Association of rs12997 variant in the *ACVR1* gene: a member of bone morphogenic protein signaling pathway with primary open-angle glaucoma in a Saudi cohort

Altaf A Kondkar ,[1,2,3] Taif A Azad,[1] Tahira Sultan,[1] Essam A Osman,[1] Faisal A Almobarak,[1,2] Saleh A Al-Obeidan[1,2]

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jim-2020-001596).

[1]Department of Ophthalmology, College of Medicine, King Saud University, Riyadh, Saudi Arabia
[2]Glaucoma Research Chair in Ophthalmology, College of Medicine, King Saud University, Riyadh, Saudi Arabia
[3]King Saud University Medical City, King Saud University, Riyadh, Saudi Arabia

Correspondence to
Dr Altaf A Kondkar, Department of Ophthalmology, College of Medicine, King Saud University, Riyadh, Saudi Arabia; akondkar@gmail.com

Accepted 1 December 2020
Published Online First 21 December 2020

To cite: Kondkar AA, Azad TA, Sultan T, *et al*. *J Investig Med* 2021;**69**:402–407.

ABSTRACT

We investigated the association between variants rs12997 in activin A receptor type I (*ACVR1*) and rs1043784 in *BMP6* located in the 3′ untranslated region, and primary open-angle glaucoma (POAG). The retrospective case-control study used TaqMan real-time PCR assay to genotype 400 subjects, including 150 patients with POAG and 250 controls. The minor 'G' allele of rs12997 in *ACVR1* showed significant association with POAG (p=0.027, OR=1.39, 95% CI=1.03 to 1.87). Likewise, rs12997 genotypes showed moderate association with POAG in recessive (p=0.048, OR=1.80, 95% CI=1.01 to 3.20) and log-additive models (p=0.030, OR=1.39, 95% CI=1.03 to 1.87), but did not survive Bonferroni correction. Rs1043784 in *BMP6* showed no associations. Furthermore, rs12997 G/G genotype significantly (p=0.033) increased the risk of POAG (twofolds) independent of age, sex and rs1043784 genotypes in regression analysis. However, clinical variables such as intraocular pressure and cup/disc ratio showed no association with both the polymorphisms. To conclude, the study shows a modest association between rs12997 in the *ACVR1* gene, a member of the bone morphogenic protein signaling pathway and POAG. However, the results need further replication in large population-based cohorts and different ethnicities to validate its role as an important genetic biomarker.

BACKGROUND

Primary open-angle glaucoma (POAG) is a multifactorial optic neuropathy of unknown etiology. One of the known mechanisms in POAG is increased proteoglycan synthesis of the extracellular matrix (ECM), deposition and remodeling in the trabecular meshwork (TM). These processes can reduce the outflow facility of the anterior chamber resulting in elevated intraocular pressure (IOP), retinal ganglion cell (RGC) death, damage to optic nerve head (ONH) and loss of vision.[1] The genetic factors responsible for TM alteration and outflow resistance are still unclear.[2]

Transforming growth factor-β (TGF-β), due to its role in ECM remodeling, has been implicated in glaucoma pathogenesis affecting both the TM outflow pathway and pressure-induced optic nerve damage.[3–5] Also, genetic loci linked to POAG in a genome-wide study were found to contribute to the regulation of TGF-β signaling, lending further credence to the involvement of the TGF-β pathway in glaucoma.[6] Besides TGF-β, the members of this superfamily of growth factors also include bone morphogenic proteins (BMPs), activins, inhibins, growth factors and other signaling molecules involved in the BMP signaling pathway.[7 8]

BMPs were initially identified as osteoinductive factors. However, their role in development, morphogenesis, cell proliferation, fibrosis and apoptosis is now established.[9] Members of the BMP family of genes, including BMPs and BMP receptors, are expressed in the human TM and ONH.[10] BMPs also inhibit TGF-β2-induced ECM changes in the TM cells.[11] Similar to TGF-β cytokine, signaling by BMP ligands involves type I and type II transmembrane serine/threonine kinase receptors.[12] Activin A receptor type I (*ACVR1*), also known as *ALK2*, encodes a BMP type I receptor of the TGF-β receptor subfamily. The receptor interacts with the BMP type II receptors to form transmembrane complexes and, on ligand binding, initiates signal transduction and transcription of target genes via SMAD1/5/8 (canonical) or p38/JNK/MAP kinase (non-canonical) signaling pathways.[12] ACVR1 is involved in several biological processes and is linked to a wide variety of pathologies, including cardiac, reproductive system and cancer.[13] Moreover, the causal role of *ACVR1* mutations has been extensively investigated in fibrodysplasia ossificans progressiva (FOP). FOP is a rare genetic disease characterized by progressive heterotopic ossification.[13 14] Interestingly, some patients with FOP with *ACVR1* mutation also exhibited childhood glaucoma,[15] supporting its role in glaucoma. Bone morphogenetic protein 6 (BMP6) is an AVCR1 ligand. It also belongs to the TGF-β superfamily and plays a critical role

in the pathogenesis of age-related macular degeneration (AMD). Patients with neovascular and early AMD reported reduced expression of BMP6.[16 17] Besides, BMP6 conferred protection to the retinal pigment epithelial cells protection against oxidative damage and apoptosis.[17]

It can thus be speculated that genes or genetic variations involved in the TGF-β/BMP signaling may play an essential role in modulating TM homeostasis and POAG pathogenesis.[12 18] Based on our recent findings in primary angle-closure glaucoma (PACG) and pseudoexfoliation glaucoma (PXG),[19] we investigated the association between two variants in the 3' untranslated region (UTR), rs12997 in *ACVR1* and rs1043784 in *BMP6* and POAG. These 3'UTR variants by affecting microRNA (miRNA)/messenger RNA (mRNA) stability[20] may alter gene expression and thereby influence TM homeostasis and the disease risk.

METHODS

Study design and participants

A retrospective case-control study was performed in 150 unrelated Saudi patients with POAG and 250 control subjects. The participants were recruited between April 2017 through September 2019 at King Abdulaziz University Hospital, King Saud University, Riyadh, Saudi Arabia. The patients met the following diagnostic criteria: (1) presence of glaucomatous optic disk damage or retinal nerve fibre layer changes, for example, thinning or notching of disc rim, progressive changes, nerve fibre layer defect; (2) presence of visual field abnormalities (eg, arcuate scotoma, nasal step, paracentral scotoma, tunnel vision) in the absence of other causes or explanation; (3) bilaterally open anterior chamber angles on gonioscopy and (4) adult-onset. Individuals with secondary causes of glaucoma, trauma or on steroids were excluded. The controls were >40 years of age, normal IOP with no presence of glaucoma on clinical examination.[21]

Genotyping of rs12997 and rs1043784

DNA samples were genotyped using the TaqMan assays, C___7545093_10 for rs12997 and C___2064624_20 for rs1043784 (catalog number: 4351379), on ABI 7500 real-time PCR (Applied Biosystems, Foster City, California, USA) as recommended by the manufacturer.[22]

Statistics

To test Hardy-Weinberg Equilibrium (HWE) and other genetic associations, χ^2 analysis was used. The continuous parameters were tested by independent samples t-test and one-way analysis of variance. Logistic regression analysis was used to test the effect of multiple risk factors on POAG. Statistical analysis was done using SPSS V.22 (IBM, Chicago, Illinois, USA) and SNPStats online software (https://www.snpstats.net/start.htm). A two-tailed $p<0.05$ was considered statistically significant, and Bonferroni corrected p value ($0.05/5=0.01$) was considered where applicable.

RESULTS

A total number of 400 participants, including 150 POAG cases and 250 controls, were evaluated in this study. As shown in table 1, there were no significant differences in age and gender distribution in the two study groups. Table 1

Table 1 Demographic characteristics and distribution of minor allele frequency of study participants

Characteristics	POAG (n=150)	Controls (n=250)	OR	95% CI	P value
Age in years (SD)	61.5 (10.9)	59.9 (6.9)	–	–	0.068
Male/Female, n	81/69	136/114	1.01	0.67 to 1.52	0.920
Minor allele frequency					
Rs12997[G]					
Total	0.41	0.33	1.39	1.03 to 1.87	**0.027**
Men	0.43	0.36	1.36	0.91 to 2.03	0.127
Women	0.38	0.30	1.43	0.92 to 2.24	0.109
Rs1043784[C]					
Total	0.15	0.15	0.98	0.65 to 1.47	0.920
Men	0.14	0.15	0.92	0.53 to 1.59	0.764
Women	0.15	0.14	1.06	0.58 to 1.47	0.920

Significant p value in bold.
POAG, primary open-angle glaucoma.

also summarizes the minor allele frequency distribution of rs12997 and rs1043784 variants in cases and controls. The control group showed no deviation from HWE (p>0.05). The rs12997[G] allele was significantly associated with increased risk of POAG (OR=1.39, 95% CI=1.03 to 1.87, p=0.027). In contrast, the rs1043784 [T/C] variant showed no significant distribution between cases and controls. Besides, gender stratification also showed no association for both the variants.

Association analysis between rs12997 and rs1043784 and POAG risk was performed in different genetic models using the SNPStats software, as shown in table 2. Rs12997 variant in the *ACVR1* gene showed a significant association with POAG risk in recessive and log-additive models (best-fit) with the lowest Akaike information criterion and Bayesian information criterion values. The p value remained significant after adjustment for age and sex, but not for Bonferroni correction. On the other hand, rs1043784 showed no significant association with POAG in any genetic models analyzed (table 2). Likewise, gender-stratified genotype analysis for rs12997 and rs1043784 also showed no gender-specific association (data not shown).

The binary logistic regression analysis showed that the G/G genotype of rs12997 conferred a significant twofold increased risk of POAG (OR=2.06, 95% CI=1.06 to 4.01, p=0.033) independent of age, sex and rs1043784 genotype, indicating that the variant rs12997 may be an independent risk factor of POAG (online supplemental table 1). Furthermore, clinical parameters such as IOP, cup/disc ratio and the number of antiglaucoma medications showed no significant association with both the variants (online supplemental figure 1).

DISCUSSION

This study evaluated associations between genetic variants rs12997 in *ACVR1* and rs1043784 in *BMP6* genes and patients with POAG. Rs1047384 in *BMP6* showed no association with POAG, suggesting that this variant/gene may not significantly impact POAG genetic etiology. In contrast, we found that the *ACVR1*-rs12997[G] allele was significantly associated with POAG having an OR of 1.39. And the homozygous GG genotype increased the POAG risk by twofolds but did not survive Bonferroni correction. Although many studies have provided evidence for the role of TGF-β in glaucoma pathogenesis, this is the first study to report a modest association between genetic variant (A>G rs12997) in the gene (*ACVR1*) involved in the BMP signaling pathway and POAG.

The regulation of *ACVR1* is still not completely understood. ACVR1, a TGF-β superfamily co-receptor, can function via the TGF-β/BMP signaling through a variety of different mechanisms. Mutant *ACVR1* displays ligand-independent BMP signaling, hyper-reactivity to specific BMP ligand stimulation in different cell-types, and cause illicit overactivation of *ACVR1* and dysregulated BMP signaling.[23–25] It has also been proposed that signaling changes may arise as a result of alteration in ACVR1 interaction with FKBP12, a cytoplasmic FK506-binding protein. FKBP12 prevents leaky activation of ACVR1 in the absence of ligand and acts as its negative regulator.[26] Interestingly, the FKBP12 ligand FK506 has been demonstrated to interfere with apoptotic mechanisms after optic nerve crush and confer neuroprotection on the RGCs.[27]

Similarly, activin A, another member of the TGF-β superfamily, can also bind to ACVR1 and induce downstream signaling.[28] However, they do not engage the receptor directly and instead compete with BMP ligands.[28] This competition may create an imbalance in the levels of expression between type I and type II BMP receptors and cause dysregulated signaling outcomes.[29] Besides, abnormal ACVR1 activity can also result in a pro-inflammatory state via increased nuclear factor-κB and p38MAPK activity, causing specific immune function alterations that may have pathological consequences.[30] *ACVR1* gene is also linked to cancer and shown to be tumor suppressive in mouse lens.[31] Likewise, an increase in copy numbers and overexpression of *ACVR1* mRNA was associated with survival in head and neck cancers.[32] The variant rs12997 has been reported to increase the risk of colorectal cancer.[20]

The exact molecular mechanism by which the *ACVR1* variant may influence the risk of POAG is currently unknown and can only be speculated. There is evidence to suggest that abnormalities in cell signaling pathways in the TM can contribute to glaucoma pathogenesis, and Wnt signaling appears to be a key player in this context.[5 11 33 34] Genes involved in canonical Wnt signaling pathways have been reported to be expressed in the human TM.[35] Wnt signaling plays a crucial role in ECM cell behavior and elasticity.[36] The function of the canonical Wnt signaling pathway in ECM regulation to maintain TM homeostasis[37] and IOP via β-catenin's effects on cadherin junctions has been well documented.[33] Studies have linked Wnt antagonism to glaucoma pathogenesis. Morgan *et al* demonstrated a link between Wnt antagonism and increased TM stiffness that may contribute to glaucoma progression.[38] Increased expression of Wnt antagonist, secreted frizzled-related protein-1 (sFRP-1), was observed in glaucomatous TM and reported to be responsible for high IOP.[39]

Interestingly, ACVR1 is an essential regulator of the BMP/Wnt signaling pathway to promote proliferation and metastasis.[40 41] Similar to sFRP-1, ACVR1 is also a Wnt signaling antagonist and has been shown to inhibit Wnt signaling in osteoblasts through Wnt inhibitors SOST and DKK1.[42] Likewise, Acvr1-deficiency was found to increase osteogenesis by activating Wnt signaling and reducing these Wnt inhibitors.[42] Taken together, these studies suggest a plausible role for *ACVR1* in TM modulation and outflow resistance via regulation of Wnt signaling. Since rs12997 is located in the 3'UTR region, *ACVR1* expression can be regulated by specific miRNAs by affecting mRNA stability.[43] In silico tools have shown that this variant may affect the binding of miR-330-3p and cause a failure of *ACVR1* regulation.[20] Therefore, the mutant allele may exhibit differential expression patterns than the wild-type allele and disrupt TM homeostasis by causing ECM abnormalities via the *ACVR1*/BMP/Wnt signaling pathway, through Wnt inhibitors, thereby showing an association with POAG (figure 1). Besides, miR-384 has been shown to negatively regulate Wnt signaling by targeting *ACVR1* in breast cancer cells, lending further support to this hypothesis.[41] Furthermore, the possibility of a linkage between rs12997 and another causative variant(s) or gene-gene interactions cannot be ruled out. We have also recently reported an association

Table 2 Association of rs12997 and rs1043784 polymorphisms with the risk of POAG compared with control under different genetic models

SNP number	Model	Genotype	Control n (%)	POAG n (%)	OR (95% CI)	P value	AIC	BIC	P value*
Rs12997	Co-dominant	A/A	110 (44.4)	54 (36.0)	1.00	0.081	528.3	540.3	0.062
		A/G	111 (44.8)	69 (46.0)	1.27 (0.81 to 1.97)				
		G/G	27 (10.9)	27 (18.0)	**2.04 (1.09 to 3.81)**†				
	Dominant	A/A	110 (44.4)	54 (36.0)	1.00	0.100	528.7	536.6	0.090
		A/G-G/G	138 (55.6)	96 (64.0)	1.42 (0.93 to 2.15)				
	Recessive	A/A-A/G	221 (89.1)	123 (82.0)	1.00	**0.048**‡	527.4	535.4	**0.035**
		G/G	27 (10.9)	27 (18.0)	**1.80 (1.01 to 3.20)**				
	Overdominant	A/A-G/G	137 (55.2)	81 (54.0)	1.00	0.810	531.3	539.3	0.830
		A/G	111 (44.8)	69 (46.0)	1.05 (0.70 to 1.58)				
	Log-additive	---	---	---	**1.39 (1.03 to 1.87)**	**0.030**‡	526.6	534.6	**0.023**
Rs1043784	Co-dominant	T/T	184 (73.6)	109 (73.2)	1.00	0.850	533	544.9	0.880
		C/T	57 (22.8)	36 (24.2)	1.07 (0.66 to 1.72)				
		C/C	9 (3.6)	4 (2.7)	0.75 (0.23 to 2.49)				
	Dominant	T/T	184 (73.6)	109 (73.2)	1.00	0.920	531.3	539.3	0.870
		C/T-C/C	66 (26.4)	40 (26.9)	1.02 (0.65 to 1.62)				
	Recessive	T/T-C/T	241 (96.4)	145 (97.3)	1.00	0.610	531	539	0.680
		C/C	9 (3.6)	4 (2.7)	0.74 (0.22 to 2.44)				
	Overdominant	T/T-C/C	193 (77.2)	113 (75.8)	1.00	0.760	531.2	539.2	0.740
		C/T	57 (22.8)	36 (24.2)	1.08 (0.67 to 1.74)				
	Log-additive	---	---	---	0.98 (0.67 to 1.45)	0.930	531.3	539.3	1.000

Significant OR and p value in bold. Bonferroni corrected p value is 0.01.
*Adjusted for age and sex.
†A/A versus G/G p value=0.024.
‡Best-fit model p value.
AIC, Akaike information criterion; BIC, Bayesian information criterion.

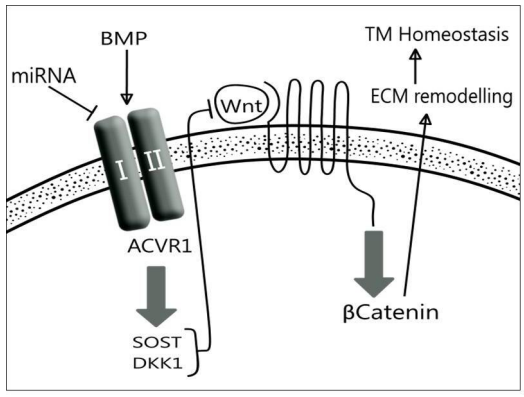

Figure 1 Schematic representation of the postulated mechanism in regulating trabecular meshwork homeostasis through ACVR1/BMP/Wnt signaling pathway. ACVR1, activin A receptor type I; BMP, bone morphogenic protein; ECM, extracellular matrix; miRNA, microRNA; TM, trabecular meshwork.

of this variant in PACG and PXG,[19] suggesting an essential role that this gene/variant may have, and a common molecular pathway underlying TM modulation and glaucoma types. Further in vitro studies and molecular investigations are needed to confirm these hypotheses.

Based on the allele frequency in our study, we had 0.90 and 0.76 probabilities of detecting significant associations between POAG and rs12997 and rs1043784 variants, respectively, with an OR of 2.0 (α=0.05). Nonetheless, much larger samples need to be examined to detect an OR of ≤1.5, which is commonly observed in genetic association studies. It will also be interesting to investigate other ligands and receptors in the BMP signaling pathway in the future. These studies may provide new insights into the pathophysiology of POAG, highlighting the role of members of the BMP signaling pathway in POAG pathogenesis and reveal potential genetic biomarkers and drug targets.

To conclude, this is the first report showing an association between polymorphism rs12997 in the *ACVR1* gene and POAG indicating that *ACVR1*/members of the BMP signaling pathway may be an essential player in the genetic etiology of the disease. However, large multicenter population-based replication studies in different ethnicities are needed to validate and draw definite conclusions on its role as a potential genetic marker.

Acknowledgements The authors would like to thank the Vice Deanship of Scientific Research Chair, Glaucoma Research Chair in Ophthalmology at the King Saud University for their support. The authors would also like to thank Mr Abdulrahman Al-Mosa for his clinical and Ms Alanoud A. Alotaibi for her technical assistance in the study.

Contributors AAK: concept, experimental design, analysis, data interpretations, wrote and edited the manuscript. TAA, TS: sample preparation, genotyping and data acquisition, manuscript editing and revision. EAO, FAA: concept, recruitment, clinical diagnosis, data interpretation, manuscript editing and revision. SAA-O: concept, experimental design, recruitment, clinical diagnosis, data interpretation, manuscript editing and revision. All authors read and approved the final manuscript.

Funding This work was supported by King Saud University through Vice Deanship of Scientific Research Chair and Glaucoma Research Chair in Ophthalmology (#08–657).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study adhered to the Declaration of Helsinki principles and was approved by the institutional review board committee at the College of Medicine, King Saud University, Riyadh, Saudi Arabia. All the participants signed informed consent.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iD

Altaf A Kondkar http://orcid.org/0000-0002-5516-6951

REFERENCES

1 Tektas O-Y, Lütjen-Drecoll E. Structural changes of the trabecular meshwork in different kinds of glaucoma. *Exp Eye Res* 2009;88:769–75.
2 Borrás T. Mechanosensitive Genes in the Trabecular Meshwork at Homeostasis. In: Tombran-Tink J, Barnstable CJ, Shields MB, eds. *Mechanisms of the glaucomas ophthalmology Research™ Humana press*. Totowa NJ, 2008: 329–61.
3 Fuchshofer R, Birke M, Welge-Lussen U, *et al*. Transforming growth factor-beta 2 modulated extracellular matrix component expression in cultured human optic nerve head astrocytes. *Invest Ophthalmol Vis Sci* 2005;46:568–78.
4 Fuchshofer R, Tamm ER. The role of TGF-β in the pathogenesis of primary open-angle glaucoma. *Cell Tissue Res* 2012;347:279–90.
5 Fleenor DL, Shepard AR, Hellberg PE, *et al*. Tgfbeta2-Induced changes in human trabecular meshwork: implications for intraocular pressure. *Invest Ophthalmol Vis Sci* 2006;47:226–34.
6 Li Z, Allingham RR, Nakano M, *et al*. A common variant near TGFBR3 is associated with primary open angle glaucoma. *Hum Mol Genet* 2015;24:3880–92.
7 Miyazono K, Ten Dijke P, Ichijo H, *et al*. Receptors for transforming growth factor-beta. *Adv Immunol* 1994;55:181–220.
8 Miyazono K, ten Dijke P, Yamashita H, *et al*. Signal transduction via serine/threonine kinase receptors. *Semin Cell Biol* 1994;5:389–98.
9 Wordinger RJ, Sharma T, Clark AF. The role of TGF-β2 and bone morphogenetic proteins in the trabecular meshwork and glaucoma. *J Ocul Pharmacol Ther* 2014;30:154–62.
10 Wordinger RJ, Agarwal R, Talati M, *et al*. Expression of bone morphogenetic proteins (BMP), BMP receptors, and BMP associated proteins in human trabecular meshwork and optic nerve head cells and tissues. *Mol Vis* 2002;8:241–50.
11 Wordinger RJ, Fleenor DL, Hellberg PE, *et al*. Effects of TGF-beta2, BMP-4, and gremlin in the trabecular meshwork: implications for glaucoma. *Invest Ophthalmol Vis Sci* 2007;48:1191–200.
12 Gomez-Puerto MC, Iyengar PV, García de Vinuesa A, *et al*. Bone morphogenetic protein receptor signal transduction in human disease. *J Pathol* 2019;247:9–20.
13 Valer JA, Sánchez-de-Diego C, Pimenta-Lopes C, *et al*. Acvr1 function in health and disease. *Cells* 2019;8:1366.
14 Bocciardi R, Bordo D, Di Duca M, *et al*. Mutational analysis of the ACVR1 gene in Italian patients affected with fibrodysplasia ossificans progressiva: confirmations and advancements. *Eur J Hum Genet* 2009;17:311–8.
15 Kaplan FS, Xu M, Seemann P, *et al*. Classic and atypical fibrodysplasia ossificans progressiva (FOP) phenotypes are caused by mutations in the bone morphogenetic protein (BMP) type I receptor ACVR1. *Hum Mutat* 2009;30:379–90.
16 Hadziahmetovic M, Song Y, Wolkow N, *et al*. Bmp6 regulates retinal iron homeostasis and has altered expression in age-related macular degeneration. *Am J Pathol* 2011;179:335–48.
17 Chen L, Liu M, Luan Y, *et al*. BMP-6 protects retinal pigment epithelial cells from oxidative stress-induced injury by inhibiting the MAPK signaling pathways. *Int J Mol Med* 2018;42:1096–105.
18 Tovar-Vidales T, Fitzgerald AM, Clark AF. Human trabecular meshwork cells express BMP antagonist mRNAs and proteins. *Exp Eye Res* 2016;147:156–60.
19 Kondkar AA, Sultan T, Azad TA, *et al*. Association analysis of polymorphisms rs12997 in ACVR1 and rs1043784 in BMP6 genes involved in bone morphogenic protein signaling pathway in primary angle-closure and

pseudoexfoliation glaucoma patients of Saudi origin. *BMC Med Genet* 2020;21:145.
20 Gong J, Shen N, Zhang H-M, *et al*. A genetic variant in microRNA target site of TGF-β signaling pathway increases the risk of colorectal cancer in a Chinese population. *Tumour Biol* 2014;35:4301–6.
21 Abu-Amero KK, Sultan T, Al-Obeidan SA, *et al*. Analysis of *CYP1B1* sequence alterations in patients with primary open-angle glaucoma of Saudi origin. *Clin Ophthalmol* 2018;12:1413–6.
22 Kondkar AA, Mousa A, Azad TA, *et al*. Analysis of polymorphism rs1900004 in atonal bHLH transcription factor 7 in Saudi patients with primary open angle glaucoma. *Genet Test Mol Biomarkers* 2016;20:715–8.
23 Shen Q, Little SC, Xu M, *et al*. The fibrodysplasia ossificans progressiva R206H ACVR1 mutation activates BMP-independent chondrogenesis and zebrafish embryo ventralization. *J Clin Invest* 2009;119:3462–72.
24 Song G-A, Kim H-J, Woo K-M, *et al*. Molecular consequences of the ACVR1(R206H) mutation of fibrodysplasia ossificans progressiva. *J Biol Chem* 2010;285:22542–53.
25 Billings PC, Fiori JL, Bentwood JL, *et al*. Dysregulated BMP signaling and enhanced osteogenic differentiation of connective tissue progenitor cells from patients with fibrodysplasia ossificans progressiva (FOP). *J Bone Miner Res* 2008;23:305–13.
26 Wang T, Li BY, Danielson PD, *et al*. The immunophilin FKBP12 functions as a common inhibitor of the TGF beta family type I receptors. *Cell* 1996;86:435–44.
27 Freeman EE, Grosskreutz CL. The effects of FK506 on retinal ganglion cells after optic nerve crush. *Invest Ophthalmol Vis Sci* 2000;41:1111–5.
28 Olsen OE, Sankar M, Elsaadi S, *et al*. Bmpr2 inhibits activin and BMP signaling via wild-type ALK2. *J Cell Sci* 2018;131:jcs.213512. doi:10.1242/jcs.213512
29 Hatsell SJ, Idone V, Wolken DMA, *et al*. ACVR1R206H receptor mutation causes fibrodysplasia ossificans progressiva by imparting responsiveness to activin a. *Sci Transl Med* 2015;7:aac4358.
30 Barruet E, Morales BM, Cain CJ, *et al*. NF-κB/MAPK activation underlies ACVR1-mediated inflammation in human heterotopic ossification. *JCI Insight* 2018;3:jci.insight.122958. doi:10.1172/jci.insight.122958
31 Wiley LA, Rajagopal R, Dattilo LK, *et al*. The tumor suppressor gene Trp53 protects the mouse lens against posterior subcapsular cataracts and the BMP receptor ACVR1 acts as a tumor suppressor in the lens. *Dis Model Mech* 2011;4:484–95.
32 Ambrosio EP, Drigo SA, Bérgamo NA, *et al*. Recurrent copy number gains of ACVR1 and corresponding transcript overexpression are associated with survival in head and neck squamous cell carcinomas. *Histopathology* 2011;59:81–9.
33 Webber HC, Bermudez JY, Millar JC, *et al*. The role of Wnt/β-catenin signaling and K-Cadherin in the regulation of intraocular pressure. *Invest Ophthalmol Vis Sci* 2018;59:1454–66.
34 Webber HC, Bermudez JY, Sethi A, *et al*. Crosstalk between TGFβ and Wnt signaling pathways in the human trabecular meshwork. *Exp Eye Res* 2016;148:97–102.
35 Mao W, Millar JC, Wang W-H, *et al*. Existence of the canonical Wnt signaling pathway in the human trabecular meshwork. *Invest Ophthalmol Vis Sci* 2012;53:7043–51.
36 Du J, Zu Y, Li J, *et al*. Extracellular matrix stiffness dictates Wnt expression through integrin pathway. *Sci Rep* 2016;6:20395.
37 Villarreal G, Chatterjee A, Oh SS, *et al*. Canonical Wnt signaling regulates extracellular matrix expression in the trabecular meshwork. *Invest Ophthalmol Vis Sci* 2014;55:7433–40.
38 Morgan JT, Raghunathan VK, Chang Y-R, *et al*. Wnt inhibition induces persistent increases in intrinsic stiffness of human trabecular meshwork cells. *Exp Eye Res* 2015;132:174–8.
39 Wang W-H, McNatt LG, Pang I-H, *et al*. Increased expression of the Wnt antagonist sFRP-1 in glaucoma elevates intraocular pressure. *J Clin Invest* 2008;118:1056–64.
40 Li L, Liu Y, Guo Y, *et al*. Regulatory MiR-148a-ACVR1/BMP circuit defines a cancer stem cell-like aggressive subtype of hepatocellular carcinoma. *Hepatology* 2015;61:574–84.
41 Wang Y, Zhang Z, Wang J. MicroRNA-384 inhibits the progression of breast cancer by targeting ACVR1. *Oncol Rep* 2018;39:2563–74.
42 Kamiya N, Kaartinen VM, Mishina Y. Loss-Of-Function of ACVR1 in osteoblasts increases bone mass and activates canonical Wnt signaling through suppression of Wnt inhibitors SOST and DKK1. *Biochem Biophys Res Commun* 2011;414:326–30.
43 Mura M, Cappato S, Giacopelli F, *et al*. The role of the 3'UTR region in the regulation of the ACVR1/Alk-2 gene expression. *PLoS One* 2012;7:e50958.