versus diffuse retinitis pigmentosa caused by different mutations of the rhodopsin gene.[18]

We have additionally been able to order the marker D3S3562 with respect to the marker D3S3590 in our family. While their order in the Genethon 1996 map is not specified, it is clear from our haplotype data that D3S3562 is telomeric to D3S3590 (fig 3). D3S2748 and D3S3642 are both uninformative in this family.

Several genes have been mapped to 3q28-qter. They include A-2HS-glycoprotein (AHSG),[19] melanoma associated antigen p97 (MIF2),[20] and tracheobronchial mucin 4 (MUC4).[21] None of these would appear to be suitable as candidate genes for OPA1. Hence, this refinement of the disease containing interval on 3q28-qter will facilitate the eventual positional cloning of the dominant optic atrophy gene. Approaches similar to those used in the positional cloning of the gene for Freidreich's ataxia[22] and X linked retinitis pigmentosa[23] will be used. These include the construction of a YAC contig spanning the critical region, generation of new microsatellite markers for further genetic mapping and refinement, and identification of expressed sequences and potential candidate genes.

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