FOXP4, a novel candidate gene for angle closure glaucoma

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Introduction

Primary angle closure glaucoma is a significant burden on vision loss worldwide, and affects 0.5% of the population[1-3]. This condition is defined by the apposition of the peripheral iris tissue to the iridocorneal angle, leading to blockage of the outflow pathways, rise in eye pressure, and subsequent irreversible damage to the optic nerve. Few genes have been identified as causative for primary angle closure glaucoma[1], and little is known about the developmental pathways leading to this condition. We have recently identified a multigenerational pedigree where high hyperopia, short axial length, plateau iris configuration, and angle closure glaucoma are transmitted in an autosomal dominant fashion. We have collected unrelated families and cases with the spectrum angle closure disorders for genetic and functional analysis.

Methods

An Ashkenazi Jewish kindred underwent pooled whole exome sequencing. Variants were prioritized and filtered using a customized pipeline[4].
Functional analysis: FOXP4 structure was modeled using 2a07 crystal structure.
Luciferase assays: HEK293T cells were transfected with wild-type: YFP-FOXP4
Cellular localization of GFP fluorescence was evaluated with an inverted confocal microscope
Mutational screening: DNA from blood or saliva samples from 40 independent probands with angle closure spectrum phenotypes (primary angle closure, plateau iris, high hyperopia with narrow angles, primary angle closure glaucoma) were collected and processed
Expression analysis: Wild-type mouse eyes were fixed in 4% PFA overnight followed by processing for paraffin sectioning. Sections were immunostained against FOXP4 protein (anti-FOXP4)[5] or RNA using an RNAscope in situ hybridization probe.

Results
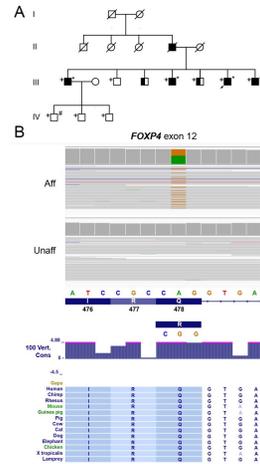
Figure 1: FOXP4 variant identified in angle closure, short axial length family. (A) Pedigree of family showing dominant inheritance pattern. B) Integrated Genomics Viewer layout showing sequence reads for *FOXP4* exon 12 demonstrating that 44% of reads contain variant (antisense strand) in the affected and not unaffected pool, and that this causes p.(Q478R) amino acid change. This residue is highly conserved by PhyloP conservation and invariant in higher organisms. +, sampled, * in affected pool, # in unaffected pool, half-shade – plateau iris, full shade – plateau iris, short axial length, angle closure/angle closure glaucoma.
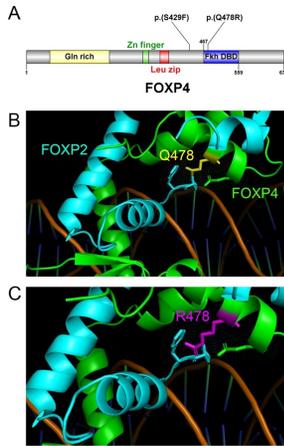
Figure 2: FOXP4 structural modeling. (A) FOXP4 protein structure showing location of variants. (B-C) Homology model of wild-type FOXP4 (B) and p.Q478R (C) heterodimer with FOXP2 and bound to DNA based on SWISS-model of crystal structure 2a07. R478 appears to cause steric disruption of critical dimerization interactions. Gln rich, glutamine rich; Leu zip, leucine zipper domain; Fkh DBD, forkhead DNA binding domain.
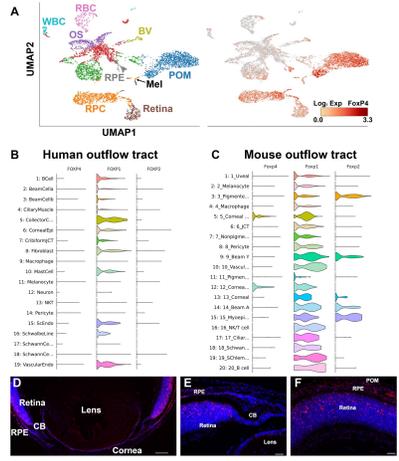
Figure 3: RNA analysis of FOXP4 expression pattern in the mammalian and human eye. (A) scRNAseq analysis from mouse E13.5 eye cup with expression pattern of *Foxp4*. UMAP projection of scRNA sequencing data from E13.5 control mice. Each dot represents a single cell, and the proximity of cells indicates similar gene expression patterns. Cell identity is based on curation of gene expression patterns within each K-means cluster. Log2 expression of *Foxp4* in single cells reveals abundant expression in retinal progenitors (RPC), differentiating retina, retinal pigment epithelium (RPE), and periocular mesenchyme. (B-C) Violin plots showing expression of FOXP4, FOXP1, and FOXP2 in the human (B) and mouse (C) outflow tracts, visualized using Spectacle[6] and based on van Zyl et. al data[7]. FOXP4 is most prominently expressed in corneal tissue, while FOXP1 is more broadly expressed. (D-F) RNAscope in situ hybridization of P0 wild-type mouse eyes with probe specific for mouse *Foxp4* with *Foxp4* transcripts shown in red and nuclei stained with DAPI in blue. Low power view (D) showing expression of *Foxp4* transcripts in the retina, RPE, cornea, and lens epithelium, with weak/absent expression in ciliary body. Scale bar 100um. High magnification views (E-F) showing expression of Foxp4 in the retina, periocular mesenchyme and retinal pigment epithelium. Scale bar, 25 μm. BV, blood vessels; WBC, white blood cells; RBC, red blood cells; Mel, melanocytes; OS, optic stalk.
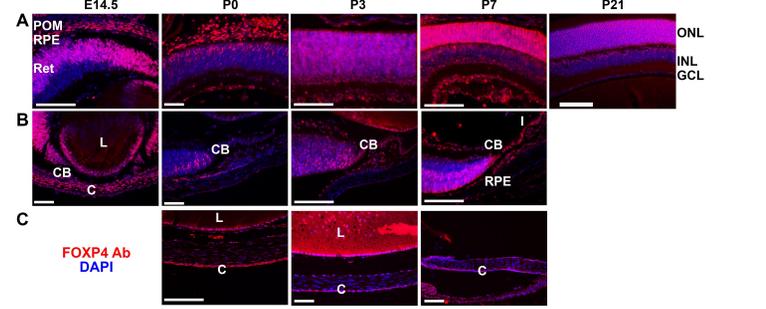
Figure 4: Expression pattern of Foxp4 across development in the mouse eye. (A-C) RNAscope in situ hybridization of P0 wild-type mouse eyes with probe specific for mouse *Foxp4* with *Foxp4* transcripts shown in red and nuclei stained with DAPI in blue. Low power view (A) showing expression of *Foxp4* transcripts in the retina, RPE, cornea, and lens epithelium, with weak/absent expression in ciliary body. Scale bar 100um. High magnification views (B-C) showing expression of Foxp4 in the retina, periocular mesenchyme and retinal pigment epithelium. Scale bar, 25 μm. (D-G) FOXP4 immunostaining of P0 mouse eyes showing nuclear localized expression in the retina, RPE, and periocular mesenchyme (POM) at low power (D) and high power (E-F) view. There is also staining in the corneal epithelium and lens epithelium (G).

Figure 5: Functional consequences of FOXP4 p.Q478R variant. A) Luciferase assays with evaluating repression of SPRX2 promoter by FOXP4 wild-type and variant proteins as described[8]. Relative luciferase values were normalized to Renilla luciferase and empty vector was set to 1.0. FOXP4 wildtype (WT) shows repression of SPRX2 promoter. This repression is lost in FOXP4 p.H517N, an established loss of function variant that causes a multisystem FOXP4 disorder[8]. FOXP4 p.Q478R retains ability to repress the SPRX2 promoter. (B) Cellular localization of FOXP4 variants, showing cytoplasmic localization of p.H517N and p.Q478R, with predominantly nuclear localization of wild-type FOXP4. ** p<0.01 Tukey's test.

Conclusions

• FOXP4 rare variant identified in a family with angle closure glaucoma, plateau iris, and hyperopia. The variant does not affect transcriptional activity *in vitro,* but appears to alter localization of the protein.
• FOXP4 is expressed in ocular tissues during development in the mouse eye including the retina, RPE, periocular mesenchyme, cornea, and anterior segment, supporting an important role in eye development.
• An additional rare coding VUS was identified in *FOXP4* p.Phe483Leu in a patient with ACG and a small eye (axial length 20.69/20.65mm)
• Our work supports a role for *FOXP4* in pathogenesis of angle closure glaucoma, though additional studies are necessary to definitively establish a causative relationship.

References

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Chr:Location	FOXP4 exon	rs#	cDNA variant	Protein change	MAF AC cases	gnomAD MAF	Fisher exact P-value	SpliceAI Max Score	GERP	CADD	REVEL	ACMG Class	ACMG Rules
6:41533573	2	rs34730847	c.533 G>A	p.Gly25Gly	0.04	0.04	1	0.01	1.4	3.959	N/A	Benign	N/A
6:41533579	2	rs2104506	c.539 C>A	p.Ala27Ala	0.34	0.32	0.7134	0.01	-10.9	0.513	N/A	Benign	N/A
6:41545807	3	rs72858984	c.746 C>T	p.Ala96Ala	0.04	0.02	0.4378	0	1.91	11.17	N/A	Benign	N/A
6:41545842	in3	N/A	c.300+23G>A	N/A	0.01	N/A	N/A	0	N/A	14.33	N/A	VUS	BP4
6:41557624	in10	N/A	c.1149+32G>A	N/A	0.03	N/A	N/A	0	1.19	-0.12499	N/A	VUS	BP4
6:41558952	in12	rs9471605	c.1435-7C>T	N/A	0.14	0.09	0.0996	0	-2.33	3.075	N/A	Benign	BP4
6:41565533	16	N/A	c.2198 G>A	p.Glu580Glu*	0.02	N/A	N/A	0.23	N/A	7.907	N/A	Likely Benign	BP4, BP7, PM2
6:41565542	16	rs562089407	c.2207 C>A	p.Phe583Leu*	0.02	0.00001	N/A	0	1.02	14.88	0.19	VUS	PM2, BP4

Chr, chromosome/location, based on GRCH37 hg19 build, MAF, minor allele frequency; AC, angle closure; gnomAD, frequency in gnomad.broadinstitute.org control population cohort; SpliceAI score; GERP, Genomic Evolutionary Rate Profiling conservation score, CADD, combined annotation dependent depletion score; REVEL, rare exome variant ensemble learner score; ACMG, American College of Medical Genetics ; VUS, variant of unknown significance, #, variant identified in the same individual

Table 1: FOXP4 sequence variants identified in 40 unrelated angle closure/plateau iris/hyperopia probands.

Acknowledgements