GDF6, a novel locus for a spectrum of ocular developmental anomalies

Citation for published version:

Digital Object Identifier (DOI):
10.1086/511280

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
American Journal of Human Genetics

Publisher Rights Statement:
Copyright © 2006 by The American Society of Human Genetics

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Colobomata represent visually impairing ocular closure defects that are associated with a diverse range of developmental anomalies. Characterization of a chromosome 8q21.2-q22.1 segmental deletion in a patient with chorioretinal coloboma revealed elements of nonallelic homologous recombination and nonhomologous end joining. This genomic architecture extends the range of chromosomal rearrangements associated with human disease and indicates that a broader spectrum of human chromosomal rearrangements may use coupled homologous and nonhomologous mechanisms. We also demonstrate that the segmental deletion encompasses GDF6, encoding a member of the bone-morphogenetic protein family, and that inhibition of Gdf6 in a model organism accurately recapitulates the proband’s phenotype. The spectrum of disorders generated by morpholino inhibition and the more severe defects (microphthalmia and anophthalmia) observed at higher doses illustrate the key role of GDF6 in ocular development. These results underscore the value of integrated clinical and molecular investigation of patients with chromosomal anomalies.

Normal ocular development entails a series of tightly choreographed events, of which fusing the edges of the optic cup’s embryonic fissure represents a key step in forming the future spherical eye. Disruption of this process results in colobomata (MIM 120200)—congenital anomalies affecting tissues in the posterior (retina, choroid, and optic nerve) and anterior (cornea, iris, and lens) ocular segments. Colobomata may, in turn, cause abnormal morphogenesis of other ocular tissues, because of the retina’s inductive role in lens formation. Consequently, colobomata are frequently associated with ocular malformations, including cataract, microphthalmia, and anophthalmia; systemic anomalies—such as renal anomalies, mental retardation, and CHARGE (coloboma, heart defects, choanal atresia, retarded growth, and genital and ear anomalies [MIM 214800])—are also observed in a small subset. Causal mutations for coloboma have been identified in only a small number of cases, and the genetic heterogeneity (OTX2 [MIM 600037],2 SHH [MIM 600725],3 MAF [MIM 177075],4 CHX10 [MIM 142993],5 CHD7 [MIM 608892],6,7 and PAX6 [MIM 607108]) reflects the diverse molecular interactions that control eye development. Although autosomal dominant, recessive, and X-linked inheritance of colobomata are observed, the absence of clear Mendelian inheritance patterns indicates the presence of a less straightforward molecular basis in some cases.2,12,13 Increasing the proportion of colobomata with a defined genetic origin would benefit the understanding of complex disease and provide novel insight for ocular development and may identify therapeutic targets for a frequently blinding disorder.

Colobomata are associated with rearrangements affecting at least 10 autosomes,3–6,8,12,14–17 including several syndromes, such as CHARGE,7,6 “Cat eye” syndrome (MIM 115470),17,18 Jacobsen syndrome (MIM 147791),19 and Wolf-Hirschhorn syndrome (MIM 194190).6 Study of chromosomal anomalies has proved a fruitful means of identifying dosage- or position effect–sensitive genes, particularly for ocular disorders, since research is facilitated by the fact that the eye is composed of an interface of embryonically distinct tissues and by accessibility to detailed phenotyping.20–23 Detection of a segmental deletion syntenic with the murine ocular malformation locus Tcm24,25 in a patient with colobomata enabled us to investigate the cause of the ocular developmental phenotype and the mechanism mediating this chromosomal rearrangement. Observation of features of nonallelic homologous recombination (NAHR) and nonhomologous end joining (NHEJ) in this segmental deletion extend the range of chromosomal rearrangements associated with human disease. Analyses of genes within and adjacent to the segmental deletion led us to identify growth differentiation factor 6 (GDF6 [MIM 601147]) as a candidate gene for the patient’s ocular phenotype. Morpholino inhibition of the function of gdf6 in zebrafish, performed to evaluate this gene’s role in retinal development, recapitulated the patient’s phenotype.

**Material and Methods**

**Patients**

Peripheral-blood samples were obtained from a family in which ocular and systemic developmental anomalies had been identi-
fied in one individual. In view of the breadth of the phenotype, karyotyping of the proband and her parents was undertaken, together with subsequent multiplex-FISH (M-FISH) analysis. This study was approved by the University of Alberta Hospital Health Research Ethics Board, and informed consent was obtained from all participants.

**Defining the Segmental Deletion**

After initial karyotyping (at the University of Alberta Hospital Cytogenetics Laboratory) revealed a chromosome 8q segmental deletion, M-FISH was undertaken using region-specific and partially overlapping chromosome probes (mBAND X Cyte8 probe set [MetaSystems]).27 Each contains a unique fluorochrome, which permits quantitative analysis of fluorescence intensity along a chromosome. The deletion’s extent was next refined by fluorescent microsatellite-marker genotyping (centromeric: D8S1697, D8S1702, D8S1838, D8S461, D8S1912, and D8S1119; telomeric: D8S1699, D8S1127, D8S1772, D8S1129, and D8S1778). Subsequently, comparative genomic hybridization (CGH) was performed with a custom array comprising isothermal, long oligonucleotide probes tiled at a 5-kb density across the repeat-masked genomic interval encompassing the segmental deletion (Nimblegen). The mean probe density was increased to 1 kb in regions predicted by microsatellite-marker genotyping to correspond to the centromeric and telomeric breakpoints. CGH was performed as described elsewhere,27 with use of the proband’s DNA labeled in the centromeric and telomeric breakpoints. CGH was performed with a custom array comprising isothermal, long oligonucleotide probes tiled at a 5-kb density across the repeat-masked genomic interval encompassing the segmental deletion (Nimblegen). The mean probe density was increased to 1 kb in regions predicted by microsatellite-marker genotyping to correspond to the centromeric and telomeric breakpoints. CGH was performed as described elsewhere,27 with use of the proband’s DNA labeled with Cy3, hybridized with a Cy5-labeled reference DNA.

**Amplification of the Junctional Fragment**

Guided by the CGH results and bioinformatic sequence information, multiple primer pairs were designed to amplify a junctional fragment that spanned the segmental deletion. One of these nine primer permutations (F2-R3) yielded an amplicon in the proband, through use of long-range PCR (Elongase [Invitrogen]), which was purified with Montage (Millipore) and was sequenced using these and internal primers (table 1), with a BigDye (v3.1) terminator kit and 3100 DNA sequencer (Applied Biosystems). Subsequent amplification of the Junctional Fragment

**Bioinformatic and Expression Analyses**

Database analyses of genes lying in the deleted region or adjacent to the breakpoints were performed to identify candidate(s) for the ocular phenotype (Ensembl, UCSC, and UniGene). FISH was also undertaken with the BAC clone RP11-516P9, to validate database predictions. The expression of one gene (gdf6a) was investigated by RT-PCR of murine (adult and embryonic) and zebrafish (18 and 42 h postfertilization [hpf]) tissue (through use of primers shown in table 1 and Superscript III RT-PCR [Invitrogen]). Ethidium bromide–stained amplicons were visualized on 1% agarose gels. Whole-mount in situ hybridization was undertaken using digoxigenin-labeled antisense RNA probes specific to retinal developmental genes that are primarily markers of regional identity. cDNA from hmx3b (nikx.1/soha1), aldh1a2 (raldh2), and foxg1 were generated, and in situ hybridization was performed as described elsewhere30 on wild-type and gdf6a-morphant zebrafish.

**Analysis of gdf6a Function in Zebrafish**

Zebrafish possess two orthologues, gdf6a (radar) and gdf6b (dynamo),31 with gdf6a expressed in developing retina, dorsal fin, dorsal neural tube, and posterior endoderm.32,33 Zebrafish embryos have two sources of gdf6a: unspliced zygotic and prespliced maternal gdf6a mRNA. Morpholino inhibition of zebrafish zygotic gdf6a function results in cranial and dorsal neural-tube apoptosis, whereas inhibition of both maternal and zygotic gdf6a (with a translation-blocking morpholino) generates an earlier dorsализed phenotype, thereby preventing analysis of eye development.34 Accordingly, two splice-blocking morpholino antisense oligonucleotides (MOS) were designed to independently target gdf6a splicing, which enables comparison of the phenotype generated by each. The first (gdf6aMO1) targets the 3′ splice site, whereas the second (gdf6aMO2) targets the intron 1–exon 2 boundary. Additional morpholinos were designed to target the translation start site of gdf6b (gdf6bMO1) and to provide a capability to reduce apoptotic cell death to the translation start site of p53 (p53MO). Oligonucleotides gdf6aMO1 (GCCATACACCTTCTTTCTTCTGTC), gdf6aMO2 (GAGATCGTCTGCAAGATAGAGA), and p53MO (GGGCCATGTGTGCAAGATAGAGA) were provided by Gene Tools; gdf6bMO1 (TCAAAGATATCCCGACAGCCACGACGGAC) was provided by Open Biosystems. The efficiency of the two gdf6a morpholinos was evaluated by RT-PCR of mRNA derived from 18-hpf un.injected and morphant embryos, by use of gdf6a primers spanning the intron (table 1). Zebrafish (AB strain) were obtained from the zebrafish stock center ZIRC and were maintained under stan-

---

**Table 1. Primers Used to Amplify the Chromosome 8q22 Junctional Fragment and for RT-PCR of Murine and Zebrafish gdf6**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Forward</th>
<th>Reverse</th>
<th>Annealing Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragment:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2-R3</td>
<td>CCGACGGGTGTTGCAACACAC</td>
<td>TCATTGTCTCTGGGTAGAGG</td>
<td>60</td>
</tr>
<tr>
<td>Chromosome 8-4</td>
<td>AGGCCAACACAGGAAATGAGGTT</td>
<td>GTGATCCCTCCTTCCTCCA</td>
<td>60</td>
</tr>
<tr>
<td>Chromosome 8-5</td>
<td>GAGACATTCTATACAGCAGCTG</td>
<td>GACCCCATCATCTATCTTGCTT</td>
<td>60</td>
</tr>
<tr>
<td>Chromosome 8-6</td>
<td>TAGTGGCTCTAGTGGTCTT</td>
<td>GGGTCTGTCTCAGTGTT</td>
<td>60</td>
</tr>
<tr>
<td>RT-PCR:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Murine</td>
<td>GGCGGCTTGCTCCACAGAGTAC</td>
<td>GGCGGCGGATAACCTACCTGCTC</td>
<td>60</td>
</tr>
<tr>
<td>Zebrafish</td>
<td>CGCGGTCTACAAGAGAGAGGAAACCTT</td>
<td>CGCGGTCTCACGAGCTCATG</td>
<td>60</td>
</tr>
<tr>
<td>gdf6a</td>
<td>GTAGACACCGGGTCCTACCTTACTC</td>
<td>GAAGTGTAGACGCGCTAGGCCC</td>
<td>55</td>
</tr>
</tbody>
</table>
Results

Phenotype

The proband exhibited multiple developmental defects, including neurodevelopmental impairment (performance IQ 74), bilateral soft-tissue syndactyly of the 2nd and 3rd toes, an atrial septal defect, and ocular malformations (fig. 1). The latter comprised bilateral retinocochoroidal coloboma with optic-nerve involvement (fig. 1A and 1B), plus a unilateral iris coloboma (fig. 1C), which reduced vision to counting fingers and 20/50 in the right and left eyes, respectively. Although the proband’s father was asymptomatic, ocular examination revealed features of much milder developmental defects that included a minor degree of optic-nerve dysplasia, anomalous retinal vascular branching, and small retinocochoroidal colobomata (fig. 1G and 1H). The changes were associated with corrected acuities of 20/20 and 20/30 (normal ~20/20).

Fine Mapping and Sequencing of Breakpoint

Karyotyping identified a chromosome 8q segmental deletion—46,XX, del (8)(q21.2q22.1)—in the proband but in neither parent (data not shown). These findings were confirmed by M-FISH, with the segmental deletion present in all 13 examined metaphase preparations (fig. 1J–1L). The informativeness of microsatellite-marker genotyping was constrained by the small pedigree size and polymorphism-information-content values of available markers. Nonetheless, it confirmed that the deletion was present in the paternally derived chromosome 8, and the semiquantitative dosage information it provided localized the centromeric and telomeric breakpoints to ~3-Mb regions. CGH accurately defined the extent of the segmental deletion (10.37 Mb) and refined the breakpoint positions to ~10-kb intervals (data not shown). Long-range PCR amplified a 4.5-kb junctional fragment in the proband but in neither parent (fig. 2C). Sequence analysis of the junctional fragment revealed the insertion of four nucleotides (AGCT) at the junction of the centromeric and telomeric breakpoints (fig. 2A and 2B). The telomeric breakpoint lies in a long terminal repeat (LTR) of family MaLR, within a 3.6-kb region of repetitive sequence (fig. 2A). The centromeric breakpoint is within an Alu element located within a 5.7-kb stretch of contiguous repeats (short interspersed nuclear elements [SINEs], long interspersed nuclear elements [LINEs], LTRs, and simple repeats).

Candidate-Gene and Mutation Analysis

The proband’s retinal phenotype and the known molecular conservation of ocular development were used to identify candidate genes in silico. Of 31 genes within and 10 adjacent to the segmental deletion, 14 were expressed in both fetal eye and retinal tissue, and, of these, 11 (CA2, CA3, WWP1, NBN, FAM82B, TMEM64, TMEM55A, UQCRB, PTDSS1, SDC2, and GDF6) had orthologues present in Fugu or Danio. Because of its involvement in bone morphogenetic protein (BMP) signaling, its developmental role, and the location of Gdf6 within the Tcm locus, GDF6 was selected for more-detailed study. Subsequent refinement of the Tcm locus from a 26-Mb to a 1.3-Mb interval permitted comparison of the five genes or transcripts (unknown, helicase-related, Crallbp-related, Asph, and Gdf6) within the Tcm locus with those encompassed by the segmental deletion. Because of a synteny break between human chromosome 8 and murine chromosome 4, Gdf6/GDF6 is the only gene common to the Tcm and chromosome 8q segmental deletion intervals (fig. 2D).

Studies of gdf6a in Zebrafish

Previous studies have examined the role of gdf6a in early dorsal-ventral axis formation, but its function during retinal development remains to be elucidated. The expression of zebrafish gdf6a was examined with in situ hybridization and was shown to be specific for the dorsotemporal region of the developing retina at 18 and 24 hpf (fig. 3A and 3B). The expression pattern of Gdf6 is evolutionarily conserved, as determined by our RT-PCR results of murine retina during early development and the published expression in the dorsal retina of Xenopus (data not shown). Splice-blocking gdf6a morpholinos were injected into one-cell–stage zebrafish embryos; RT-PCR confirmed that >70% of gdf6a mRNA was aberrantly spliced in both gdf6aMO1- and gdf6aMO2-injected morphants (fig. 3C). Morpholino inhibition of gdf6α function revealed the axial vasculature, dorsal fin, hypochord, and dorsal neural-tube anomalies (reported elsewhere). In situ hybridization with probes to aldh1a2, hmx3b, and foxg1 demonstrated that gdf6a morpholinos profoundly altered retinal patterning (fig. 3D–3L). Expression of aldh1a2, a marker of dorsotemporal retina, was completely eliminated in 90% and 97% of gdf6aMO1- and gdf6aMO2-injected embryos, respectively (n = 113) (fig. 3D–3F). Expression of hmx3b, an early marker of dorsal retina, was strongly reduced in 81% and 85% of gdf6aMO1- and gdf6αMO2-injected embryos, respectively (n = 110) (fig. 3G–3I). In contrast, expression of foxg1, a marker of ventral-nasal retina, was expanded in 77% and 88% of gdf6aMO1- and gdf6αMO2-injected embryos, respectively (n = 118) (fig. 3J–3L). Lenticular expression of hmx3b was unaltered in morphant embryos (fig. 3G–3I). Overall, these results demonstrate that gdf6a functions...
Figure 1. A–F, Phenotypes of proband with chromosome 8q segmental deletion who has colobomatous developmental anomalies affecting the anterior and posterior ocular segments (A). Note the junction between the normal and abnormal retina. B, Retinochoroidal colobomata, with extensive optic nerve involvement. C, Iris coloboma (right eye). D, Normal optic nerve and retina (for comparison). E and F, Syndactyly affecting the 2nd and 3rd toes. G–I, Phenotypes of the proband’s father, demonstrating milder ocular colobomata and syndactyly. G, Dysplastic optic nerve with anomalous vascular pattern and small inferior retinochoroidal coloboma. H, Similar but milder changes (left eye). I, Soft-tissue syndactyly. M-FISH and summary of microsatellite marker genotyping results confirmed chromosome 8q segmental deletion and approximate breakpoint positions. J, 4′,6-Diamidino-2-phenylindole staining demonstrating length difference between the undeleted and the segmentally deleted chromosome. K, Merged fluorescence signals from five M-FISH probes illustrating the segmental deletion. L, “False color” counterstaining, to illustrate with greater clarity the effect of the segmental deletion on length of chromosome 8q. M, Montage illustrating normal hybridization pattern for each probe, decreased hybridization for diethylamino-coumarin (DEAC) and SpO, the approximate extent of the segmental deletion (dotted blue line), and the microsatellite markers used to refine the breakpoint position. Undeleted markers are shown in bold, deleted markers are underlined, and uninformative markers are shown in plain type.
Figure 2.  A, Schematic representation of the proband’s segmental deletion, with coordinates of the centromeric and telomeric breakpoints shown above the representation. GDF6 is located at positions 97223736–97244196. B, Sequence of junctional fragment comprising TCCTGG from the centromeric end, fused to a 4-bp insertion (AGCT), and AGGTTT from the telomeric breakpoint 10 million bp away. The repetitive sequences adjacent to the breakpoints comprise 5.7 kb of repeats centromerically and 3.6 kb of repeats telomerically. (Some repeats extend beyond the depicted region.) C, Amplification of a 4.5-kb deletion-junction fragment in the proband that is not present in the unaffected parents (1.7-kb control fragment amplified in all subjects). F = father; M = mother; P = proband. D, Murine Tcm locus on chromosome 4, a 1.17-Mb region encompassing five genes (A = unknown; B = helicase-related; C = Crabp-related; D = Asph; E = Gdf6) and their orthologues on human chromosome 8. Because of a synteny break, four genes are ~35 Mb centromeric to the segmental deletion, with only GDF6 common to both.

to specify dorsotemporal retinal identity and to repress ventronasal identity.

In addition, as is evident (fig. 4), gdf6a morphants exhibit a range of ocular anomalies that recapitulate the patient phenotype (fig. 1). These features, apparent as early as 18 hpf, include ventral colobomata (35%; n = 214, at 48 hpf), persistent dorsal-retinal groove (17%; n = 214, at 48 hpf), and decreased ocular and lenticular size (30%; n = 214, at 48 hpf) (figs. 4A, 4B, and 5). In contrast to the declining proportion of morphants observed with colobomatous defects at later stages (ventral coloboma [10% at 72 hpf], dorsal-retinal groove [2% at 72 hpf]) (fig. 4A–4D), the frequency of lens extrusion and microphthalmia (9% and 60%, respectively, at 120 hpf) increased over time (figs. 4E–4H and 5). Injection of higher doses of gdf6a morpholino cause ocular regression to vestigial structures that resemble human anophthalmia (fig. 4I). To investigate retinal development at the cellular level, we examined gdf6a morpholino–treated zebrafish embryos histologically. This revealed a consistent reduction in ocular, lenticular, and retinal size, together with prominent defects in both retina and lens (fig. 4I–4L). Although all cell types are visible, the retina is disorganized, indicating a critical role for gdf6a in regulating neural retina cell behavior. Further-
more, we find that the optic nerve is hypoplastic and occasionally absent in morphant embryos (10% of embryos are affected) (fig. 4J and 4L). In stark contrast to our results with gdf6a/radar, inhibition of gdf6b/dynamo function revealed no ocular phenotype, which clearly demonstrates the specific role of gdf6a in ocular morphology.

Discussion

Although colobomata represent a relatively common developmental anomaly (2.6 in 10,000 births)\(^3\) that account for up to 11% of pediatric blindness,\(^3\) knowledge of the genetic basis remains limited. The identification of additional colobomata-causing genes is thus of value; it improves the understanding of function in both normal development and disease and provides the realistic prospect of elucidating non-Mendelian pathways that contribute to ocular-disease phenotypes. The present study characterizes a novel chromosomal anomaly associated with ocular colobomata, provides strong evidence of the key role of GDF6 in ocular development, and illustrates the interrelated nature of several ocular-disease phenotypes.

GDF6 is a member of the BMP subfamily of transforming growth factor-beta (TGF\(\beta\)) signaling ligands, which are involved in the creation of dorsal-ventral axes, specification of neural crest, bone formation, and organogenesis.\(^3,4\) Evidence of involvement of BMP proteins in retinal development is provided by the laminar organization and retinal axon pathfinding defects caused by overexpression of the BMP inhibitor gremlin.\(^4\) However, despite studies of model organisms, the role of BMP signaling in retinal development remains insufficiently characterized, and, in particular, the contribution that BMP mutations make to human ocular disease has not been determined.

Ascribing phenotypic causation to a single candidate gene in a large deleted interval presents challenges. Accordingly, a variety of techniques—including CGH deletion mapping, expression analysis, and study of a zebrafish
Figure 4. Larval zebrafish retinal defects, caused by injection of splice-blocking gdf6a morpholinos. Wild-type images at 48 hpf (A) and 72 hpf (C), compared with morphant counterparts (B and D). Decrease in lenticular and ocular size is evident at later stages (C and D). Ventral colobomata and dorsal groove are highlighted in panel B (arrows). E–H, Images of 5-dpf zebrafish larvae, illustrating phenotypic variability observed with higher doses (10 ng) of gdf6a morpholino (injected at the one-cell stage). E, Uninjected control. F, Milder phenotype, with unilateral coloboma and lenticular extrusion. G, Unilateral coloboma and microphthalmia. H, Severe retinal degeneration, resembling human anophalmia. Transverse histological sections from wild-type (I and K) and gdf6a morphant (J and L) 3-d-old embryos, with use of phalloidin-Alexa488 and Richardson’s stains. Phalloidin-stained images are composites of 12 equally spaced 1-μm sections throughout the eye (I and J). Note reduction in ocular and lenticular size, loss of normal retinal lamination, and vacuolation of the lens that protrudes anteriorly (L). Scale bar = 100 μm.

model of reduced gdf6a function—were undertaken, which indicate GDF6’s responsibility for the ocular malformations. Gdf6/gdf6’s expression is temporally and spatially appropriate for a developmental retinal phenotype, and support for its involvement in the observed eye phenotypes is provided by its role in ventral patterning—a process that, when disrupted by SHH mutations, also results in colobomata.3 This accords with decreased GDF6 copy number (figs. 1 and 2), the eye’s exquisite sensitivity to altered gene dosage,23,42–46 the precise dosage requirement of TGFβ-signaling ligands, and the results of gdf6a-morpholino inhibition.

To validate these findings, gdf6 gene function was inhibited in a zebrafish model. The recapitulation of the proband’s phenotype—which generated posterior (dorsal or retinochoroidal) and anterior segment (ventral or iris) colobomata as well as microphthalmia—verifies the key role of gdf6 and GDF6 in ocular development. The specificity of these effects is demonstrated by the unaffected lens expression of hmx3b, decreased retinal expression of aldhl1a2 and hmx3b, and expanded retinal expression of foxg1 (fig. 3), plus the phenotypic spectrum induced by progressively higher morpholino doses (microphthalmia and posterior-segment colobomata, anterior-segment colobomata, and, finally, anophthalmia) (fig. 4). Furthermore, observation of retina-specific defects at a stage (18 hpf) before retinal vascularization precludes the ocular phenotypes being attributable to nonspecific effects, such as morpholino-induced retinal hypovascularization. Finally, existence of the irradiation-induced Tcm mutant, mapping to a small interval containing Gdf6, complements the human and zebrafish phenotypes. In view of the fact that only Gdf6/GDF6 is present in both the Tcm and segmentally deleted intervals and that Gdf6-coding mutations were not observed in Tcm mice,25 the possibility that Tcm is allelic and attributable to a segmental anomaly affecting a Gdf6 regulatory element should be critically evaluated.

To summarize, the effects of zebrafish gdf6a inhibition illustrate the interrelated nature of certain ocular phenotypes. In patients, failure of embryonic fissure closure is associated
Figure 5. Quantitative ocular phenotypic data from wild-type and gdf6a morphant (5 ng gdf6aMO1 plus 3 ng p53MO) embryos at 2–5 dpf.

with variation in ocular size, including microphthalmia, axial length <20 mm (normal 23.5 mm), and anophthalmia (absent eyes or rudimentary ocular remnants). Complementing these clinical observations, in our zebrafish model, more-severe phenotypes were induced with progressively lower gdf6 levels. In addition to the ocular anomalies, the syndactyly accords with the identical phenotype generated by mutation in the BMP-antagonist NOGGIN and Gdf6’s role in skeletal development, where null gdf6 mutations cause carpal and tarsal bone fusion. The same but milder phenotypes present in the proband’s father could be compatible with gonosomal mosaicism or chromosomal inversion, and future cytogenetic analyses may differentiate between these possibilities.

Segmental deletions (and duplications) are usually generated by low-copy repeats (LCRs) that provide a substrate for NAHR. Less frequently, NHEJ is responsible, and such rearrangements are characterized by insertion of additional bases at the junction and at breakpoints lying within Alu elements or LINEs. The segmental deletion identified in this study exhibits features of both mechanisms. The presence of LTRs on both sides of the deletion indicates intrachromosomal NAHR, whereas the breakpoint’s centromeric end’s location in an Alu, together with insertion of 4 bp, suggests NHEJ. The combination of NAHR and NHEJ has recently been reported in X-chromosomal segmental duplications. Observation of NAHR and NHEJ with a segmental deletion extends the range of chromosome rearrangements associated with human disease and indicates that a broader spectrum of human chromosomal rearrangements may use coupled homologous and non-homologous mechanisms.

In summary, it is evident that GDF6 has an essential role in retinal development and that altered gene dosage results in a diverse spectrum of ocular malformations. The human and zebrafish phenotypes confirm interspecies conservation of the requirement for precise GDF6/gdf6 dosage in normal development. The studies we have undertaken so far have identified genetic variants that cause substantial functional defects. These ocular phenotypes range from optic-nerve hypoplasia to coloboma/microphthalmia and anophthalmia, which raises the possibility that milder sequence changes contributing to other phenotypes remain to be determined. Availability of zebrafish and murine models, especially Gdf6/H11001/H11002 mice in which detailed analysis of the retina has yet to be reported, will facilitate testing of this hypothesis. Equally exciting is the potential for determining the phenotypes associated with reciprocal chromosomal anomalies, exogenous gdf6’s ability to rescue induced phenotypes, and the role of other members of the same clade in ocular development and disease. Such investigations, which are just beginning, stem from clinical observation of a broader phenotype in a patient with chorioretinal colobomata and may improve understanding of and the outcome for a frequently blinding disorder.

Note Added in Proof.—While this manuscript was under
review, Hanel and Hensey demonstrated inhibition of *Xenopus gdf6* results in ocular developmental phenotypes, providing further confirmation of this gene’s important role in vertebrate eye development.

Acknowledgments

We thank the patients for their help with this study; Federic Rosa, Samuel Sidi, Mora Robu, and Stephen Ekker, for sharing unpublished information; Dr. Stephen Bamforth, for helpful discussions; and Dr. Garry Drummond, for referring the proband. This work was supported by the Canadian Institutes of Health Research (to A.J.W. and O.J.L.), Alberta Heritage Foundation for Medical Research (to O.J.L.), Canadian Foundation for Innovation (to O.J.L.), and National Scientific Engineering Council and Alberta Ingenuity Fund (to A.J.W.). A.J.W. and O.J.L. are recipients of Canada Research Chairs.

Web Resources

The accession number and URLs for data presented herein are as follows:

- Mouse Genome Informatics, http://www.informatics.jax.org/ (for *Gdf6* [accession number MGI:3604391])
- UCSC Genome Browser, http://genome.ucsc.edu/

References


