A Gene Causing Autosomal Recessive Cataract Maps to the Short Arm of Chromosome 3

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Abstract

Background: Fourteen loci have been associated with autosomal dominant cataract, but only one with the recessive form of the disease.

Objectives: To find the chromosomal location of a gene causing autosomal recessive cataract in three inbred Arab families.

Methods: A single nucleotide polymorphism-based genome-wide search, with the Effymetrix GeneChip HuSNP genotyping array, was performed on a pooled DNA sample from six affected family members in a search for regions showing homozygosity. Using conventional microsatellite markers, regions of homozygosity were further analyzed in all the families.

Results: A region on chromosome 3p spanning 43 megabases showed homozygosity with 13 consecutive SNPs. Three microsatellite markers from this region yielded lod scores > 3.00. A maximal two-point lod of 4.83 was obtained with the marker D3S1298 at $\theta = 0.004$. Haplotype analysis placed the disease gene in a 20 Mb interval between D3S1768 and D3S2409.

Conclusions: A gene causing autosomal recessive cataract maps to the short arm of chromosome 3.

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Congenital cataracts constitute a major cause of blindness in newborns and infants, with an estimated frequency of 1–6 per 10,000 live births [1]. About half of the cases are inherited as a monogenic non-syndromic trait, or as part of a complex genetic disease [2]. While autosomal dominant transmission is by far the most common mode of inheritance, autosomal recessive and x-linked inheritance have also been reported. Inherited congenital cataract is an extremely heterogeneous disorder.

The various types of opacities described in the different compartments of the lens are consistent with the large number of genetic loci recently described. Autosomal domininant non-syndromic cataract has been linked to 14 loci. These include mutations in five different crystallin genes, CRYAA, CRYAB, CRYBA, CRYBB and the gamma crystallin cluster at chromosome 2q34 [3–7], mutations in two gap junction genes, GJA8 and GJA3 [8,9], and mutations in AQP0, encoding a water channel protein [10]. Six additional loci have been mapped by linkage analysis but the disease-causing gene has not yet been identified [11–16]. The genetics of autosomal recessive cataract is less well understood. We recently described an inbred Jewish Persian family in which the autosomal recessive form of the disease was caused by a non-sense mutation in the CRYAA gene [17]. Interestingly, a missense mutation in the same gene (R116C) was shown to cause an autosomal dominant disease, suggesting a dominant negative effect [18]. In another family of Arab Muslim origin we excluded CRYAA as the disease-causing gene as well as the other 13 loci involved in the dominant form of the disease, thus providing proof for further genetic heterogeneity [17].

In this report we present the results of a genome-wide search for an additional gene causing autosomal recessive cataract in three highly inbred Arab Muslim families.

Methods

Families and DNA specimens

The families were recruited at the Sheba Medical Center, Tel-Hashomer, the Sapir Medical Center, Kfar Saba, and HaEmek Medical Center, Afula, Israel. The study protocol was approved by the Institutional Review Board. The participants or their guardians signed an informed consent in accordance with the tenets of the Helsinki Declaration. Affected individuals were

SNP = single nucleotide polymorphism

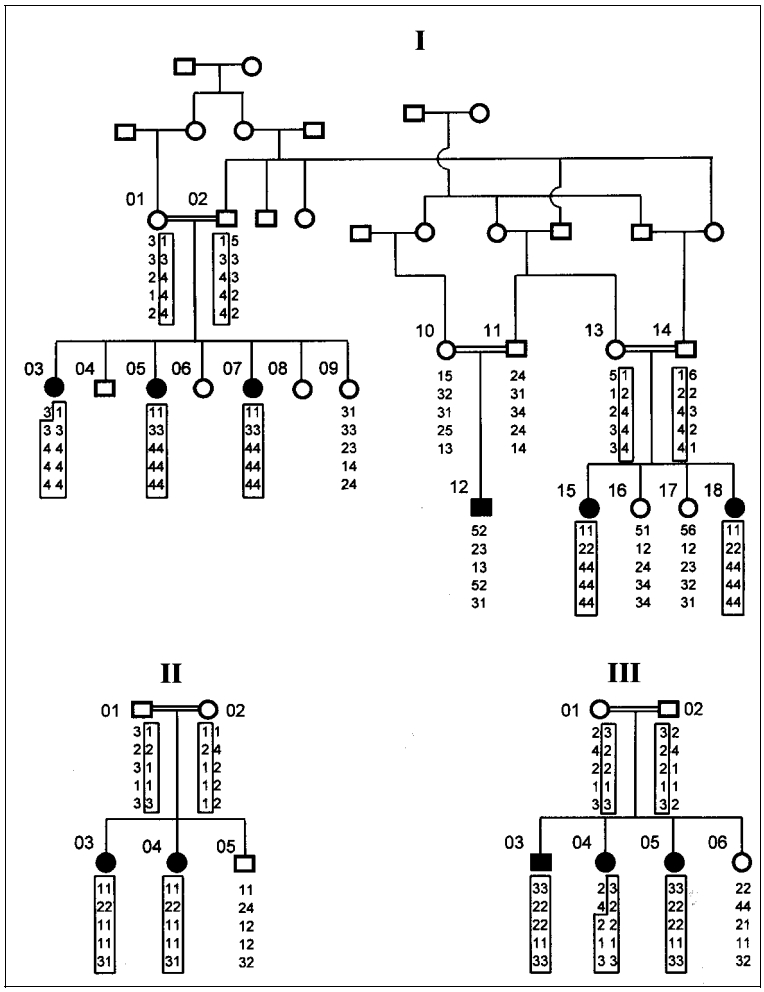

Figure 1. Typings for five chromosome 3p markers (from top to bottom): D3S2432, D3S1768, D3S1298, D3S2407 and D3S2409. Carrier chromosomes in the parents and affected individuals are presented in boxes.

diagnosed within a few weeks after birth, and operated upon during the first 3 months of life. No details were available regarding the histological findings in the lens. Ophthalmologic examination of the parents and unaffected siblings in both families did not reveal any ocular abnormalities. The parents of the affected sibs are first cousins in all three families [Figure 1]. Family 1 has been previously described [17]. Families 2 and 3 live in the same village and are distantly related.

Twenty milliliters of heparinized blood were drawn from each participant and DNA was extracted using a commercial kit (Gentra Systems, Minneapolis).

SNP genome-wide search

We pooled 50 ng of DNA from the six affected sibs of family 1 and sent it to Research Genetics (Huntsville, AL, USA) for SNP genotyping. This procedure was performed with the Effymetrix GeneChip HuSNP genotyping array. Results were provided as A, B or AB genotype, in each of 1,494 genetic markers.

Microsatellite genotyping

Markers described in this study included: D3S2432, D3S1768, D3S1298, D3S2407 and D3S2409. Amplification was carried out in a 10 µl reaction volume containing 50 ng of DNA, 13.4 ng of each unlabeled primer, 1.5 mM dNTPs, 0.08 µg ^{32}P-labeled primer in 1.5 mM $MgCl_2$ polymerase chain reaction buffer, with 1.2 U of *Taq* polymerase (Bio-Line, London, UK). After an initial denaturation of 5 min at 95°C, 31 cycles were performed (94°C for 2 min, 52°C for 3 min, and 72°C for 1 min), followed by a final extension time of 7 min at 72°C. Samples were mixed with 10 µl of loading buffer, denaturated at 95°C for 5 min and electrophoresed on a 6% denaturing polyacrylamide gel.

Linkage and haplotype analysis

Linkage was calculated with the LINKAGE (version 5.1) package of computer programs [19], assuming an autosomal recessive model of inheritance, 100% penetrance in both sexes and a gene frequency of 0.021 [20]. The marker order and distance was taken from published sources (http://cedar.genetics.sorton.ac.uk/pub/), and equal allele frequencies were assumed. Linkage was calculated separately for each of the three branches in family 1. Haplotypes were constructed so as to minimize recombinants.

Results

Due to consanguinity in these families, the disease gene in each family is most likely inherited from a common ancestor. Therefore, affected family members are expected to show homozygosity for the mutation and for polymorphic markers in the vicinity of the disease gene. A pooled DNA sample from the six affected sibs in family 1 was screened by 1,494 SNPs in the search for regions showing homozygosity. Of the 1,239 SNPs with known location on the 22 autosomes, 939 SNP markers fully amplified, yielding a success rate of 76%. A 43 Mb region on chromosome 3p showed homozygosity with 13 consecutive SNPs. Further analysis of this region with microsatellite repeats revealed three markers that yielded a lod score > 3.00.

Table 1. Two point LOD scores between autosomal recessive congenital cataract and chromosome 3p markers

Marker / θ	0.00	0.05	0.10	0.15	0.20	0.25	0.30	Max θ	Max Z
D3S2432	-infinity	2.38	2.46	2.24	1.90	1.52	1.13	0.09	2.48
D3S1768	-infinity	1.80	1.79	1.60	1.34	1.05	0.77	0.07	1.83
D3S1298	4.83	4.53	3.98	3.37	2.74	2.12	1.53	0.004	4.83
D3S2407	3.13	3.06	2.72	2.31	1.88	1.47	1.07	0.02	3.17
D3S2409	3.40	3.45	3.15	2.73	2.26	1.77	1.28	0.03	3.51

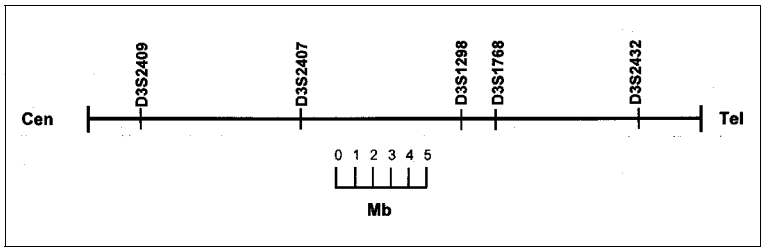

Figure 2. Schematic map of the region of linkage on chromosome 3p.

Pair-wise lod scores between congenital recessive cataract and 5 chromosome 3 markers are shown in Table 1. A maximal two-point lod score of 4.83 was obtained with the marker D3S1298 at $\theta = 0.004$. The relative position of these markers is shown in Figure 2. Typing results for the autosomal recessive cataract families and 5 chromosome 3p markers are presented in Figure 1. Patient 1-12 does not share the carrier haplotype found in his affected cousins and therefore was unhelpful in setting the boundaries of the gene interval.

Two affected individuals, 1-03 and 3-04, are recombinant for the marker D3S2432, but only one of them, 3-04, is also recombinant for the D3S1768, defining this marker as the telomeric boundary. Except for 1-12, no informative recombinations were observed at the centromeric boundary of the gene locus. However, loss of homozygosity in individuals 2-03 and 2-04 at D3S2409 helped us define this marker as the centromeric gene boundary. As in all the other families, the parents in family 2 are first cousins and therefore we may expect markers from the region of linkage to show homozygosity in the affected individuals. Indeed homozygosity in family 2 patients could be observed for all markers except D3S2409. This finding probably represents a recombination that had occurred in one of the grandparents, or in one of the great-grandparents.

Discussion

In this report we have shown that a gene causing autosomal recessive cataract maps to the short arm of chromosome 3. Recombination and homozygosity analysis place this gene in a 20 Mb interval between D3S1768 and D3S2409.

Using SNP genotyping, we performed a genome-wide search on a pooled sample of DNA from six affected sibs of a large inbred family. Over 30% of the SNPs showed homozygosity, a much higher percentage than originally anticipated, probably because of the high coefficient of inbreeding (0.021) in this community [20]. However, contiguous homozygosity of 10 or more SNPs was much less common, and the evaluation of such regions with microsatellite markers eventually disclosed linkage. The extensive region of SNP homozygosity around the disease-causing gene in family 1 suggests a relatively recent introduction of the cataract mutation into this family. Without this large area of homozygosity around the disease gene, the pooled sample analysis would not have been helpful in mapping this disease. Indeed, pooled samples from two other genetic diseases that we analyzed in the same way were unsuccessful in localizing the disease genes (E. Pras, unpublished data).

Interestingly, linkage to chromosome 3p was detected despite the fact that patient 1-12 has completely different haplotypes in this region, compared to the other patients. We assume that the signal produced by a single sample out of six in a pool, under the conditions used by the GeneChip probe array, is too weak to be detected. An alternative possibility is that the DNA of this individual failed to amplify within the pooled sample.

Three explanations could account for the haplotypes observed in patient 1-12. Firstly, this patient may represent a double recombination event, occurring both in the father and in a maternal predecessor. Secondly, congenital cataract in this patient is due to a different cause (phenocopy or genetic heterogeneity); and thirdly, cataract in this individual may have been misdiagnosed. In view of the high consanguinity rate among this population and the considerable size of the linked chromosomal segment, the first possibility seems by far the most likely. In this case, saturation of the interval with microsatellite repeats will help to define the recombination site. This in turn will considerably narrow the gene interval. Since families 2 and 3 are distantly related it is most likely that all affected individuals in these two families share the same mutation. Therefore, markers very close to the disease gene will exhibit a common allele in all the carrier chromosomes. Currently such a common allele is detected only for the marker D3S2407. Typing these two families with additional markers from the interval may help clarify this point.

Ehling et al. [2] estimated that about 30 loci are involved in autosomal dominant human congenital cataract. In mice, 40 such loci have already been mapped (http://www.ncbi.nlm.nih.gov/Homology/). The estimations of the number of loci involved in autosomal recessive congenital cataract are much smaller [2]. To date, proteins implicated in human cataract include the crystallins that were shown to have multiple functions including chaperone, autokinase, and glutathione S-transferase-like activity, in addition to their structural importance; aquaporines that serve as water channels; and gap junction proteins through which cells communicate [8–10]. Lens transparency also depends upon various enzymes such as catalase, enzymes from the glutathione redox cycle, and the mercaptopurinic pathway, all of which are critical for maintenance of a reducing environment in the lens [21,22]. Developmental regulation by factors such as Pax-6 is critical for the formation of the lens architecture [23].

Theoretically, any molecule found in the lens – whether structural, functional or developmental – may be involved in cataract formation. Using currently available databases, we did not identify any appealing candidate genes from this region. The recent completion of the human genome sequencing is bound to detect new genes from within this interval and will be of great help in the future cloning of this gene.

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