Refinement of the MYP3 locus on human chromosome 12 in a German family with Mendelian autosomal dominant high-grade myopia by SNP array mapping

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Abstract. Myopia, or short-sightedness, is the most common form of vision disorder worldwide. Higher levels of myopia, usually defined as an axial eye length of >26 mm or a refractive error of < -5.00 diopters are often designated as 'pathologic' myopia, because of the predisposition to develop further eye disorders such as retinal detachment, macular degeneration, cataract, or glaucoma. Many distinct forms of autosomal dominant non-syndromic high-grade myopia are described in humans. While the underlying chromosomal locations and critical disease intervals have been identified and located to physical map positions, the gene defects and causative mutations responsible for autosomal dominant myopia remain elusive to date. Examination of a German six-generation kindred by 10K whole genome chips led to the identification of a 19-cM map segment as being the most likely familial myopia candidate region spanning from chromosomal band 12q14.3 to 12q21.31 (MYP3). In our family, a maximum multi-point LOD score of 3.9 was obtained between rs1373877 and rs717996. The recombination breakpoints in this family and the interval of the originally reported German/Italian family defining the MYP3 locus on chromosome 12 (OMIM 603221, two-point LOD score 3.85 for markers *D12S1706* and *D12S327* at 12q21-23) allowed us to significantly refine a minimum consensus region. This new composite region is located between microsatellite marker *D12S1684* at 75.8 K and SNP_A-1509586 (alias rs717996) at position 82,636,288 bp, and narrows the original 30.1 cM of the MYP3 interval to 6.8 cM. The refined MYP3 interval allowed us to restrict the list of database-indexed genes to 25, several of which are promising MYP3 candidates based on similarities with genes and proteins involved in vision physiology and eye disease. While autosomal dominant high-grade myopia is recognized to be genetically heterogeneous, our results suggest genetic homogeneity of the MYP3-based condition in families that share the same ethnic and geographical background. The future identification of this MYP3 gene may provide insights into the pathophysiology of myopia and eye development.

Introduction

Refractive errors are by far the most common ocular disorders in humans, with myopia (short-sightedness) alone accounting for ~25% of cases of uncorrected impaired vision in populations of the Western world (1-3). High-grade myopia (≤ -5 diopters) affects 4.5% of the U.S. population 40 years of age and older (4), and is a major cause of legal blindness in many developed countries (5-7), as it predisposes individuals to premature cataracts, glaucoma, retinal detachment, and macular degeneration (8-12). While in most cases refractive errors pose a minor restriction that can be corrected with glasses, contact lenses or refractive surgery, in some parts of the world myopic refractive errors have become a significant public health problem. Myopia is especially common in some urban Asian regions, such as Singapore, Taiwan, Japan and Hong Kong, where 60-80% of young adults are myopic (13). Moreover, a continuous increase in the worldwide prevalence and severity of myopia has been observed over the past decades, a trend that appears to be dramatically affecting some developed Asian countries (14).

Myopia represents a highly heterogeneous disorder with varying severity of phenotypes and patterns of inheritance, but

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an autosomal dominant heredity is the most frequent mode of inheritance observed in families. The lack of molecular and biochemical data hinders the establishment of successful schemes for therapy including approaches such as gene therapy. Based on genetic studies in families and/or unselected single patients with inherited myopia, up to 15 loci responsible for or involved in non-syndromic myopia have been mapped on human chromosomes to date. Despite the considerable number of recognized loci, no disease genes, either for X-chromosomal recessive ('MYP1' OMIM 310460, MYP13 OMIM 300613) (15-17) or autosomal dominant disease phenotypes (MYP2 OMIM 160700, MYP3 OMIM 603221, MYP4 OMIM 608367, MYP5 OMIM 608474, MYP11 OMIM 609994, MYP12 OMIM 609994, and myopia locating to chromosome 10q21.1) (18-24) have been identified. This also holds true for the genes involved in common myopia where six independent loci have been determined in the recent past (MYP6 OMIM 608908, MYP7 OMIM 609256, MYP8 OMIM 609257, MYP9 OMIM 609258, MYP10 OMIM 609259, and MYP14 OMIM 610320) (25-27).

In a large German/Italian family with non-syndromic pathological high-grade myopia, the candidate locus was linked to 12q21-23 (OMIM 603221), i.e. the critical interval of the MYP3 locus (19). Here, we report on a second family from Germany mapping also to the MYP3 region. To the best of our knowledge this is the first SNP chip-based study on high-grade pathological myopia where a significant refinement was achieved by eliminating 75% of the original interval. By analysing the family members with whole genome 10K SNP chips and by reviewing available genetic and physical maps we narrowed the critical region, originally 30.1 cM, to only 6.8 cM.

Despite this significant reduction of the MYP3 locus, the physical distance was still substantial to allow a directly accessible search and straightforward selection of candidate genes with suitable expression profiles of a predictive gene causing short-sightedness in humans. Our new MYP3 family which is unrelated to the previously reported German/Italian family suggests genetic homogeneity of the MYP3-based myopia on chromosome 12q21. Linkage details and SNP-based haplotype mapping data were assessed and compared to the originally described interval that was established from microsatellite analyses (19) to construct a so-called consensus region matching the data of both multi-generational families. In addition, the microsatellite-based data from a non-whole genome UK study on the MYP3 locus (including MYP2 and 17q) is discussed in this context (28).

Materials and methods

Subjects. Twenty-eight members (8 affected) of a large six-generation family from Kiel in northern Germany (Pedigree MYPfam40, Fig. 1) consented to participate in the study. Written informed consent was obtained from all study participants and from parents of patients younger than 18 years of age according to the requirements of the Ethics Committee of the University of Tübingen. The diagnosis of myopia was made on the basis of a complete ophthalmic examination including recording of the spherical equivalent obtained from cycloplegic refraction. Our family (46 individuals in total, Fig. 1) was selected based on the presence of affected individuals (12 in total) in multiple generations (1-4 individuals per generation). Individuals with high-grade myopia, defined as a spherical refractive error of ≤ -5.00 diopters in at least one eye, were considered 'affected' when adults. Individuals with a spherical refractive error between -2.00 and -5.00 diopters in at least one eye were also considered 'affected' when they were still in childhood/adolescence; e.g., this was the case for the young individual 4-46 (Fig. 1). All other individuals (spherical refractive errors > -5.00 diopters in both eyes in adults, and > -2.00 diopters in children) were considered 'not affected'. No participant had a known ocular disease or insult that could predispose to myopia or any known genetic syndrome associated with myopia. Glaucoma (only in individual 4-21), keratoconus, corneal thinning, lenticonus, and lens dislocation were absent in the study participants. Cataracts were present in members 4-24 and 4-25. The family did not display any other systemic genetic traits. Details of the patients and the ophthalmic examinations are summarized in Table I. DNA was isolated from peripheral blood lymphocytes using standard techniques (Gentra Systems, Minneapolis, MN).

Genome-wide linkage analysis. We genotyped 28 DNA samples from a German myopic kindred consisting of 46 family members. We analysed 8 affected individuals (4-21, 4-23, 4-32, 4-36, 4-41, 4-42, 4-43, 4-46), 13 unaffected persons (4-25, 4-33, 4-34, 4-310, 4-44, 4-45, 4-47, 4-48, 4-49, 4-410, 4-411, 4-412, 4-413), 6 spouses (4-24, 4-26, 4-31, 4-35, 4-38, 4-39), and one phenotypically unknown person (4-37) using the Affymetrix GeneChip Human Mapping 10K Array, ver. 2.0 (Affymetrix, Santa Clara, CA). This version of the Mapping 10K array comprises a total of 10,024 SNPs with a mean intermarker distance of 258 kb, equivalent to 0.36 cM. Genotypes were called by the Affymetrix GeneChip DNA Analysis Software (GDAS) ver. 2.0. Genders of samples were verified by counting heterozygous SNPs on the X chromosome. Relationship errors were evaluated with the help of the program Graphical Relationship Representation (29). The program PedCheck was applied to detect Mendelian errors (30), and data for SNPs with such errors were removed from the data set. Non-Mendelian errors were identified by using the program Merlin (31) and unlikely genotypes for related samples were deleted. Non-parametric linkage analysis using all genotypes of a chromosome simultaneously was carried out with Merlin. Parametric linkage analysis was performed by the program Allegro (32). In each calculation we used alternating and overlapping sets of short intervals consisting of 50 SNP markers thereby scanning the chromosomes like a 'moving window'. We assumed dominant inheritance with complete penetrance; 0.0001 disease allele frequency. Haplotypes were reconstructed with Allegro and presented graphically with HaploPainter (33). All data handling was performed using the graphical user interface Alohomora (34).

Results

A multi-generational family consisting of 46 individuals from the northern part of Germany, termed MYPfam40, with

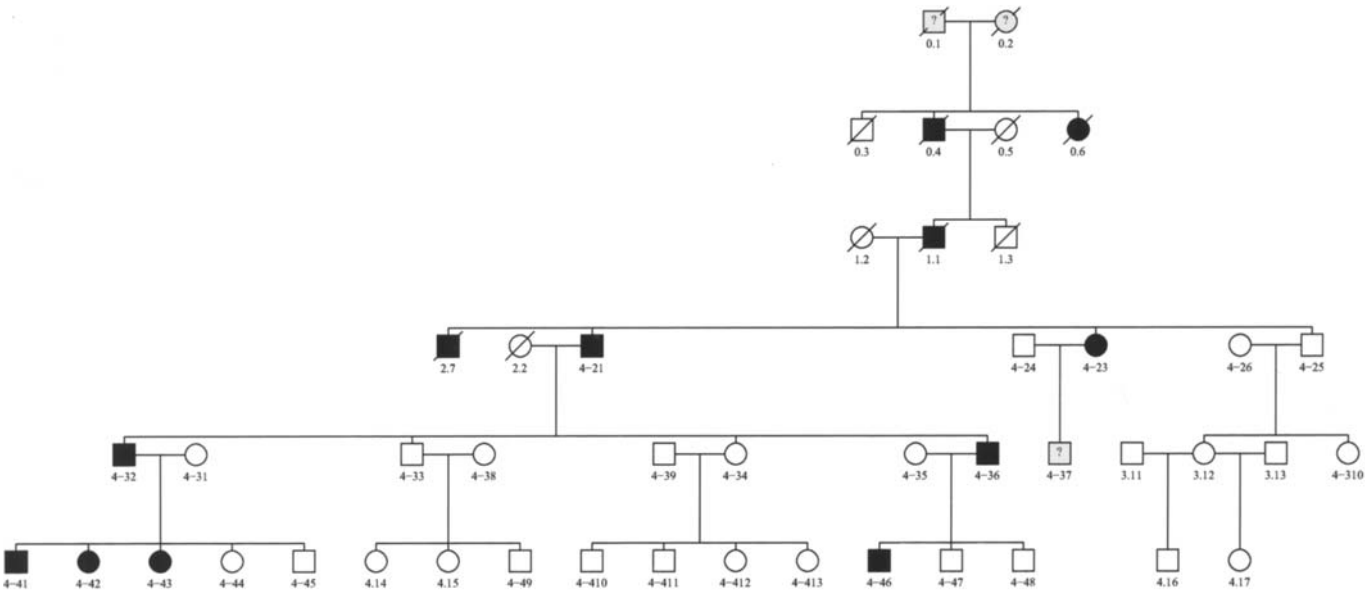

Figure 1. Family MYPfam40, with familial autosomal dominant high-grade myopia. Circles and squares denote females and males, respectively; blackened symbols denote affected individuals. A questionmark within the symbol denotes that the affection status is unknown.

Table I. Clinical characteristics of pedigree MYPfam40 family members from northern Germany with high-grade myopia.

No.	Subject	Gender	Year of birth	First exam (spherical equivalent)	Second exam
1	0.4	Male	<1850	~ -8/-9 diopters[a]	Deceased
2	1.1	Male	1877	~ -15/-15 diopters[a]	Deceased
3	2.7	Male	1915	~ -4/-5 diopters[a]	Deceased
4	4-21	Male	1917	-14.0/-19.0 diopters	-13.0/-19.0 diopters
5	4-23	Female	1926	-4.0/-5.25 diopters	-3.75/-5.25 diopters
6	4-32	Male	1954	-10.5/-9.5 diopters	-9.0/-10.0 diopters
7	4-36	Male	1966	-7.75/-5.5 diopters	-9.0/-7.0 diopters
8	4-41	Male	1980	-6.75/-5.5 diopters	-7.0/-6.0 diopters
9	4-42	Female	1981	-7.25/-8.0 diopters	-7.5/-7.5 diopters
10	4-43	Female	1984	-6.5/-6.5 diopters	-6.25/-6.25 diopters
11	4-46	Male	1994	-1.4/-1.4 diopters	-2.5/-2.0 diopters

[a]Spherical equivalents were not considered for the estimation of mean values.

high-grade myopia was identified and characterized. All affected persons reported poor uncorrected vision from infancy with a progression in severity during school age. The mode of inheritance was in concordance with an autosomal dominant inherited model of myopia (Fig. 1). The average spherical equivalent of affected individuals of pedigree MYPfam40 was -8.32 diopters (range, -3.75 to -19.00) (Table I). The range was from -2.00 to -19.00 diopters when considering the child 4-46 in the latest generation (additional information in Materials and methods). In two patients with retinal detachment we were able to obtain pre-operation refractive error information (individuals 4-21 and 4-36, Table I). All patients designated as affected individuals were therefore included in the calculation for the mean value of the spherical equivalent. Two measurements of cycloplegic refraction were performed for each individual in an interval spanning from 2 to 5 years to determine the progressive status of the disorder (Table I). None of the patients from family MYPfam40 were anisometropic, i.e. with one highly myopic and one moderately myopic eye. However, two of the healthy persons were anisometropic due to cataract formation in one of the eyes, i.e. person 4-24 (-1.25/-7.75 diopters) and family member 4-25 with moderate anisometropia (+0.25/-2.25 diopters). The spherical refractive error of individual 4-37 was not obtained and thus designated 'unknown' in the pedigree. Since we performed a microarray-based whole-genome analysis we were able to exclude all known syndromic myopia loci (e.g. Stickler syndrome type 1 on chromosome 12, Stickler syndrome type 2 on chromosome 1, Stickler syndrome type 3 on chromosome 6, Marfan syndrome on chromosome 15, juvenile glaucoma on chromosome 1, Knobloch syndrome on chromosome 21) in this family on the

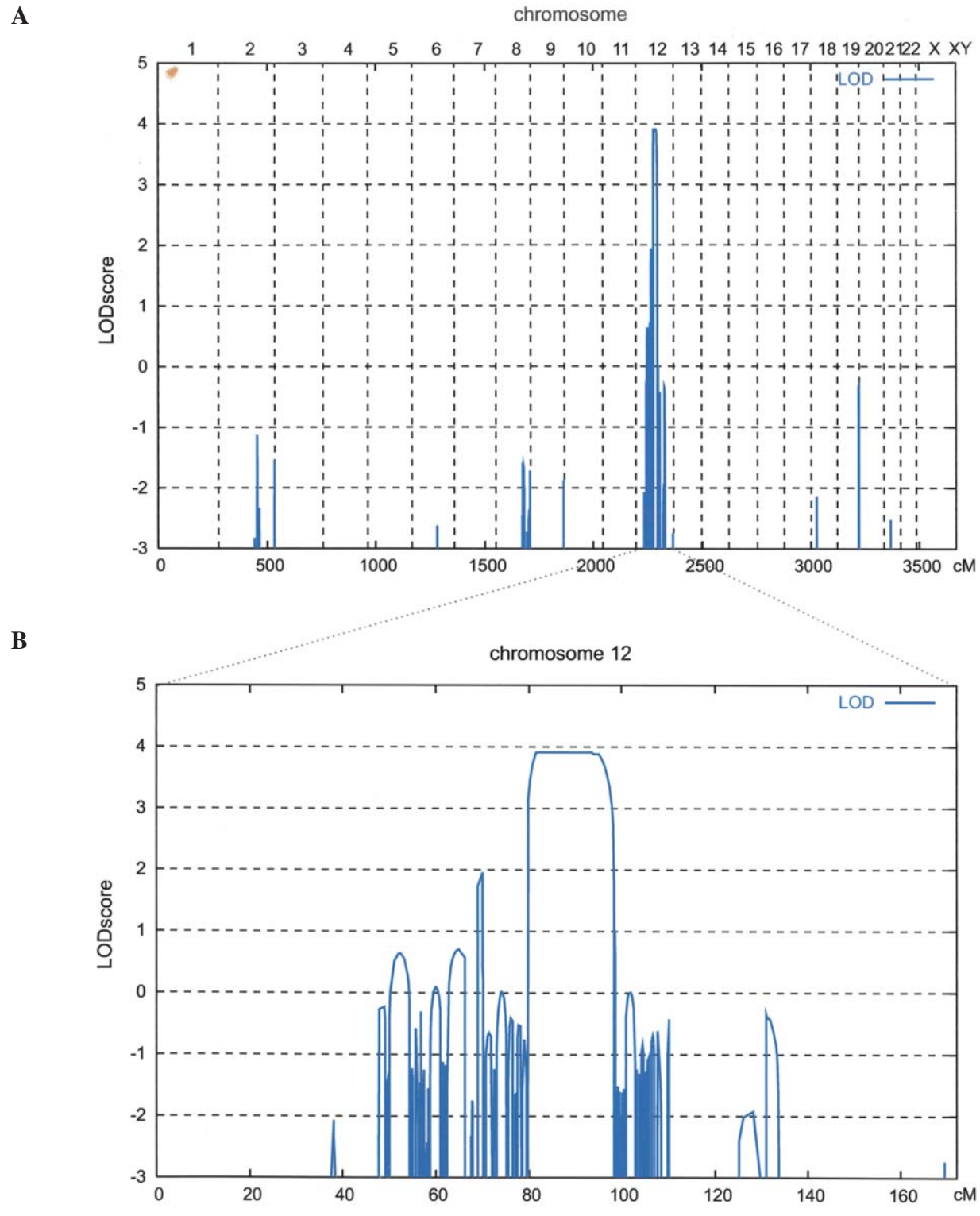

Figure 2. Mapping of the smallest MYP3 region in the German family MYPfam40 with high-grade myopia. The program ALLEGRO (32) was used with overlapping SNP sets consisting of 50 markers. (A) Overview of the genome-wide data of parametric linkage analysis. The highest LOD scores were obtained with chromosome 12 markers. (B) Magnification of chromosome 12: a maximum LOD score of 3.9 for the region between 78.43 and 97.3 cM (according to the deCODE map) is shown.

basis of non-significant LOD score values far smaller than zero (Fig. 2A). Furthermore, all known non-syndromic loci for common and high-grade myopia forms were excluded by determining LOD scores < -2 with the exception of the MYP3 locus on chromosome 12 (Fig. 2A and B). A subsequent fine-analysis of chromosome 12 data identified a maximum LOD score of 3.9 between 78.43 cM and 97.3 cM (Fig. 2B). Haplotype analysis of the significant LOD score region spanning from 12q14.3 (SNP_A-1507739) to 12q21.31 (SNP_A-1509586) revealed a consistent affected haplotype in all branches of the family (Fig. 3A). As mentioned earlier, this safely excludes the possibility of mutations in the

collagen type II α-1 gene (*COL2A1*) on chromosome 12 that has been shown to be connected to a syndromic myopia phenotype called Stickler syndrome type 1 (*D12S85*). This syndromic locus has been mapped to region 12q13.11-q13.2 which resides proximal to the MYP3 locus and is thus not overlapping with our familial interval of MYPfam40.

Haplotype analysis in our family members revealed that recombination events limited our familial 19-cM interval to position 63,662,789 bp proximally and to position 82,636,288 bp distally (Fig. 3B). In comparison, the region originally described for the MYP3 locus was defined as a 30.1-cM interval with recombination events that identified markers *D12S1684* and *D12S1605* as flanking markers on chromosome 12q21-23. Both flanking markers matched position 75,787,368-75,787,609 bp (*D12S1684*: 89.85 cM deCODE, 86.40 cM Marshfield) and position 107,227,972-107,228,172 bp (*D12S1605*: 122.79 cM deCODE, 116.66 cM Marshfield) respectively. The resulting overlap between the MYP3 locus of the original German/Italian pedigree (19) and our German MYPfam40 interval is therefore between position 75,787,368 and 82,636,288 bp. Thus, the new composite minimal interval consists of the proximal marker of the original MYP3 locus in 12q21 and the distal marker in 12q21.31 which derives from our family (Fig. 4). This new interval has an extension of <7 cM, i.e. in the corresponding physical maps this equals a region of ca. 6.9 Mb. The original interval described by Young and coworkers (19) containing 285 genes could be narrowed to a composite region with only 25 genes. We attempted a straightforward selection of myopia candidate genes and identified *synaptotagmin I* as a promising peptide that may be involved in the pathogenesis of myopia. Of note, tentative candidates encoding extracellular matrix proteins *keratokan* (*KERA*), *lumican* (*LUM*) and *decorin* (*DCN*), and mapped to the original MYP3 interval and being intensely discussed by certain authors (19,35-39) are now located distally to our new composite consensus interval of autosomal dominant high-grade myopia on chromosome 12.

Discussion

Tight genetic determination is a hallmark of Mendelian genetics in which the principle of genetic linkage analysis is applied to identify gene location and subsequently mutations causing hereditary disease. The familial occurrence of myopia has been described in numerous pedigrees as a discrete, segregating phenotypic trait, mostly based on the distinction of low and high grades of myopia. All types of Mendelian modes of inheritance of familial myopia have been described; autosomal dominant modes being the most frequently documented. Though the definition of 'high' is arbitrary in genetic terms, high refractive errors are more likely to result from a major effect mutation than low grades of refractive error (40).

The identification of genetic factors contributing to the development of high-grade myopia has been mainly complicated by both the genetic and aetiological heterogeneity of the disorder. The latter implies the strong influence of environmental effects on myopia development besides genetic factors. Furthermore, as a quantitative trait, myopia lacks the ease of being classified into a few discrete classes that are meaningful in order to decipher the disease origin. In addition, myopia, in physiological terms, is the end point in the interplay of a number of quantitative ocular traits (e.g. corneal curvature and axial length). Despite these limitations in defining the disease phenotype, the distinction of affected and unaffected individuals based solely on clinical grounds, i.e. refractive error and propensity to develop further ocular complications, has allowed considerable progress in the study of myopia development. Two X-linked recessive forms of myopia/cone dysfunction and sole myopia have been identified (MYP1, OMIM 310460 and MYP13, OMIM 300613) on chromosome Xq28 (15,16) and Xq23-25 respectively (17). The vast majority of studies revealed families with high-grade myopia and an autosomal dominant mode of inheritance. Significant linkage was reported for the following chromosomal positions: 18p11.31 (MYP2, OMIM 160700) (18), chromosome 12q23.1-24 (MYP3, OMIM 603221) (19), chromosome 7q36 (MYP4, OMIM 608367) (20), chromosome 17q21-23 (MYP5, OMIM 608474) (21), chromosome 4q22-q27 (MYP11, OMIM 609994) (22), chromosome 2q37.1 (MYP12, OMIM 609994) (23), and at chromosome 10q21.1 (24).

However, determination of genetic loci for familial high-grade myopia has generally been based on just a few families with little replication of linkage by other investigators (41,42). Based on a two-point LOD score 3.85 for markers *D12S1706* and *D12S327* a locus for fully penetrant non-syndromic high-grade myopia, MYP3, was found in a single large German/Italian family at 12q21-23 (19).

In one microsatellite-based UK study of 245 individuals from 51 families, the 12q locus MYP3 was also found to be responsible for high-grade myopia in the UK in more than 25% of cases with autosomal dominant transmission (28). No significant contribution of either the 18p or 17q locus was found, suggesting genetic heterogeneity in familial high-grade myopia. However, in another study on the genetically isolated population of Ashkenazi Jews with less severe forms of myopia, an association with the previously assigned chromosome 12 (MYP3) and 18 (MYP2) regions was excluded, but evidence was reported for a new locus on chromosome 22q (25).

We examined a large family with pathological myopia by molecular means of whole genome chips with a screening density of 10K. We were able to exclude linkage to the candidate gene regions/loci for disorders such as juvenile glaucoma, Stickler syndrome, and Marfan syndrome to ensure that our family did not exhibit a mild phenotypic expression or phenocopy of high-grade myopia for any of these autosomal dominant, early-onset disorders. The German multi-generation family investigated in this study now provides the smallest critical interval reported so far for autosomal dominant myopia on human chromosome 12. A common disease haplotype of 19 cM is seen for an SNP marker set spanning from 63,662,789 bp down to position 82,636,288 (Figs. 3B and 4). However, the interval derived from the study of the original German/Italian MYP3 family (19) extends distally, but limits the interval of our German MYPfam40 family proximally. Thus, the so-called composite consensus interval between the proximal marker *D12S1684* at position 75,787,368-75,787,609

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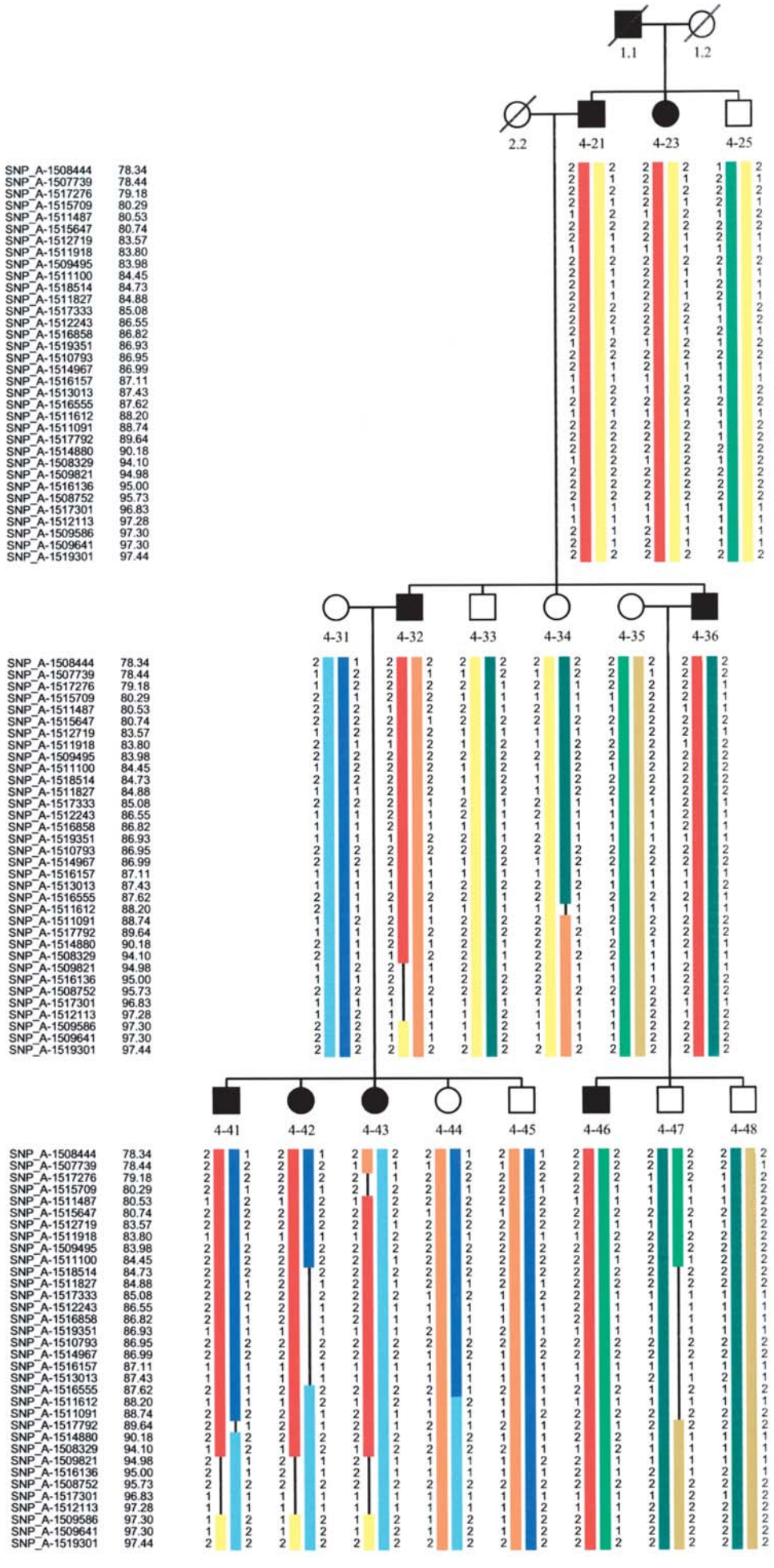
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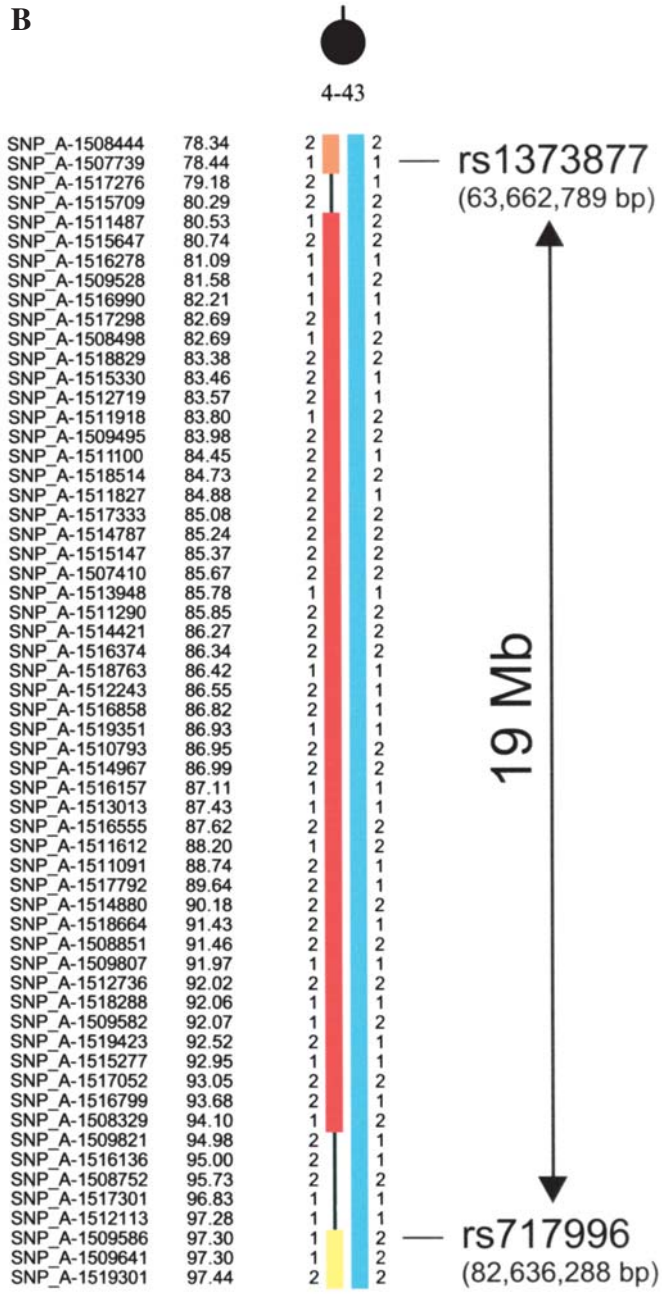

Figure 3. Segregation analysis in family MYPfam40 using microarray technology. (A) The alleles for some of the SNPs are shown (please note that only the informative branches of the pedigree are shown). Haplotypes were constructed on the basis of the minimum number of recombinations between these markers. The chromosome assumed to carry the inherited disease allele is depicted as a red bar while other colours represent the non-disease haplotypes. Only essential matings are shown; non-participating family members are not shown. (B) Exemplary MYP3 haplotype of the affected female individual 4-43 showing the total number of SNP markers tested. The minimal interval is defined as a 19-Mb region. A thinner black line denotes identical parental marker-allele assignment.

(19) and the distal single nucleotide polymorphism rs717996 (alias SNP_A-1509586) at position 82,636,288 is the smallest one reported so far for the MYP3 locus (Fig. 4).

In this refined 6.8-cM candidate region the databases revealed the location of only 25 genes; however, expression data for the eye, and in particular for the scleral tissue were available for only a few of them. Since, on theory, any of the genes within a candidate region could be involved in the disease phenotype, additional criteria for distinction were required such as a possible function within the target tissue, membership to a well-known protein family already shown to be involved, or a specific expression pattern. In our MYP3 interval, several candidates appeared to be highly important. *Synaptogamin I* (*SYT I*), for instance, a ubiquitous synaptic membrane protein, that is important in vesicular trafficking and exocytosis, and expressed in the retina and visual cortex, has been ascribed a role in neurite growth during development of the visual system. *SYT I* may be involved in the molecular framework responsible for myopia development, since monocular deprivation, a common stimulus in experimental myopia, has been shown to induce changes in *SYT I* expression in the visual cortex (43,44).

The genetic intervals identified by linkage analysis in families with segregating high-grade myopia harbour a number of loci encoding structural tissue proteins that could be involved in scleral tissue remodelling during myopia development. For example, four genes have been localized to the vast genetic region of 30.1 cM on chromosome 12q21-q23 (MYP3) each encoding a member of the family of structural proteins known as small leucine-rich repeat proteoglycans (SLRPs). Owing to their extracellular matrix expression in the sclera, two of these *SLRP* genes, *decorin* (12q21-22) (45) and *lumican* (12q21.3-q22) (46,47), have been suggested as candidate genes in the aetiology of familial high-grade myopia. Both interact with collagen and limit the growth of the fibril diameter (48-52). The association of structural gene mutations with high-grade myopia is well established from disease entities such as Stickler, Marfan and Ehlers-Danlos syndromes and hereditary ocular diseases sch as congenital stationary night blindness type I, where high-grade myopia is part of the phenotype. These disorders, which are associated with gene mutations in principal structural components of various extracellular matrices, provide the strongest support for the scleral theory of myopia development (53). According to this theory, an alteration in the structure and composition of the sclera occurring on axial eye elongation may be a critical determinant of the genesis of myopia. Results from animal studies investigating the role of *decorin*, one of the candidate proteins for familial high-grade myopia mapped to 12q21-22, have been equivocal. While a nearly steady *decorin* synthesis in response to experimentally induced myopia was noted in neonatal primates (54), reduced proteoglycan synthesis was observed when adolescent primates were investigated (55). Independent studies on the role of *lumican* in experimentally induced myopia produced more consistent results (56,57). Lumican-deficient mice were shown to have a disrupted collagen fibril formation of the sclera, which, based on volumetric estimations, is associated with larger eyes (58). However, *lumican* has been excluded as the causative gene in a family with 12q21-22-linked high-grade myopia (36,37). It has been suggested that the SLRPs *decorin* and *lumican* may play a role in scleral collagen fibril formation and organization of the extracellular matrix through inhibition of spontaneous collagen molecule assembly (50). The recognition of the key role played by SLRPs in myopia development has prompted investigators to search for a

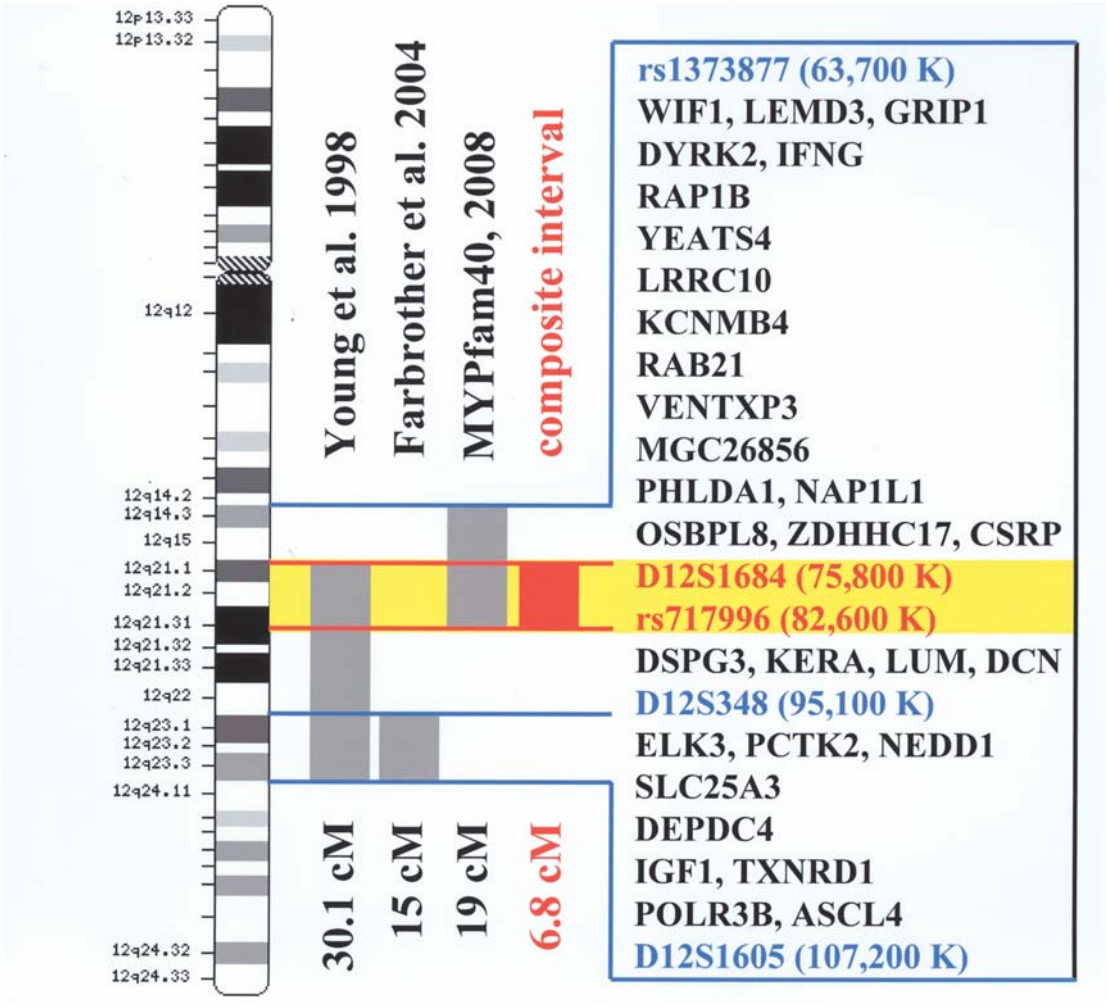

Figure 4. Schematic representation of a linkage map of microsatellite (19,28) and SNP markers (own data) located at the MYP3 region. The mapping order and genetic distances [in centiMorgans (cM)] were primarily obtained from the human genetic map of deCODE. The grey-scale segments denote the linked intervals determined by multipoint linkage, SNP and haplotype analysis. The new composite interval is shown as a bold red segment refining the original 30.1 cM region to only 6.8 cM. The cytogenetic sketch of chromosome 12 was taken from NCBI. To note, unlike the other studies, the study of Farbrother and colleagues (28) did perform a 40-cM screen on three selected chromosomal regions rather than a whole genome scan.

genetic association between common myopia and other SLRP family members [keratocan, fibromodulin, biglycan, dermatan sulfate proteoglycans (DSPG 3)] (38). To date, none of these candidate proteins or loci has been found to be causally linked to common, juvenile-onset myopia (59). Indeed, a mutational screening of selected SLRP genes (*LUM*, *FMOD*, *PRELP* und *OPTC*) mapped to chromosomes 1 and 12 (39) as well as the X-chromosome (nyctalopin, *NYX*) (60-62) revealed unfrequent alterations, some of them segregating with the disease, and some of them not. However, for our research on familial autosomal dominant myopia only the genes mapping to chromosome 12 were of interest. These promising candidates described afore, however, reside outside the newly refined 6.8-cM composite interval. The distal border of this interval is at position 82,636,288 in 12q21.31, and cen-*DSPG3-KERA-LUM-DCN*-tel on the long arm of chromosome 12 maps to a distant genomic region spanning from position 89,881,591 to 90,100,937.

Another linkage study from the UK suggested that approximately one quarter of 'seemingly' autosomal dominant families were linked to the MYP3 locus (28). These subjects were genotyped for microsatellite markers spanning ~40 cM of regions on 18p, 12q and 17q. Their 95% confidence interval suggested that it was unlikely that either none of the families, or more than half of the families were linked to this locus. Assessment of haplotypes demonstrated that 21 of the 42 families were consistent with linkage, but of these only one pedigree (i.e. number 24) had a multipoint LOD exceeding 1.0. Recombination events in these families suggested that the disease gene is harbored within the narrowed interval (ca. 15 cM) between markers *D12S348* and *D12S1605*. This region vastly matches with the most distal portion of the original MYP3 interval, i.e. mapping to 12q23.1-q23.3 (95,100 to 107,200K). Notably, this interval determined in the study does not overlap our interval which locates to the more proximal part of the original MYP3 locus (Fig. 4). This may be explained by the fact that the LOD score obtained in the UK study was not significant, that the family was too small for obtaining an informative number of necessary meioses, that the mode of inheritance was only assumed to be autosomal dominant and/or that they did not perform a whole genome scan, but a screen of selected regions

of three chromosomes known to be involved in pathological myopia. On the other hand, while rather unlikely, the finding may point to genetic heterogeneity at the MYP3 locus.

In summary, we mapped a German family to the proximal part of the 30.1-cM critical MYP3 region (LOD score of 3.9). By combining the microsatellite data of the pioneer family for the MYP3 locus (19) and our whole genome SNP chip data we refined the locus to a 6.8-cM region harbouring only 25 genes. We continue in our efforts to further characterize the region for MYP3 high-grade myopia, through analysis of other families and by conducting a straightforward DNA sequencing of the entire MYPfam40 region in 12q21 by the Genome sequencer 20 system to identify the gene responsible for this myopia phenotype. Construction and analysis of scleral cDNA libraries may enhance the throughput in identifying the myopia gene(s), hopefully providing insights into the molecular mechanisms underlying the regulation of eye growth.

This is a pioneer gene chip-based study on familial high-grade myopia, and it contributes new data to the research of short-sightedness in humans.

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