Sequence Variants in the Transforming Growth b-Induced Factor (TGIF) Gene Are Not Associated With High Myopia

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Background

PURPOSE: Transforming Growth Beta-Induced Factor (TGIF) is a TALE homeobox gene that maps within the MYP2 high myopia locus interval at chromosome 18p11.31. In a recent report, TGIF was implicated as the MYP2 gene by SNP association studies of exon 3 in 1 transcript variant. (1) We systematically screened for nucleotide variations in the entire TGIF gene, examining an additional 7 transcript variants.
METHODS: TGIF was screened for sequence variants in the 7 original MYP2 families by direct DNA sequencing. The TGIF model used was from the NCBI human genome reference sequence (4/20/03), which has 10 exons and encodes for 8 transcript variants. Polymorphic sequence changes were compared to the previous report.
RESULTS: We observed 13 polymorphisms in 8 transcript variants. 3 were missense mutations and 4 were silent, 4 untranslated and 2 were deletions. The 3 missense variances localized to exon 10 at positions 235C? T(Pro? Leu), 243C? T(Pro? Ser), and 244C? T(Pro? Leu). Silent mutations were observed on exon 10 at positions 177A->G, 333C->T, 414T->G, and 799G->A. Five polymorphisms were novel. Of the 25 SNPS published in the earlier report, only one - 177A? G (804A? G as previously noted) - was observed in our investigation. No sequence alterations co-segregated with the affected disease phenotype.
CONCLUSIONS: Mutation analysis of the full TGIF gene for MYP2 autosomal dominant high myopia did not identify sequence alterations associated with the disease phenotype. Further studies of MYP2 candidate genes are needed to determine the gene causative for this potentially blinding

Introduction

Our laboratory identified the MYP2 locus using 7 families with non-syndromic autosomal dominant high myopia of –6.00 diopters or greater. We demonstrated significant linkage to chromosome 18p11.31 with a maximum cumulative LOD score of 9.59 at theta=0.00 [2]. The 7.6cM recombinant interval was defined distally by marker D18S59 and proximally by marker D18S1138. These 7 pedigrees, represent the group of MYP2 families we have screened for mutations at the MYP2 locus.

Markers D18S52 and D18S1138 show the strongest statistical association with the disease phenotype suggesting that the MYP2 gene is likely within a 2.2 cM interval between D18S52 and D18S481.

One gene that maps within the chromosome 18p11.3 interval is transforming growth β-induced factor (TGIF). TGIF is a DNA-binding homeo-domain protein that belongs to the TALE family homeobox. [4,5]. It is a transcription repressor with multiple actions including a role in retinoid-responsive transcription [4]. TGIF mutations are associated with holoprosencephaly, a congenital craniofacial and brain anomaly disorder. [6,7]

Recently Lam et al. reported a possible association of TGIF exon 3 polymorphisms with MYP2 associated high myopia in a cohort of 6 Hong Kong Chinese families [1]. Of note, the TGIF genetic structure studied by Lam et al was 3 exons. The current build of the human reference sequence provides evidence for alternate splicing of 10-exons. Exons 1, 2, and 3 studied previously correspond with exons 5, 9, and 10 respectively, which corresponds to transcript variant 4 (Figure 1).

We sought to determine if the TGIF gene is causally related to MYP2-associated high myopia by direct DNA sequencing, using DNA samples from the original MYP2 pedigrees.

Methods

Patients: Probands from the initial 7 MYP2 families affected by an autosomal dominant form of high myopia were studied. Clinical details regarding the complete pedigree were published previously. [2] Controls were obtained from marry-ins, non-myopic family members, and unrelated subjects.
DNA Amplification and Mutation Screening: The genomic structure of TGIF, as reported in MapViewer (build 33) of the reference human genome sequence is outlined in Figure 1. The genomic structure for TGIF contains 10 exons spanning 46kb, and has 8 transcript variants encoding four proteins of 402 residues (variant 1), 287 residues (variant 2), 273 residues (variants 3 and 4), and 253 residues (variants 5-8).
Twelve oligonucleotide primer pairs were designed to amplify the exonic sequences including 50-200 base pairs beyond the intron-exon boundary. PCR amplicon sequences were trimmed for quality, BLASTed and aligned. Sequence alterations were annotated and compared between normal and affected individual DNA samples.
Reverse Transcription– Polymerase Chain Reaction:
One microgram of total RNA from sclera, optic nerve, retina and cornea, as well as commercially prepared poly-A RNA from various human organs were used as a template for first-strand cDNA synthesis. The RT-PCR products, along with the amplicon products of the housekeeping gene b-actin were visualized on 2% agarose gels (Figure 2) after electrophoresis and staining with ethidium bromide

Results I

A total of 12 polymorphisms were observed in the 10 exons screened. (Table 1) Of these, 2 were missense variances, 4 were silent, 4 were not translated, and 2 were deletions. The 2 missense variants localized to exon 10 at positions 236C →T(Pro→Leu) and 244C→T(Pro→Leu). Silent mutations were observed on exon 10 at positions 177A→G, 245T→C, 332C→T and 413T→G. One variation was consistent with previous published results, 11 were novel. The two deletions were observed in exon 6 at positions 3442213 and 3442220 on the scaffold sequence NT_010859.12. Both deletions cause early termination of the protein, yielding proteins of 132 and 141 residues, respectively. No sequence variants co-segregated with the affected phenotype.

Figure 1 Genomic Structure and Transcript Variants of TGIF

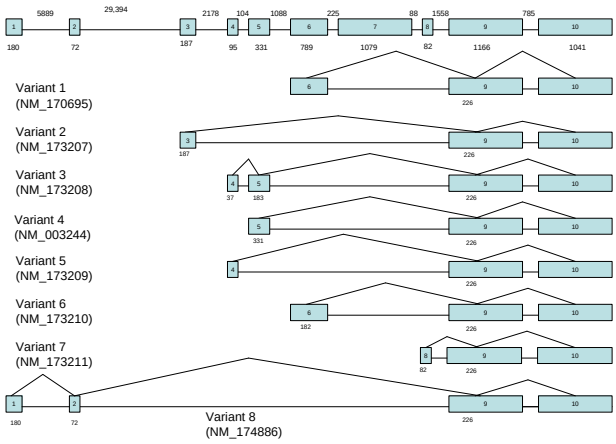


Table 1 TGIF Polymorphisms

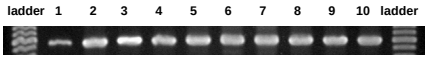
Position on NT_010859	Wild Type	Observed	Sample # (RED=Affected)	Amino Acid Change	Exon
3440452	C	C/T	5472, 2852, C0009	5'UTR	bp 284 of exon 5
3441759	C	T/T	2698, C0183	5'UTR	bp172 of exon 6
3441759	C	C/T	2700, 2852	5'UTR	bp172 of exon 6
3441893	G	A/G	2700, 2852	5'UTR	bp306 of exon 6
3441893	G	A/A	2698	5'UTR	bp306 of exon 6
3442213	T	DELETED	1932, 5071, 2141, 2679		bp633 of exon 6
3442215	C	DELETED	5071		bp 635 of exon 6
3447536	A	A/G	2852, 2700, C0183, 2679, 5472	NONE	bp177 of exon 10
3447536	A	G/G	2698	NONE	bp177 of exon 10
3447595	C	C/T	5071	PRO>LEU	bp 236 of exon 10
3447603	C	C/T	2679	PRO>LEU	bp 244 of exon 10
3447604	C	C/T	2700, 2852	NONE	bp 245 of exon 10
3447692	C	C/T	5071, C0183	NONE	bp333 of exon 10
3447773	T	G/T	5071, C0183	NONE	bp 414 of exon 10
3448158	G	G/A	1932	3'UTR	bp 799 of exon 10

Results II

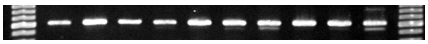
Of the 25 SNPS previously studied in the Lam et. al. report [1], only one at position 804A→G was observed in our investigation. This was the only polymorphism found in our patient sample cohort that was previously reported in the public SNP databases.

RT-PCR results confirmed TGIF expression in all ocular tissues. (Figure 2)

Figure 2. Expression of TGIF in Ocular and Systemic Tissues



b-ACTIN (Standard Invitrogen Control) 353bp



TGIF 360bp

1 – Sclera	6 - Skeletal Muscle
2 – Cornea	7 - Heart
3 - Optic Nerve	8 -Trachea
4 – Retina	9 - Kidney
5 - Lung	

Discussion

We sequenced the full TGIF gene in our patient samples of individuals from pedigrees with MYP2- associated high myopia. No DNA sequence variants were noted that implicated TGIF as the causative gene. TGIF exon 10 (exon 3 in the initial build of this gene) did not show the same level of polymorphic variants in our cohort, as we only observed one common SNP at mRNA position 804. This may be due to the ethnic differences in our two sample sets, although one family of the MYP2 pedigrees studied was of Chinese descent.

In conclusion, TGIF is a potential candidate gene for the MYP2-associated high myopia based on its mapped location within the MYP2 interval. Mutation analysis of the full TGIF gene did not identify sequence alterations associated with the disease phenotype. Further studies of MYP2 candidate genes are needed to determine the gene causative for this potentially blinding disorder.

References

1. Lam DSC, Lee WS, Leung YF, Tam POS, Fan DSP, Fan BJ, Pang CP. TGFB-Induced Factor: A Candidate Gene for High Myopia. Invest Ophthalmol Vis Sci. 2003;44:1012-1015.
2. Young TL, Ronan SM, Drahozal LA, Wildenberg SC, Alvear AB, Oetting WS, Atwood LD, Wilkin DJ, King RA. Evidence that a locus for familial high myopia maps to chromosome 18p. Am J Hum Genet 63(1): 109-119; 1998.
3. Young TL, Atwood LD, Ronan SM, Dewan AT, Alvear AB, Peterson J, Holleschau, King RA. Further refinement of the MYP2 locus for autosomal dominant high myopia by linkage disequilibrium analysis. Ophthalmic Genetics 22:69-75;2001.
4. Bertolino E, Reimund, B, Wildt-Perinic D, Clerc RG. A novel homeobox protein which recognizes a TGT core and function interferes with a retinoid-responsive motif. J Biol Chem. 1995;52:31178-31188.
5. Wotton D, Lo RS, Swaby LAC, Massague J. Multiple modes of repression by the Smad transcriptional corepressor TGIF. J Biol Chem. 1999;274:37105-37110.
6. Gripp KW, Wotton D, Edwards MC, et al. Mutations in TGIF cause holoprosencephaly and link NODAL signaling to human neural axis determination. Nat Genet. 2000;25:205-208.
7. Chen C-P, Chern S-R, Du S-H, Wang W. Molecular diagnosis of a novel heterozygous 268C>T (R90C) mutation in TGIF gene in a fetus with holoprosencephaly and premaxillary agenesis. Prenatal Diagnosis. 2002,22:5-7.

Acknowledgements:
NIH-EY00376, Research To Prevent Blindness Inc., Mabel E. Leslie Research Funds.