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ARTICLE

Domain disruption and mutation of the bZIP transcription factor, *MAF*, associated with cataract, ocular anterior segment dysgenesis and coloboma

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Human congenital cataract and ocular anterior segment dysgenesis both demonstrate extensive genetic and phenotypic heterogeneity. We identified a family where ocular developmental abnormalities (cataract, anterior segment dysgenesis and microphthalmia) co-segregated with a translocation, t(5;16)(p15.3;q23.2), in both balanced and unbalanced forms. We hypothesized that this altered the expression of a gene of developmental significance in the human lens and ocular anterior segment. Cloning the 16q23.2 breakpoint demonstrated that it transected the genomic-control domain of *MAF*, a basic region leucine zipper (bZIP) transcription factor, first identified as an oncogene, which is expressed in vertebrate lens development and regulates the expression of the eye lens crystallins. The homozygous null mutant *Maf* mouse embryo demonstrates defective lens formation and microphthalmia. Through mutation screening of a panel of patients with hereditary congenital cataract we identified a mutation in *MAF* in a three-generation family with cataract, microcornea and iris coloboma. The mutation results in the substitution of an evolutionarily highly conserved arginine with a proline at residue 288 (R288P) in the basic region of the DNA-binding domain of MAF. Our findings further implicate *MAF/Maf* in mammalian lens development and highlight the role of the lens in anterior segment development. The 16q23.2 breakpoint transects the common fragile site, *FRA16D*, providing a molecular demonstration of a germline break in a common fragile site.

INTRODUCTION

Cataract is one of the major causes of treatable blindness in humans. While congenital forms are less common, as are abnormalities of ocular anterior segment development, both are difficult to treat and represent a significant cause of childhood blindness (1,2). A large proportion of congenital isolated cataract, perhaps up to 50%, is inherited with the majority in an autosomal dominant pattern. Mutations in many genes have now been identified in a small percentage of congenital cataract patients, underlining the genetic heterogeneity within this group (3). The majority encode lens fibre-specific proteins such as crystallins or lens fibre transmembrane molecules, whereas two are encoded by the transcription factors PAX6 and PITX3 (4,5).

The lens and the anterior segment are linked in development with a commonality of origin in surface ectoderm components, a necessity for separation of these components for anterior chamber formation and a requirement for signalling from the lens for anterior segment organization (6). This suggests that whereas some cases in the heterogeneous group of conditions known as anterior segment disorders may arise due to abnormalities in neural crest migration, it is likely that others arise due to abnormalities in lens development. In humans, Peters anomaly is a form of anterior segment dysgenesis characterized by central corneal opacity, where cataract may also be present (7), and there may

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be a spectrum of these anomalies within the one family (8). Mutations in *Pax6* (*small eye*)/*PAX6* and *Pitx3* (*aphakia*)/*PITX3* can result in such anomalies of the lens and anterior segment in murine and human ocular development (5,9–11). In humans, mutations in these genes are also described in individuals with isolated cataract (4,5). Peters anomaly may also be associated with other ocular malformations, such as microphthalmia, microcornea and coloboma (12,13). These findings suggest that a gene known more specifically for its involvement in either lens or anterior segment development may be a candidate as a disease-causing gene for either cataract or anterior segment dysgenesis, and that one such gene may be associated with a spectrum of lens and anterior segment anomalies.

Maf is a basic region leucine zipper (bZIP) transcription factor expressed in the lens placode, vesicle and, later, the primary lens fibres (14–17). The key role of *Maf* in eye development is highlighted by the fact that homozygous null mutant mouse embryos exhibit defective lens formation and microphthalmia, and show decreased expression of crystallins (15,17,18). Both homodimers and heterodimers formed by Maf bind to two known Maf response elements (MAREs), with varying affinities and transactivation potentials (19,20). MARE elements are found in the promoters of the crystallin genes and *Pitx3* (10,21). The Maf family of bZIP transcription factors was first identified through the *v-maf* oncogene, an avian retrovirus transforming gene, and *maf* is the cellular homologue (22). It is a member of the 'large Maf' family (23), which also includes Krml (the product at the mutant *kreisler* locus, now renamed *Mafb*), important in hindbrain and otic development in the mouse (24) and Nrl which functions in the neural retina (25). To date, *NRL* is the sole member of the Maf family associated with a human disease phenotype, autosomal dominant retinitis pigmentosa (26).

We have identified two families where abnormalities of the lens and the anterior segment of the eye are segregating in association with likely abnormality of MAF function in early ocular development. In the first family, congenital cataract co-segregates with a translocation, t(5;16)(p15.3;q23.2), in balanced and unbalanced forms, and Peters anomaly is also present in unbalanced cases. Our molecular analysis in this family shows that the translocation breakpoint on 16q23.2 lies close to the lens-development gene, *MAF*, and transects the common fragile site, *FRA16D*. In the second family, congenital cataract segregates in a three-generation family, and microcornea and iris coloboma are also associated. In this family, we have identified a mutation in the DNA-binding domain of *MAF*. These findings implicate *MAF* as a disease gene in the complex pathway of transcriptional regulatory genes involved in human lens development and related ocular anterior segment development.

RESULTS

Clinical findings

In family 1 (Fig. 1A), individuals (I.1 and II.3) with a balanced translocation, 46,XY,t(5;16)(p15.3;q23.2) had juvenile-onset progressive cataracts. The cataracts were widespread, cortical pulverulent opacities with anterior and posterior sutural densities (Table 1). These individuals were otherwise well. Individuals with an unbalanced derivative karyotype, 46,XX,der(5),t(5;16)(p15.3;q23.2) also had congenital cataracts but had more severe ocular phenotypes. In one case, there was progression to total cataracts and severe myopia (II.2), while two (III.2 and III.3) had opaque corneas. III.3 had bilateral Peters anomaly, with small cataractous lens remnants, rims of abnormal iris and microphthalmia (Fig. 1B). Those with the unbalanced karyotype were also developmentally delayed, had dysmorphic facial features and one (III.2) died soon after birth due to laryngeal stenosis. Two individuals with cataracts and developmental delay (II.1 and III.1), who did not have karyotypes performed before their deaths, had similar dysmorphic and physical features to those with this unbalanced karyotype.

In family 2, juvenile-onset cataract segregated in three generations (Table 1; Fig. 1C). The cataracts were cortical, pulverulent lens opacities in a lamellar distribution. Nuclear, pulverulent opacities were present in two cases (I.1 and III.3). There was later progression with posterior subcapsular opacification that necessitated surgery in adult life. In addition to cataract, two affected individuals (II.2 and II.3) had microcornea and one (III.3) also had bilateral iris colobomas (Fig. 1D).

Other individuals, from affected families and isolated cases, with congenital cataract (139 cases), anterior segment anomalies (50 cases) and microphthalmia (28 cases) were also studied.

Translocation breakpoint cloning in family 1

Within family 1, early-onset cataracts were present in all individuals carrying rearranged chromosomes, whether in balanced or unbalanced configuration. The presence of the phenotype in individuals with a balanced translocation strongly suggests that the phenotype is a direct consequence of altered expression of a gene at or close to one of the breakpoints. Individuals with the unbalanced karyotype have a derivative 5 chromosome, der(5), and are trisomic for 16q23.2-ter and monosomic for 5p15.3-ter. Neither of these partial aneuploidies, particularly the well described 5p deletions, is usually associated with cataract or abnormalities of the anterior segment of the eye (27–29). This supports the hypothesis that the phenotype relates to an abnormality at one of the breakpoints and implicates specifically the breakpoints of the der(5) chromosome. The Marner cataract, which is described in an autosomal dominant pedigree, has similar lenticular features to those seen in our family and is linked to the long arm of chromosome 16 (30). There are no reports of cataract families linked to 5p15.3. We hypothesized that of the two breakpoints contained on the der(5) chromosome, the gene of interest was more likely to lie on the long arm of chromosome 16, and breakpoint localization was conducted at both 16q23.2 and 5p15.3.

Initially, we used highly polymorphic genetic markers to detect the presence or absence of trisomy of distal 16q in patient III.3 (Fig. 2A). Thereafter more detailed analysis utilized somatic cell hybrids, each containing one of the two derivative chromosomes from balanced translocation patient, II.3 (Fig. 2B). A high resolution physical map of the 16q23.2 breakpoint was constructed and is shown in Figure 2C. The 16q23.2 breakpoint lies in BAC RP11-264L1 (GenBank accession no. AC046158) and sequencing across the breakpoints shows only a 3 bp loss. This lies within the eighth intron of the FOR II product of the *WWOX* gene (31,32) (Fig. 2C) and is within the 200 kb region spanning the *FRA16D* common fragile site (33).

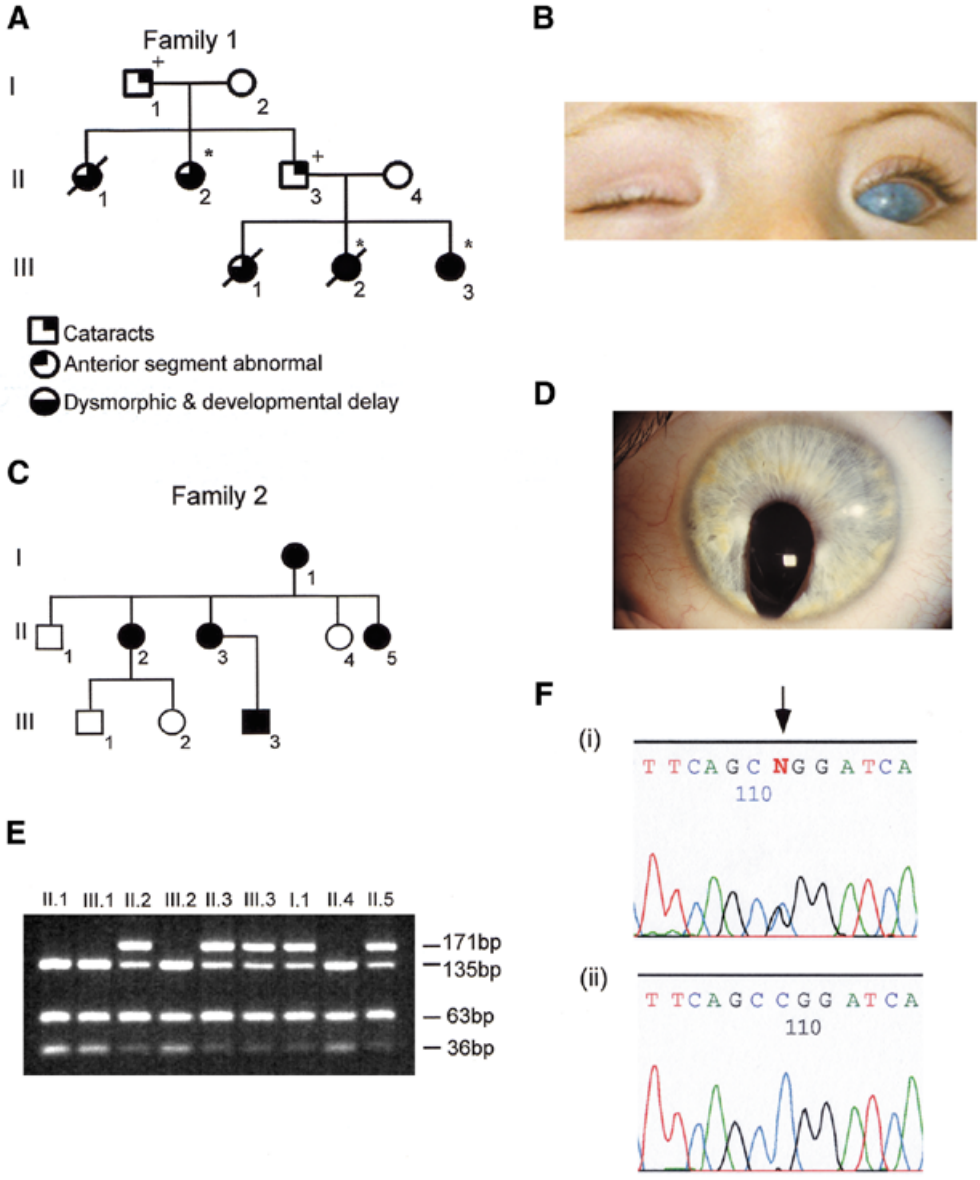

Figure 1. Pedigrees and clinical presentations of the *MAF* translocation and mutation families. (**A**) In family 1, individuals with the balanced karyotype, 46,XY,t(5;16)(p15.3;q23.2), are identified by a plus sign and those with the unbalanced karyotype, 46,XX,der(5),t(5;16)(p15.3;q23.2), are identified by an asterisk. (**B**) III.3 in family 1 at 8 months of age. The right eye is phthisical following rejection of corneal graft at 2 months of age. The left eye shows an opaque cornea due to rejection of corneal graft performed at 4 months of age. (**C**) Family 2 with autosomal dominant congenital cataract and R288P MAF mutation. (**D**) Left eye of III.3 in family 2 demonstrating iris coloboma. (**E**) Family 2 after *Msp*I digestion of PCR products. The R288P mutation abolishes an *Msp*I site. In unaffected individuals, digestion of the 234 bp wild-type DNA PCR product generates fragments of 135, 63 and 36 bp owing to the presence of two *Msp*I sites in this fragment. In heterozygotes with abolition of one of the *Msp*I sites by the mutation an additional 171 bp fragment is also present. (**F**) (i) Direct sequencing of PCR product from affected patient III.3 in family 2. Heterozygous mutation, a G→C transition at nucleotide 1670 of the mRNA (GenBank accession no. AF055376) (reverse sequence shown here), which results in an arginine to proline substitution at amino acid 288 of the protein product. (ii) Control sequence.

The 16q23.2 translocation breakpoint is within a region of recurrent *de novo* somatic translocations in B cells of multiple myeloma patients (Fig. 2C). These are associated with dysregulation of expression of *MAF* which can function as an oncogene (34), and this is thought to be due to translocated proximity of *MAF* to the IgH enhancer(s) active in B cells. This suggests that in family 1, the breakpoint lies in the genomic-control domain of *MAF*, and that translocations at large distances from *MAF* are able to alter its expression. BLAST analysis of the available sequence from the breakpoint to *MAF* revealed no other known genes or ESTs expressed in the eye or with features of a gene involved in ocular abnormality. The intragenic disruption of the *WWOX* gene by the chromosome 16 breakpoint would be expected to lead to a 50% reduction of the FOR II product in the balanced translocation carriers, but should have no effect in the unbalanced individuals. Since both

Table 1. Ocular phenotypes in translocation and MAF mutation families

	Lens opacities	Age at operation (years)	Anterior segment	Other
Family 1 (translocation)[a]				
I.1 [B]	Pulverulent cortical and sutural	Adult		
II.2 [Ub]	Total cataract	30		Severe myopia
II.3 [B]	Pulverulent cortical and sutural	24		
III.2 [Ub]	Not known	–	Opaque corneas	
III.3 [Ub]	Cataractous lens remnants	Infant	Peters anomaly	Microphthalmia
Family 2 (R288P MAF)				
I.1	Pulverulent cortical and nuclear. Later, posterior subcapsular	25		
II.2	Pulverulent cortical. Later, posterior subcapsular	28	Microcornea	
II.3	Pulverulent cortical. Later, posterior subcapsular	28	Microcornea	
II.5	Pulverulent cortical. Later, posterior subcapsular	27		
III.3	Pulverulent nuclear	–	Right and left iris coloboma	

[a][B], balanced translocation: 46,XY,t(5;16)(p15.3;q23.2); [Ub], unbalanced translocation: 46,XX,der(5),t(5;16)(p15.3;q23.2).

categories have eye defects, it is unlikely that *WWOX* is implicated. The presence of control elements for one gene within an unrelated neighbouring gene has been observed repeatedly (35).

The 5p15.3 breakpoint was localized to a 3.4 kb region in the partially sequenced BAC CTD-2299B9 (GenBank accession no. AC025775). Analysis of a 750 kb region surrounding this breakpoint revealed no known genes expressed in the eye. An EST cluster identified at ~250 kb centromeric to the breakpoint shows similarity to murine *Irx2* (*iriquois related homeobox 2*) and is likely to be the human homologue of this gene. The murine expression pattern of this gene suggests that it is not a likely candidate gene for isolated lens and anterior segment abnormality (36).

MAF mutation in family 2 with dominant cataract and coloboma

MAF mRNA exists in single- (4.4 kb) and two-exon (2.3 kb) forms due to alternative RNA processing. The resultant proteins are identical in their first 372 amino acids which include all known functional domains, have similar expression patterns in human tissues and differ by the addition of 30 amino acids at the C-terminal end in the second form (34) (Fig. 2D). We screened the coding regions of both forms for mutations using single-strand conformation polymorphism/heteroduplex analysis. We identified a mutation in *MAF* in a family where autosomal dominant juvenile-onset cataract is segregating in three generations (Fig. 1C). One individual also has iris coloboma and two of five affected individuals have microcornea. Analysis of genomic DNA revealed a G→C transition at nucleotide position 1670 of *MAF* (GenBank accession no. AF055376) (Fig. 1F). This mutation substitutes a highly conserved arginine with proline at amino acid position 288 (R288P) in the basic region DNA-binding domain of MAF. The mutation also destroys an *Msp*I restriction enzyme site, which confirmed full co-segregation between the mutation and the disease phenotypes (Fig. 1E). It was not seen in 217 other subjects with a range of eye anomalies or in 496 normal control chromosomes.

Arg288 of the MAF protein is the first residue of the basic region involved in DNA binding in this bZIP protein (Fig. 3A). This region is preceded by an extended homology region that is also critical for DNA binding (37). The arginine residue is conserved in all known large Mafs (Fig. 3B). Replacement of this hydrophilic basic residue with proline is expected to lead to abnormality of helical conformation.

DISCUSSION

MAF is a highly attractive candidate gene for human ocular developmental abnormalities; it is expressed during lens differentiation, homozygous loss of function in the mouse leads to abnormal lens development and microphthalmia, and it regulates crystallin expression (15,17,18,38,39). Our two families show a combination of cataract and anterior segment anomalies, and this is recognized in a few cases that have been associated with mutations in *PAX6*, *PITX3*, *EYA1* and *FOXE3* (4,5,40,41). Family 1 members with a spectrum of features, including cataract and Peters anomaly, carry a t(5;16)(p15.3;q23.2) translocation in balanced and unbalanced forms. They have a break in a region on 16q23.2 which is associated with altered regulation of *MAF* in oncogenic translocations (34). In family 2, a mutation in *MAF* cosegregates with a dominant form of cataract, and microcornea and iris coloboma are also associated. The mutation results in the substitution of a conserved residue of the DNA-binding domain of MAF and is absent from 496 control chromosomes. The findings in these two families implicate *MAF* as a disease-causing gene in the lens leading to cataract formation and also suggest a broader role for *MAF* in anterior segment development.

Through breakpoint cloning in the t(5;16) family with lens and anterior segment developmental anomalies, we have

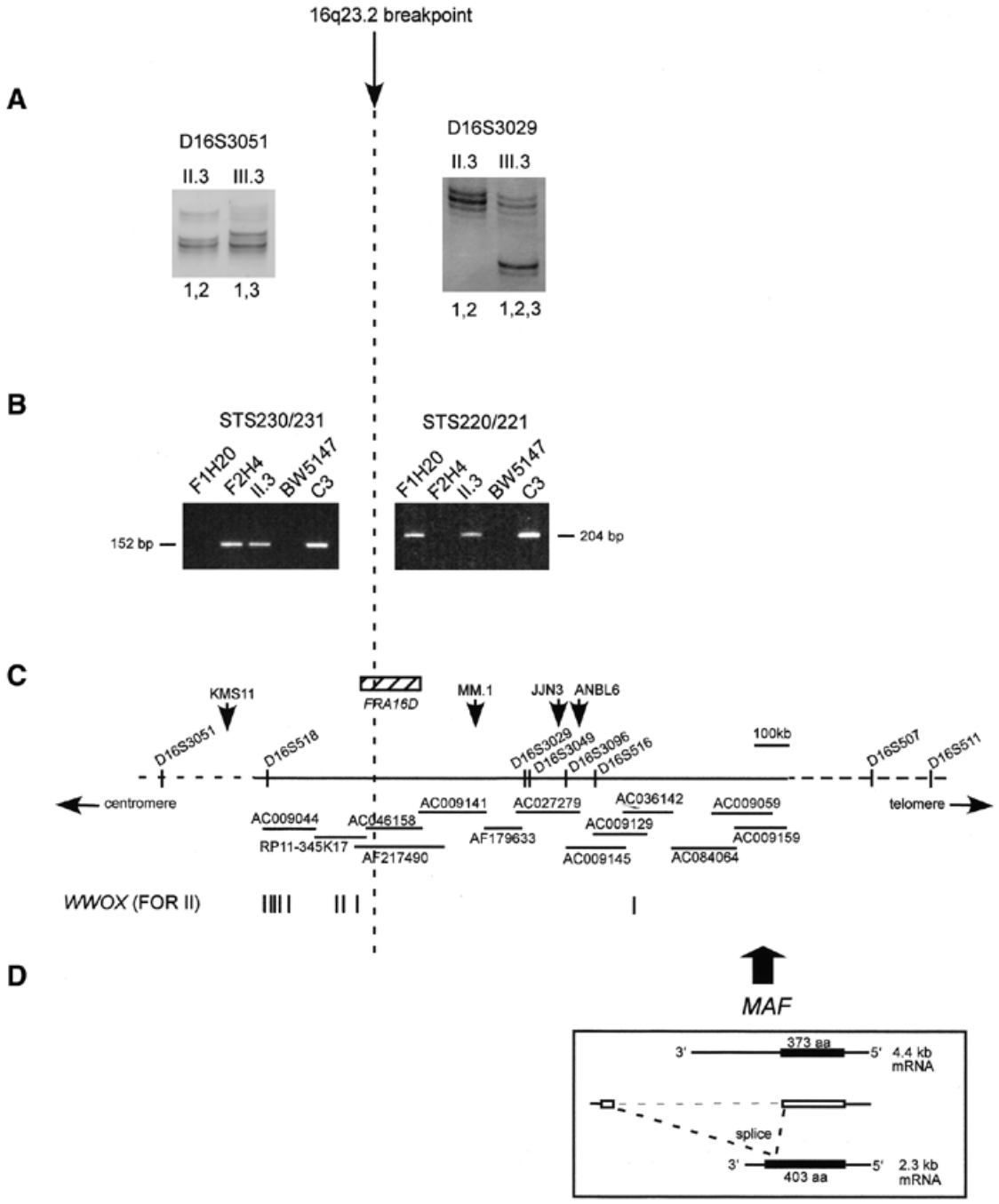

Figure 2. Physical map around family 1 translocation breakpoint on 16q23.2. (**A**) Microsatellite analysis of balanced translocation carrier (II.3) and his unbalanced translocation daughter (III.3), with the genotype indicated beneath each panel. At D16S3051, the daughter (III.3) has two alleles, one from her father and a different one from her mother placing this marker centromeric to the breakpoint. At D16S3029, the daughter has three alleles, two from her father and one from her mother placing this marker telomeric to the breakpoint. (**B**) Somatic hybrid analysis with STS markers flanking the 16q23.2 breakpoint. Lane 1, F1H20 somatic cell hybrid [(der)5 chromosome]; lane 2, F2H4 somatic cell hybrid [(der)16 chromosome]; lane 3, II.3, genomic DNA balanced translocation carrier; lane 4, BW5147, mouse lymphoma cell line; lane 5, C3, normal control. Primer pair 230/231 does not amplify from F1H20, but does amplify from F2H4, placing this marker centromeric to the breakpoint. Primer pair 220/221 amplifies from F1H20 but not from F2H4, placing this marker telomeric to the breakpoint. (**C**) The breakpoint maps within AF217490, AC046158 and the *FRA16D* fragile site (33). The breakpoint lies between two multiple myeloma translocation breakpoints (KMS11 and MM.1) and lies in the last intron of the FOR II splice form of the *WWOX* gene (32), as do multiple myeloma breakpoints MM.1, JJN3 and ANBL6 (34). The BAC contig is contiguous from AC009044 to AC009159. The scale bar is indicated. (**D**) Alternative splicing of *MAF* gene transcripts, demonstrating the single exon (373 residues) and the two-exon (403 residues) forms of the protein.

discovered a translocation in the likely genomic-control domain of the lens development gene *MAF*. Long-range disruption is thought to affect the expression of *Mafb* in the original *kreisler* mouse where an inversion breakpoint is

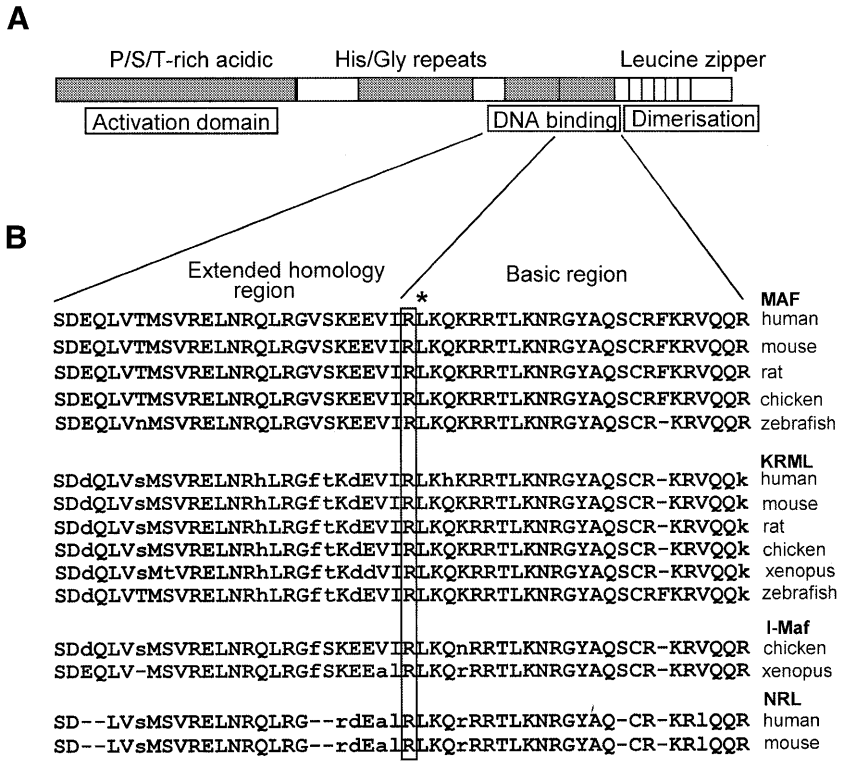

Figure 3. Schematic representation of large Maf proteins and amino acid sequence of DNA-binding domains. (**A**) Functional domains including N-terminal transactivation domain, the DNA-binding domain and the leucine zipper required for dimerization (23). The DNA-binding domain is composed of a basic region with an N-terminal extended homology region. (**B**) Sequence conservation of DNA-binding domain amongst the large Maf proteins [MAF, KRML (also known as MAFB), l-Maf and NRL] and across species. The R288 position (indicated by an asterisk) is conserved in all known large Maf proteins. Identical residues are shown in upper case letters, residues in the same polarity group in lower case letters and differences are dashed.

situated ~30 kb from the *Mafb* transcription unit (24). Position effects have also been observed at several loci in humans, including *PAX6* and *SOX9* (35,42). In family 1 the breakpoint is ~1 Mb from *MAF*. Position effects resulting from genomic disturbance at distances of ≥1 Mb from the affected gene have been described for *SOX9* in both humans and mice (42,43). In multiple myeloma cell lines, translocation breakpoints at similarly large distances from *MAF* have previously been demonstrated to dysregulate *MAF* expression (34). This suggests that the *MAF* genomic-control domain is very large (Fig. 2C) and that *MAF* dysregulation, in the absence of other lens-related genes in the breakpoint regions, is likely to be responsible for the cataract and anterior segment abnormality in this family.

The substitution mutation of *MAF* in family 2 occurs in a conserved arginine (R288) residue at the start of the basic region DNA-binding domain, immediately C-terminal to the extended homology region DNA-binding domain (Fig. 3A and B). A substitution mutation in the basic region of the bZIP domain of Mafb has been described in mice with the *kreisler* phenotype (24). Analysis of deletion and some substitution mutation constructs of both the basic and extended homology regions of Maf demonstrate *in vitro* impairment of DNA-binding ability on gel shift analysis (20,37). Sequence comparisons show that R288 is the ninth residue of a 15 amino acid sequence that resembles a helix–turn–helix DNA-binding motif (44). The R288P mutation replaces a hydrophilic residue with a proline in a region involved in α-helical conformation and DNA binding. We performed electrophoretic mobility shift assays (EMSAs) using constructs from normal MAF and the R288P mutant and a labelled MARE oligonucleotide. We could not discern abolition of DNA binding on the gel shift assay in the R288P mutant (data not shown). We note that for other substitution mutations in the DNA-binding domain of MAF, functional effects have been shown by alterations in α-helicity upon binding to MAREs using circular dichroism spectroscopy, alterations in trypsin cleavage (44) and by an increase in transforming activity in the absence of a change on gel shift analysis (45). At least three missense mutations in the DNA-binding domains of FOXC1 and PITX2 have been shown to confer normal or near-normal DNA-binding activity on gel-shift analysis but markedly abnormal (both decreased and increased) transactivation activity (46,47). We suggest that the proline substitution does not cause a simple loss of DNA-binding ability, but changes MAF activity in another way such as dysregulation of DNA-binding ability, change in transactivation potential or a change in binding to other proteins with which MAF interacts. Given the conserved nature of the R288 amino acid in the large Mafs from zebrafish to humans, its position in the helix–turn–helix of the ancillary DNA-binding domain, the cosegregation of the R288P mutation with the disease phenotype in family 2, the particular amino acid substitution of a hydrophobic proline for

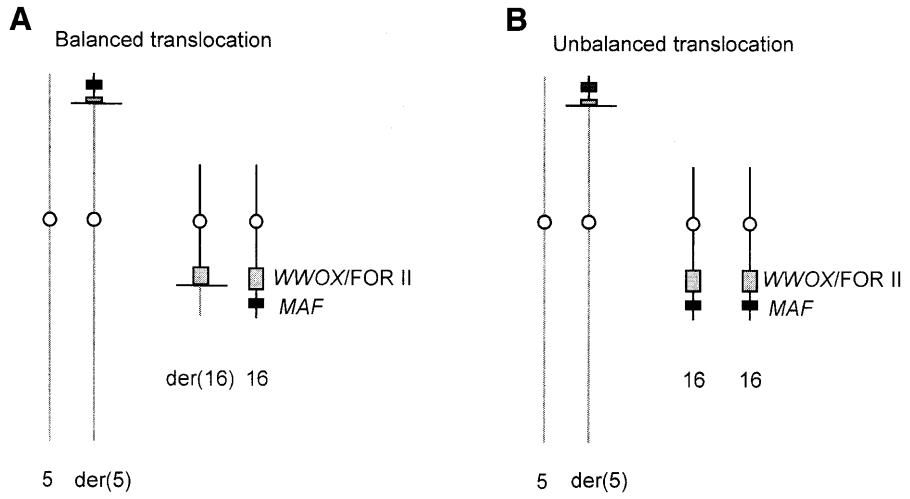

Figure 4. Diagrammatic representation of balanced and unbalanced forms of the 5;16 translocation in family 1. Breakpoints on 5p and 16q, with relative positions of *MAF* and *WWOX* (FOR II) are indicated. (**A**) In the balanced translocation, *MAF* is telomeric to the breakpoint on 5p with the 3′ end of the *WWOX* (FOR II) gene. (**B**) In the unbalanced translocation, the translocated *MAF* is present as well as two normal copies of *MAF*. There are two normal copies of *WWOX* in addition to the 3′ end of FOR II on the der(5) chromosome.

a hydrophilic arginine and the absence of this mutation in 496 independent chromosomes from normal controls, we consider that mutation of this arginine residue is likely to have important functional consequences for MAF. The finding of one mutation in a panel of 140 congenital cataract patients is not surprising given the marked heterogeneity in causation of congenital cataracts (48). Similarly, in a panel of 161 anterior segment dysgenesis patients with and without cataract, one mutation affecting a mother and daughter was found in *FOXE3* and none was found in two other recognized anterior segment and cataract disease genes, *PITX2* and *PITX3* (40).

As in the *MAF* mutation family, our analysis of the t(5;16) family suggests that the functional effect of this translocation is to lead to altered gene expression rather than downregulation or haploinsufficiency. All individuals carrying the der(5), as either balanced or unbalanced translocations (Fig. 4), have ocular abnormalities. This suggests that the rearrangement is giving rise to a dominant rather than a haploinsufficient effect, since individuals with der(5) in the presence of two normal copies of chromosome 16 have the eye phenotype, and deletions of distal 5p are not associated with these ocular abnormalities (28,29). Since simple 16q duplication is not usually associated with the ocular phenotypic abnormalities seen in this family (27), this alteration is most likely in timing and/or tissue specificity of expression. This is supported by our finding that the two *MAF* alleles in family 1 balanced translocation patient, II.3, show bi-allelic expression in his adult fibroblasts (data not shown). It is proposed in the translocation multiple myeloma cell lines that *MAF* overexpression occurs because of translocation to a region with a strong IgH enhancer. This is of specific relevance in the B cell lineage where the IgH enhancer is active, but there is no evidence that *MAF* would be overexpressed in other tissues where the IgH enhancer is not active. Similarly, in our family it is logical to expect that the different genomic and chromatin context in which *MAF* is placed by the translocation is of relevance for the spatiotemporal expression of *MAF* and its role in tissue-specific differentiation. In the eye, this would be expected to be during lens differentiation, when the ocular transcription factors that normally control *MAF* expression would face, on the translocation chromosome, a different chromosome domain tertiary structure and enhancer context than normal. This tissue specificity of the likely effect of the translocation is supported by the finding in our translocation family that no individuals with balanced or unbalanced karyotypes have developed multiple myeloma or other forms of cancer, including the 73-year-old paternal grandfather (family 1, I.1). Since the specific translocation effect on *MAF* in eye development is likely to occur in early embryogenesis, confirmation of this conclusion will have to come from studies of animals transgenic for *MAF* control elements in order to assay the effects in a developing mammal. Our analyses of both our families suggest that it is dysregulation of *MAF* rather than haploinsufficiency that is leading to abnormal lens and anterior segment development. *Maf* haploinsufficiency is also not pathogenic in the mouse, since heterozygous null mutants show normal ocular development (17,18). It is noteworthy that the individuals with the unbalanced translocation (Fig. 4), who have two normal copies of *MAF* as well as the rearranged one, show more severe ocular abnormalities. The extra copy of *MAF* may contribute to the more severe ocular phenotype as seen with increased dosage effects of *Pax6* and *FOXC1* in the eye (49,50).

There is a spectrum of eye abnormalities present in these families which links lens and anterior segment development. The cataract formation is likely to occur through aberrant MAF interactions with other lens development genes, including *PITX3*, *PAX6* and the crystallins. *Pitx3* and the crystallins have MAREs in their promoters (10,15,17,18) and Pax6 may have a synergistic role in crystallin gene regulation (39,51). Since *Maf* expression in the eye occurs in the developing lens, this suggests that the anterior segment abnormalities in our families

arise primarily due to defects in development of the lens and ectodermal derivatives, and emphasizes the importance of the lens in anterior segment development. Iris coloboma is caused by defective closure of the optic fissure or abnormality of development of the iris stroma and epithelium, and is generally considered a separate entity from cataract. Interestingly, *Pax6* is also associated with coloboma in the *small eye* heterozygous mouse (52). This is intriguing, as *in vitro* studies show that Pax6 can activate *Maf* expression (53), as well as interact with Maf at the protein level (54). The finding of iris coloboma in family 2 broadens the possible role of MAF in development of the anterior segment.

In conclusion, we have shown that the genomic-control domain of the *MAF* gene is disrupted by a translocation in a family with lens and anterior segment abnormalities, and that a mutation affecting a conserved residue of the ancillary DNA-binding domain of this bZIP transcription factor co-segregates with affection status in family 2. Our analyses suggest that in both cases MAF gain or abnormality of function in the developing lens at a key stage of embryogenesis can lead to abnormal lens and anterior segment development. These findings further implicate *MAF*/*Maf* in lens development and suggest an associated role in formation of the anterior segment.

MATERIALS AND METHODS

Construction of somatic cell hybrids, family 1

From family 1, lymphoblastoid cell lines were established from whole blood from the balanced translocation patient (II.3) and his daughter with the unbalanced translocation (III.3). Somatic cell hybrids were created from the lymphoblastoid cell line II.3 as described previously (55).

Marker typing, family 1

PCR amplification for the analysis of microsatellite polymorphisms (Genethon) was performed on genomic DNA from II.3 and III.3. Genomic DNA (40 ng) was suspended in a 20 µl reaction containing 10 pmol of each forward and reverse primers, 0.75 mM dATP, dGTP, dCTP, dTTP, 67 mM Tris–HCl pH 8.0, 3.7 mM $MgCl_2$, 6.7 µM EDTA, 16 mM $(NH_4)_2SO_4$, 0.085 mg/ml BSA and 0.1 U *Taq* DNA polymerase. Samples were processed through 30 cycles of amplification consisting of 45 s at 94°C (denaturation), 45 s at the appropriate annealing temperature and 1 min at 72°C (extension). The final step was lengthened to 10 min. Amplification products were electrophoresed on 8% polyacrylamide gels at 400 V for ~2 h. The bands were detected by silver staining. Marker typing for hybrid identification and physical mapping was performed using reaction conditions as described above, with electrophoresis on 2% agarose gels. Custom primers for the physical mapping were designed based on available sequence data. All primers were synthesized commercially (MWG-Biotech AG). Primer sequences (5′–3′): 230, GCAAGCGATGTGTGTCTGGATTAAG; 231, GCAGATTCCAGATTTTACCAGCG; 220, GCCTCTAGTAATGCCTGGAGTGAG; 221, GTCTCTTCCATCTCAGAGTGTCCTG.

Physical mapping of breakpoint regions and flanking sequence analysis, family 1

For breakpoint localization, BACs were identified by using sequence tagged sites (STS) within each region. Sequenced data were analysed using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/index.html). Contigs were developed using the Washington University human genomic physical map tracking database (http://genome.wustl.edu/cgi-bin/ace/ctc_choices/ctc.ace), the Los Alamos National Laboratory BAC Mapping Project (http://www-ls.lanl.gov/) and BLAST comparison of sequenced BACs. In regions of unordered BAC sequences, fragments were assembled in relation to each other and flanking BACs. The UK HGMP Resource Centre (http://www.hgmp.mrc.ac.uk/) NIX suite of programmes, which includes GRAIL, Fex, Hexon, MZEF, Genemark, Genefinder, Fgene, BLAST against many databases (including Ecoli, EST, STS, EMBL, trEMBL, SWISS-PROT), Polyah, RepeatMasker and tRNAscan, was used to analyse the sequence surrounding the breakpoints for genes.

The breakpoints on 16q23.2 and 5p15.3 were refined using long-range PCR on genomic DNA from II.3. Primers close to either side of the breakpoints on the two derivative chromosomes were used in the Expand 20 kb^{Plus} PCR System (Roche). Amplified products were purified on a centricon-100 column (Amicon). Direct sequencing of PCR products was performed using the BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) on a fluorescent sequencer (ABI 377).

Mutation analysis and confirmation

PCR amplification of the coding regions of both forms of the *MAF* gene was performed (primers on request). Genomic DNA from one affected individual from each pedigree was amplified. PCR reactions and cycling times were performed as above. Due to the high GC content, some reactions required the addition of enhancing agents: either DMSO at 10% or both DMSO at 10% and betaine at 1 M final concentration. For SSCP/heteroduplex analysis, 1 vol PCR product was mixed with 1 vol formamide loading dye and denatured at 96°C for 5 min prior to loading on an 8% acrylamide/bis-acrylamide gel. Gels were run at 350 V overnight at 4°C and silver stained. In cases where an SSCP shift was observed, direct sequencing of PCR products was performed as above. For restriction enzyme analysis, PCR products were subject to digestion using *Msp*I (New England BioLabs) at 37°C for 1 h. Resulting fragments were analysed by electrophoresis in a 3% agarose gel, stained with ethidium bromide and visualized under UV light.

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