Association of TGFBI variants with Congenital and Juvenile onset open angle glaucoma.

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Abstract

Purpose: To describe a novel association of *TGFBI* variants with congenital glaucoma in a family with GAPO (growth retardation, alopecia, pseudoanodontia, and progressive optic atrophy) syndrome as well as among unrelated cases of Juvenile onset open angle glaucoma (JOAG) along with the mechanistic impact of the variants on the protein. **Methods:** This study of one family of GAPO with congenital glaucoma and three unrelated patients of JOAG analysed a common link to glaucoma pathogenesis. We report ocular features of 3 girls with GAPO syndrome born of consanguineous marriage in a multi- generation consanguineous family. The proband (a 4year old girl) and her younger sibling (1year old girl) were operated for bilateral congenital glaucoma in both eyes. The elder sibling (10year old female) had features of GAPO syndrome without glaucoma. **Results**: A genetic evaluation using whole exome sequencing revealed a homozygous *ANTXR1* mutation in all three affected siblings with GAPO. No other mutations were detected in the genes associated with glaucoma. A rare missense variant in the *TGFBI* gene was shared in the two siblings with congenital glaucoma and GAPO syndrome. We further found three other unrelated patients with JOAG with no known glaucoma causing gene mutations but having three different missense variants in the *TGFBI* gene. One of these JOAG patients had familial granular corneal dystrophy. Molecular dynamic simulations of the *TGFBI* and 3-D structural models of three of its variants showed significant alterations, which could influence *TGFBI* function. **Conclusions:** Variations in the *TGFBI* gene could have a possible role in the pathogenesis of congenital and Juvenile onset open angle glaucomas that needs further evaluation.

INTRODUCTION

Primary congenital glaucoma (PCG) and Juvenile onset open angle glaucomas (JOAG) are generally monogenic, with genes having a strong biological effect playing a role in their pathogenesis. Apart from*MYOC, CYP1B1* and *LTBP2* (Abu-Amero et al. 2011; Alsaif et al. 2019; Bayat et al. 2008; Chakrabarti et al. 2006; Chen et al. 2008; Gupta et al. 2018; Saeedi et al. 2018) newer genes associated with either form of glaucoma include the *CPAMD8* , *GPATCH,*and *EFEMP1* .(Ferre-Fernández et al. 2017; Mackay et al. 2015; Siggs et al. 2020) However, a large number of PCG and JOAG patients do not have a known gene mutation among these genes. (Knight et al. 2021) There are chromosomal loci where the putative genes associated with PCG and JOAG are still to be discovered. (Cascella et al. 2015) With the advent of next-generation sequencing, new gene discovery, especially for diseases where genetic variations have significant biological effect on disease causation, is being undertaken.

In this study, we describe a family of 3 siblings with GAPO syndrome, in whom we found two of them

having congenital glaucoma. A WES analysis pointed towards the Transforming Growth Factor Beta Induced (*TGFBI*) gene variant common to both siblings. Incidentally, *TGFBI* variants were also seen in three unrelated patients with JOAG, one of whom had familial granular corneal dystrophy.

GAPO syndrome is an extremely rare genetic disorder, with only about sixty cases reported to date. The ocular features of GAPO syndrome include puffy eyelids, sparse eyelash hair, poliosis, hypertelorism, ptosis, strabismus, nystagmus, megalocornea, keratoconus, keratopathy, optic atrophy, myelinated retinal nerve fiber layer and congenital glaucoma (buphthalmos) (Bozkurt et al. 2013). The diagnosis of GAPO is usually made early due to the characteristic premature 'geriatric' appearance of the patients, the presence of cardinal features like alopecia and pseudoanodontia, as well as the associated features like dwarfism, prominent supraorbital ridges, frontal bossing depressed nasal bridge, long philtrum, and a wide open anterior fontanelle. Late onset alopecia has also been frequently documented (Goloni-Bertollo et al. 2008; Rim & Marques-de-Faria 2005). Patients with GAPO have a reduced life span and usually die in their third or fourth decades of life due to generalized interstitial fibrosis of the lungs and atherosclerosis (Gagliardi et al. 1984; Mullaney et al. 1997).

The invariable consanguinity in these families with GAPO points towards an autosomal recessive mode of inheritance caused by mutations in *ANTXR1* on chromosomal position 2p14, a protein essential for intracellular actin assembly (Bayram et al. 2014; Stranecky et al. 2013). Defective production of this protein leads to altered cell adhesion properties leading to excessive deposition of extracellular matrix (ECM) secondary to reduced turnover, resulting in the various phenotypic features of this syndrome (Stranecky et al., 2013; Wajntal et al. 1990). While there are reports of the occurrence of glaucoma among patients with GAPO syndrome, (Goyal et al. 2014; Ilker et al. 1999; Kocabay & Mert 2009; Manouvrier-Hanu et al. 1987; Mullaney et al., 1997; Sayli & Gul 1993) it is not known whether mutations in *ANTXR1* gene itself or some other (glaucoma associated) genes, lead to the development of glaucoma. This report analyses the genetic background of two (out of three) children with GAPO syndrome and congenital glaucoma.

SUBJECTS AND METHODS

A multi generation consanguineous family presented to us, where four girls, three of whom were affected with GAPO, out of whom two also had congenital glaucoma (**Figure 1**). The family belonged to North India with a strong tradition of consanguinity. The three cases of the family described here are of the three girls with features of GAPO syndrome.

Case 1 (Proband)

A 4year old girl, born of a consanguineous marriage, presented to our glaucoma clinic with chief complaints of watering and an asymmetric corneal enlargement of the right eye (RE). Physical examination revealed progressive frontal alopecia with a receding hairline, frontal bossing, high forehead, prominent supraorbital ridges, depressed nasal bridge, anteverted nostrils, long philtrum, umbilical hernia, sparse eyebrows, and an appearance of premature aging (**Figure 2A1-4**). Intraoral examination revealed the absence of a regular dentition pattern (**Figure 2A3**); a radiograph of the teeth revealed impacted dentition, confirming pseudoanodontia. Her weight (12kg) and height (92cm) were below the 3rd percentile. The patient's mental status was found to be normal, and there was no developmental delay.

Examination under anesthesia (EUA) revealed RE enlarged corneal diameters with an IOP of 30mmHg, with corneal edema precluding a detailed anterior and posterior segment evaluation. Her left eye (LE) had a clear cornea with an IOP of 16mmHg. The patient was taken up for RE trabeculectomy with mitomycin. On subsequent EUA (post-operatively), when the cornea had cleared up, the anterior segment evaluation revealed the presence of Haab's striae and peripheral keratopathy with irido-corneal adhesions that were seen on an ASOCT examination in the RE (**Figure 2A3, 2A4**). Her optic disc examination showed a vertical cup disc ratio (CDR) of 0.4:1 with temporal disc pallor in RE; while LE showed a healthy disc with a CDR of 0.2:1. Eventually, she developed increased IOP in her LE, for which she was taken up for LE trabeculectomy with mitomycin, three years after the RE surgery. Her IOPs have been controlled in both eyes (BE) ever since.

A USG abdomen revealed the presence of normal kidneys and ovaries. An MRI of the brain did not detect any intracranial abnormality. Other investigations were normal, including complete blood counts, serum and urine biochemistry, hormonal assays and echocardiography,.

Case 2

The younger sibling of the proband presented at 1 year of age with complaints of watering BE. She had lush black hair until six months of age, followed by progressive hair loss. There was a prominent supraorbital ridge, depressed nasal bridge, high forehead, wide anterior fontanelle, and a prematurely aged look (**Figure 2B1-4**). She also had pseudoanodontia. Just like her elder sister, she had growth retardation, with her weight and length below the 3rd percentile for her age.

An EUA revealed BE horizontal corneal diameter of 13.5mm, along with the presence of a diffuse corneal haze in LE. She also had a central keratopathy in RE (**Fig 2B2**). Her IOP was 28mmHg in RE and 32mmHg in LE. An ultrasound biomicroscopy (UBM) revealed central corneal thickening and a thinned out iris and ciliary body. (**Fig 2B4**). She had a cup disc ratio of 0.6:1 in BE. For her BE trabeculectomy with ab externo trabeculotomy with mitomycin was performed. The surgery was uneventful, and she maintains normal IOP.

Case 3

The 10year-old elder sister of the proband was also evaluated for GAPO features. Just like the proband, she was observed to have all the phenotypic features of this syndrome and reduced eyelashes (**Figure 2C1-4**). The intraoral examination also revealed pseudoanodontia, and her height (120cm) and weight (20kg) were below the third centile for her age. She had no developmental delay, and her mental status was normal. Her visual acuity was 6/6 in both the eyes, and IOP was 14mmHg and 16mmHg for the RE and LE, respectively. Her corneae were clear, and gonioscopy revealed wide open angles without any abnormality. Her optic nerves were found to be normal.

Table 1 shows the ophthalmic features of the three siblings with GAPO syndrome. None of the other family members had any features of GAPO or glaucoma.

Whole Exome Sequencing (WES)

Blood samples were withdrawn from the four children, the parents, and the paternal grandmother after informed consent was taken from the parents of the children. The study was carried out according to the tenets of the declaration of Helsinki, and Ethics committee approval was obtained from our Institutes ethics committee. A signed informed consent according to the guidelines of the Institute Ethics Committee was obtained.

Genomic DNA was extracted from peripheral blood lymphocytes using PAXgene Blood DNA Kit (Qiagen, Germany) for genetic evaluation. DNA was run on 0.8% agarose gel to check for quality and quantified by Nanodrop. WES capture was performed using the Sure Select Clinical Research Exome V2 kit (Agilent Technologies, Santa Clara, CA). Variant analysis was performed using the GenomeAnalysisTK-3.6 toolkit. The variant call files (VCF), containing the variant call results, generated were analyzed using Golden Helix VarSeq Software v.1.2.1 (Bozeman, MT). VarSeq variants with read depth <15 and genotype quality score <20 were excluded. To identify rare mutations, variant frequency databases were used to remove variants that are present at high frequencies among large population groups. The remaining variants were filtered according to minor allele frequency (MAF) <0.001 in multiple databases, including Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/), 1000 Genomes Project (http://browser.1000genomes.org) and gnomAD (http://gnomad.broadinstitute.org/).

Variants specifically in the two sisters with glaucoma were filtered based on only those exonic variants (Non-synonymous missense variants, frameshift and indels and splice region variants) that were present exclusively in the two sisters and absent in the other family members and 20 healthy controls. Inherited variants that were present in both the affected sisters with congenital glaucoma and one of the parents were also looked at, for any association with the known glaucoma genes. Copy number variant (CNV) analysis

was performed to look for CNV in the known genes for glaucoma or others with a strong association with glaucoma pathogenesis. The CNV's were annotated with RefSeq gene annotations and Database of Genomic Variants v107. The CNV's were then classified to assess the pathogenicity using classify CNV. The CNV's common in both affected individuals were visually inspected using IGV.

We used Homozygosity mapping to identify long stretches of homozygous haplotypes in genes associated with glaucoma. Regions of Homozygosity (ROH) were identified by using the AutoMap software (Quinodoz et al., https://automap.iob.ch/) on WES data (Quinodoz et al. 2021).

Further functional impact of the protein was predicted by bioinformatic tools; variant effect predictor (VEP), Mutation Taster, Polyphen, and FATHMM. The identified variants were confirmed by Sanger sequencing.

In silico **3D-modeling and Molecular dynamic analysis of*TGFBI* and its variants:**

The nucleotide sequence of *TGFBI* protein was retrieved from NCBI (ID), and the structure was modeled through comparative modeling using the Robetta server (Song et al. 2013).The structure of full-length *TGFBI* was modeled using the coordinates of PDBID:5NV6(Garcia-Castellanos et al. 2017). For further refinement, the model was subjected to molecular dynamics (MD) simulation for 200ns. The refined structure was validated using MolProbablity Server(Williams et al. 2018). The last frame of the 200ns simulation was used as a template for modeling the mutant forms of TGFBI, including A323E, A323V, and 649 frameshift. The modeled mutants were then simulated for 200ns each in order to understand the impact of variations on the structure and dynamics of the TGFBI protein.

MD simulations of the modeled proteins were done employing GROMACS software version 2021.5(Van Der Spoel et al. 2005). CharmM36 forcefield was used for generating the topology, and the system was solvated using the TIP3P water model(Vanommeslaeghe et al. 2010). Neutralization of the system was done by the addition of the appropriate number of sodium ions. Equilibration of the system was then performed under successive NVT and NPT ensembles for 500ps each. The production simulation was run using a leap-frog dynamics integrator and a step size of 2fs with the consideration of periodic boundary conditions in all three dimensions. The trajectory was analyzed. Principal component (PC) analysis was performed to understand the global motions of the TGFBI and the three mutant forms. The covariance matrix was generated from the Cα atom coordinates and diagonalized to obtain the eigenvectors. Since the first two eigenvectors described the major proportion of the trace of the covariance matrix, the trajectory was projected along the first two principal components. Free energy landscape was further generated using the 2-dimensional projection of the trajectory along PC1 and PC2.

RESULTS

We found a missense homozygous *ANTXR1* gene mutation in all three children affected with GAPO (NM_-032208.2:c.572T>C, p.Ile191Thr). This change is novel and predicted to be a disease causing by mutation taster (0.99), PolyPhen-2 (1.0), and PROVEAN (-4.0). Another change at this position, c.572T>G, p.Ile572Ser is reported in the COSMIC database (COSV100362660).

Homozygosity mapping applied to WES data of the consanguineous family, detected one large (11Mb) homozygous region involving the *TEK* gene (i.e., chromosome 9) in only one of the two sisters with congenital glaucoma, among the genes known for glaucoma. However, neither CNV nor intronic splice region variant was detected in the *TEK* gene in either of the sisters.

No pathogenic variants or CNV were detected that co-segregated with glaucoma in the two girls with congenital glaucoma (Case 1 and 2) at any of the known loci for Mendelian forms of glaucoma (GLC1A, GLC1B, GLC1D-Q) or PCG (GLC3A-D) including the known causative genes namely,*MYOC* (GLC1A), *CYP1B1* (GLC3A), *WDR36* (GLC1G),*ASB10* (GLC1F), *OPTN* (GLC1E), *NTF4* (GLC1O),*TBK1* (GLC1P), *LTBP2* (GLC3C), *FOXC1, PITX2* ,*TEK,* and *PAX6.* Exonic variants specific to the two sisters with congenital glaucoma and the variants they inherited from either of the parents are listed in **Suppl Table 1.**

Four missense variants exclusive to the two sisters with GAPO and congenital glaucoma (case 1 and case 2);

PTPN4 , *PCDHB4, GGT2* and *TGFBI,* were observed. The variants on *PTPN4* and*PCDHB4* were rejected as they were found to be benign. The*GGT2* variant was not considered as it was earlier reported that hGGT2 does not encode a functional enzyme and therefore is unlikely to play a role in glaucoma pathogenesis. A missense heterozygous variant in*TGFBI* , NM_000358.3:c.968C>T NP_000349.1:p.Ala323Val **(Fig 1.a)** was found, which is reported to be damaging by the variant prediction tools PROVEAN (-2.5), FATHMM-MKL (0.825), PolyPhen-2 (0.89) and disease causing by Mutation Taster (0.99). The variant is highly conserved with a Genomic evolutionary rate profiling (GERP) value of 5.65. Sanger sequencing confirmed heterozygous missense p.Ala323Val mutation in both case 1 and case 2 and was absent in case 3 and other family members. The gnomAD frequency of the variant was found to be 0.001450. The *Clinvar*star rating score, extracted for the variant, was found to be 1/4 with two submissions, one for corneal dystrophy.

We then looked for the presence of *TGFBI* gene variants in a cohort of 40 patients with JOAG (diagnosed before 25 years of age) in whom WES was previously done. We found two *TGFBI* variants present in two unrelated JOAG patients. Neither of these 2 patients had any other mutations in the known glaucoma genes. In one patient, a*TGFBI* heterozygous missense variant was found in the same codon observed in the described cases of GAPO, but with a different amino acid residue.; NM_000358.3:c.968C>A, NP_000349.1:p.Ala323Glu. The second was a novel variant in the other JOAG patient. This was a null variant (frameshift mutation); NM_000358.3:c.1944del, NP_000349.1:p.Ser649LeufsTer22. These variants were of uncertain significance with minor pathogenic evidence and likely pathogenic respectively, as per ACMG classification. The former was damaging by Mutation taster (0.99), PolyPhen-2 (0.89), and PROVEAN (-2.5). The gnomAD frequency of NM_000358.3:c.968C>A was found to be 0.00003269, and the frequency of (NM_000358.3):c.1944del is not reported in gnomAD. A third patient with granular corneal dystrophy referred to the glaucoma clinic was also found to have JOAG. This patient had familial granular dystrophy of the cornea with 2 other first degree relatives (a brother and his daughter) also affected with granular dystrophy but without having glaucoma or raised IOP (**Fig 3**). WES analysis of this patient revealed a heterozygous*TGFBI* mutation NM_000358.3:c.1663C>T, NP_000349.1:p.Arg555Trp which is reported to be pathogenic in*Clinvar* with 3 submissions associated with granular corneal dystrophies (GCD). (**Table 2**)

A comparative modeling of the *TGFBI* variants (p.Ala323Val, p.Ala323Glu and p.Ser649LeufsTer22) was performed since the available crystal structures lack the C-terminal region of the protein. The modeling results showed α-helical region in the C-terminal, as shown in**Figure 4** . The model was subjected to 200ns MD simulations for refinement. During this simulation, the C-terminal region of the protein was gradually stabilized through interactions with the FAS1-4 and FAS1-3 domains. The last frame of this simulation was therefore used as a template for the generation of the variants A323E, A323V, and 649 frameshift, which were then subjected to MD simulations to understand the effect of mutations on the local and global structure of*TGFBI* and its variants. Structural deviation, compactness of the proteins, and residue fluctuations for 200ns simulations were calculated to assess the root mean square deviation (RMSD), radius of gyration (RG), and root mean square fluctuation (RMSF), respectively (**Supplementary Figure 1**). MD simulations followed by Principal Component Analysis (PCA) were performed to study the overall motions of the *TGFBI* and these 3 variants. Projection of the trajectory along PC1 and PC2 depicted correlated motions of various domains of the protein, as shown in **Figure 5** . We found that *TGFBI*fluctuated between an elongated and a banana-like shape due to bending between FAS1-2 and FAS1-3 domains. The bending movement was more prominent in the variants compared to wild-type (WT) *TGFBI,*majorly in the FAS1-2 and FAS1-3 domains. The impact of the three variants was also evident on the Free Energy Landscape (FEL) since the free energy well was shallow and distorted compared to the deeper well of WT *TGFBI* (**Figure 6**).

DISCUSSION

This family of three children affected with GAPO, two of whom presented with bilateral congenital glaucoma, allowed us to study the genetic association of glaucoma with GAPO syndrome.

Glaucoma as an ophthalmological manifestation of GAPO syndrome has been reported in ten cases to date (Goloni-Bertollo et al., 2008; Goyal et al., 2014; Ilker et al., 1999; Kocabay & Mert 2009; Manouvrier-Hanu et al., 1987; Mullaney et al., 1997; Rim & Marques-de-Faria 2005; Sayli & Gul 1993; Wajntal et al.,

1990). Glaucoma in five of these patients, was early onset open angle glaucoma (Goloni-Bertollo et al., 2008; Manouvrier-Hanu et al., 1987; Rim & Marques-de-Faria 2005; Sayli & Gul 1993). Primary congenital glaucoma (PCG) is described in five patients (Goyal et al., 2014; Ilker et al., 1999; Kocabay & Mert 2009; Manouvrier-Hanu et al., 1987; Mullaney et al., 1997; Sayli & Gul 1993) presenting with Haab's striae and buphthalmos. Asymmetric glaucoma, as seen in Case 1 of GAPO syndrome, at presentation has also been documented (Goyal et al., 2014; Mullaney et al., 1997), while others have described a late presentation with end stage glaucoma (Goyal et al., 2014; Ilker et al., 1999; Sayli & Gul 1993). The pathogenesis of congenital glaucoma in children with GAPO syndrome is not clearly understood. Mutations in the *ANTRX1* gene have been postulated to be the cause of glaucoma and the variable expression of *ANTRX1* mutation could be the reason why the elder sister (case 3) with *ANTRX1* mutation and GAPO syndrome did not have glaucoma. Even if we consider a variable expressivity in *ANTRX1* as the reason for one sibling with GAPO syndrome not developing congenital glaucoma in this family, we believe the presence of the *TGFBI* variant may have influenced this variable expression.

The abnormal expression of *TGFBI* is related to the occurrence and development of some types of cancers as well as different types of corneal dystrophies; lattice corneal dystrophies (LCD) and granular corneal dystrophies (GCD) (Lakshminarayanan et al. 2014), while the role of *TGFBI* in glaucoma is not known. The human protein TGFBIp encoded by *TGFBI* has four FASI domains, and the mutated amino acid observed in our two patients corresponds to the FAS1-2 domain of TGFBIp. However, the *TGFBI* mutations known for various corneal dystrophies are located in the FASI-1 and FASI-4 domains (Klintworth et al. 2004). The *TGFBI* gene is also within the linkage area mapped as a glaucoma locus 5q22.1-q32. (Pang et al. 2006). A gene expression profile of human trabecular meshwork(TM) tissue by SAGE (serial analysis of gene expression) has shown a higher expression for *TGFBI* along with other glaucoma causing genes and genes involved in typical TM maintenance functions (Liu et al. 2011). A study by Kim et al., using Alb-hßigh3 transgenic mice showed that over expressed human βigh3/ (hßigh3)/ TGFBIp in the blood might be involved in anterior segment morphogenesis and eye development in mice. The phenotype observed in transgenic mice is similar to human eye disorders such as anterior segment dysgenesis and Peters' anomaly (Kim et al. 2007). These observations support the role of *TGFBI* in congenital glaucoma as observed in the two sisters with GAPO. A string analysis also showed that *TGFBI* and *LTBP2* (associated with congenital glaucoma) co-express, possibly interacting with *MYOC* and *CYP1B1* . There is also a report of glaucoma observed in a patient with *TGFBI,* R124H mutation, though the phenotypic details of this patient are not known (Jiang & Zhang 2021).These observations suggest an important role of TGFBI in glaucoma pathogenesis, in conjunction with other genes.

Further, we also found 3 unrelated JOAG patients with *TGFBI* variants in a separate cohort who had no other known glaucoma gene mutations. One of the patients had GCD. There is no report in the literature of an association between JOAG and GCD.

These 5 patients, with different diagnosis but all with developmental glaucoma, having variants in the same *TGFBI* gene support the role of *TGFBI* in glaucoma pathogenesis. A high degree of phenotypic variability and incomplete penetrance is known for *TGFBI* mutations however the fundamental reason, why different gene variations cause morphologically distinct phenotypes remains elusive. Our study is the first to report an association of *TGFBI* with congenital and juvenile glaucoma.

The modeling studies of the *TGFBI* wild type, along with the three variants A323E, A323V, and 649-frameshift, provided insights into the effect of these variations on the full-length structure and its dynamics. While specific aggregation mechanisms cannot be deduced at this stage, the alteration of the protein dynamics was clearly observed in the simulation studies. The A323 is located at the interface of CROPT, and FAS1-1 domains, and perturbation at this position can alter inter-domain interactions and even global protein structure. For A323V, the presence of hydrophobic residue valine on the surface can destabilize the protein, and the same was observed in molecular dynamics studies. In the case of A323E, the glutamic acid was observed to form strong intra-domain interactions with K72 and N207 from the CROPT domain and FAS1-1 domain, respectively. In the case of the frameshift variant of *TGFBI* , the sequence at the C-terminal is

greatly altered compared to the WT. The comparative modeling studies revealed that the C-terminal region forms two alpha-helices packed against each other with hydrophobic residues, further interacting with the FAS1-4 and FAS1-3 domains. Due to the frameshift, packing of the helices was disturbed, leading to increased solvent exposure of the C-terminal tail.

Limitations of our study include a small number of patients and our inability to genotype the parents and siblings of JOAG patients. Also, gene expression and functional studies using cell-based assays and transgenic animal models of the mutant are further needed to examine the effect of these variants on the protein and the downstream targets to establish a cause-and-effect relationship. Moreover, an interaction between *TGFBI* and other genes in the causation of glaucoma also needs to be explored. Nevertheless, based on the findings of this preliminary report, *TGFBI* could be well considered a candidate gene for glaucoma; however, more studies in a larger patient population as well as functional studies may be needed to further elucidate its role in glaucoma pathogenesis.

Data Availability: Data supporting the findings of the study are available from the corresponding author (VG) on request**.**

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Legends:

Figure 1 : (a)Pedigree chart of the family with three children affected with GAPO syndrome showing at least three generations of consanguinity. The half shaded circle depicts the child having only GAPO syndrome, but not glaucoma. The younger two siblings had glaucoma with GAPO syndrome depicted as full shaded circles. The ages mentioned in the pedigree are those at their last follow up.

(b) Chromatograms showing heterozygous,*TGFBI;* NM_000358.3:c.968C>T; p.Ala323Val mutation found in the two girls with congenital glaucoma and GAPO syndrome.

Figure 2 : Clinical photographs of Case 1 (A1-4), Case 2 (B1-4) and Case 3 (C1-4). Front facial profile and side facial profile of the patients illustrate the presence of a characteristic geriatric appearance, with alopecia, sparse eyebrows, frontal bossing, prominent supraorbital ridges, depressed nasal bridge, anteverted nostrils and a long philtrum. Clinical photograph of the oral cavity shows impaction of teeth with a reduction in its number. Radiograph of the face (lateral view with open mouth) reveals a normal number of teeth, confirming pseudoanodontia. Anterior segment photograph of Case1 (A2) reveals the presence of megalocornea with prominent iridocorneal adhesions and keratopathy (blue arrow). Corneal opacities are also seen (black arrows; magnified inset). AS-OCT image of the same patient (A4) confirms the iridocorneal adhesions (white arrow). Central keratopathy can be seen in the anterior segment photograph of RE of Case 2 (B2). An ultrasound biomicroscopy (UBM) of the same Case revealed central corneal thickening with thinned out iris and ciliary body (B4).

Figure 3 :

(A) Anterior segment photo of the patient with granular corneal dystrophy and JOAG. (B) Goniophotograph of the same patient showing open angles.(C) Fundus photograph of the patient showing advanced glaucomatous optic atrophy (D) Pedigree chart of the patient. Black shaded square depicts the proband having glaucoma and granular dystrophy. Grey shaded square and circle indicates affected status with only granular corneal dystrophy. The ages mentioned in the pedigree are those at their last follow up.

Figure 4:

Modeled structures of full length *TGFBI* and its variants are depicted in cartoon representation. The residue substitution at 323 position is magnified along with the C-terminal helices in wild type and 649 frameshift *TGFBI* .

Figure 5 :

Porcupine plots showing correlated movements of the protein throughout the 200ns simulations along PC1. The mutant forms of *TGFB* I were more dynamic compared to the wild type *TGFBI.*

Figure 6:

Free Energy Landscape (FEL) generated from the projection of trajectory on PC1 and PC2. The FEL for wild type *TGFBI* was deeper compared to the shallow FELs of the mutant forms due to decreased stability of the protein.

Supplementary Figure 1: (A) RMSD plots showing structural deviation of the wild type and mutant *TGFBI* proteins**(B)** Radius of gyration plots showing compactness of the protein. **(C)** RMSF plot showing residue fluctuation throughout the 200ns simulations

Table 1: Ocular features of the 3 girls(with Pedigree notation) with GAPO Syndrome

	Case 1 (VI iii)	Case 2 (VI iv)	Case 3 (VI ii)
Glaucoma	+	+	-
Optic Atrophy	-	-	-
Keratopathy	-	+	-
Frontal bossing	+	+	+
Sparse eyebrows	+	+	+
Sparse eyelashes	+	+	+
White eyelashes	-	-	-
Prominent globes	+	+	+
Swollen eyelids	+	+	+
Prominent supraorbital ridges	+	+	+
Epiblepharon	+	-	+

Table 2: *TGFBI* variants identified in Congenital glaucoma and JOAG patients.

Subjects	Variant	rs number	Transcript HGVS coding	Amino acid change	gnomAD South Asian frequency	ACMG Classification	Clinical significance
Case 1 & Case 2 GAPO with glaucoma	5:135388650 C/T	rs201210696	NM_-000358.3:c.968C>T	p.Ala323Val	0.001111	PP2, PP3, BS2	US

Subjects	Variant	rs number	Transcript HGVS coding	Amino acid change	gnomAD South Asian frequency	ACMG Classification	Clinical significance
JOAG	5:135388650-C-A	rs201210696	NM_-000358.3:c.968C>A	p.Ala323Glu	0.00003269	PM1,PM2, PP2	US with minor pathogenic evidence
JOAG	5:135397226-C-	-	NM_-000358.3:c.1944del	p.Ser649LeufsTer22	NA	PVS1,PM2	Likely Pathogenic
JOAG with GCD	5:135392469-C-T	rs121909208	NM_-000358.3:c.1663C>T	p.Arg555Trp	0.0002070	PP5,PM1, PM5,PM5, PM2, PP2,PP3	Pathogenic

ACMG : American College of Medical Genetics

US: Uncertain Significance

NA: not available

GAPO: Growth retardation, alopecia, pseudoanodontia, and progressive optic atrophy

JOAG: Juvenile onset open angle glaucoma

GCD: Granular corneal dystrophy

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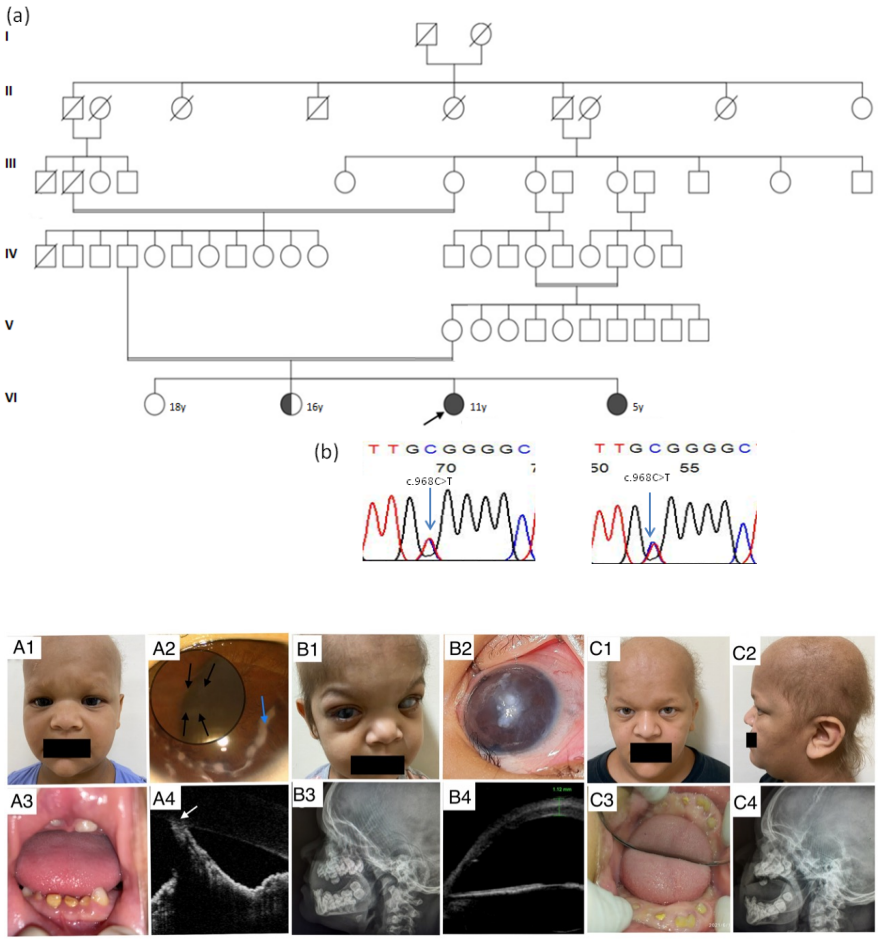

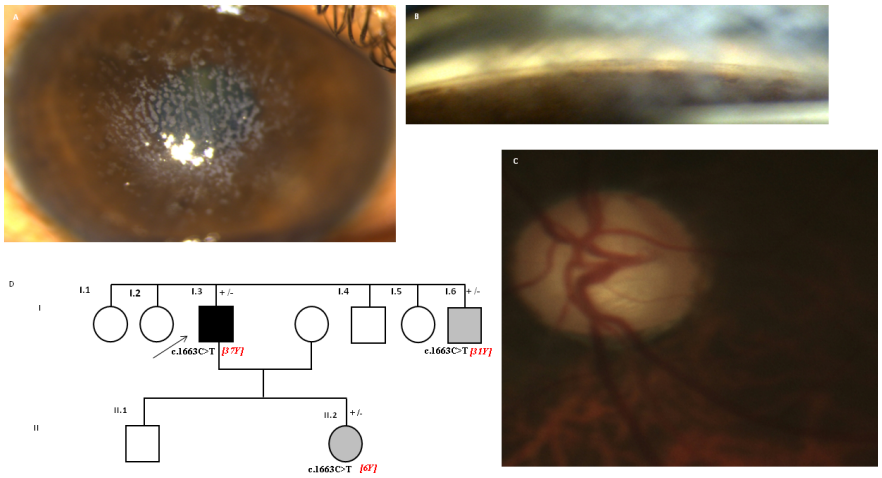

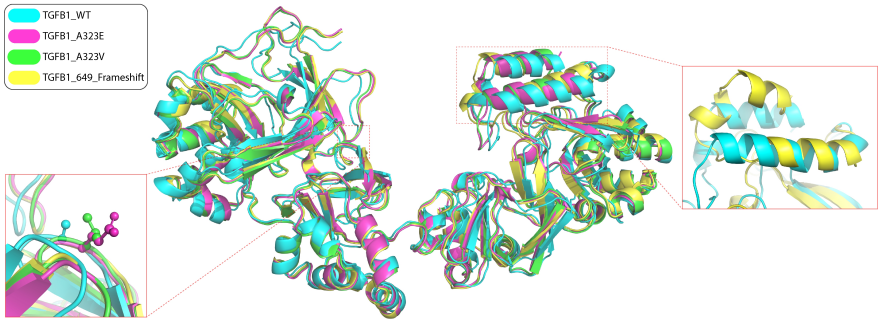

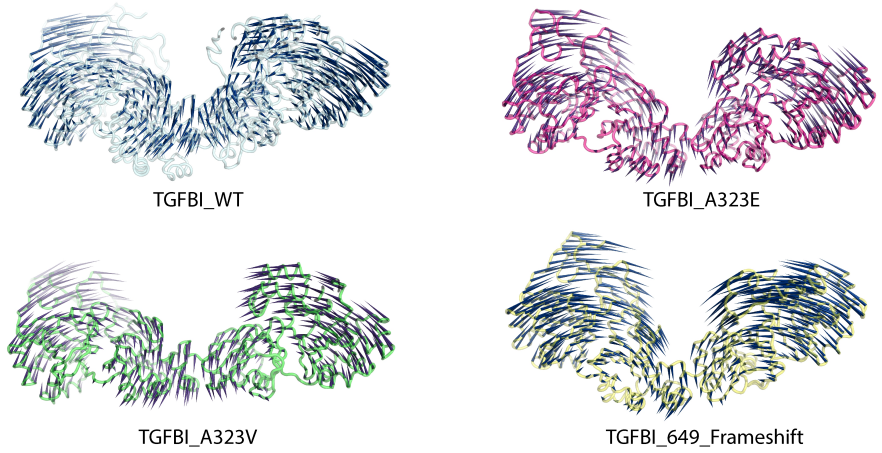

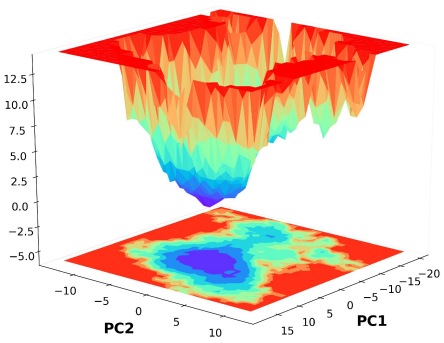

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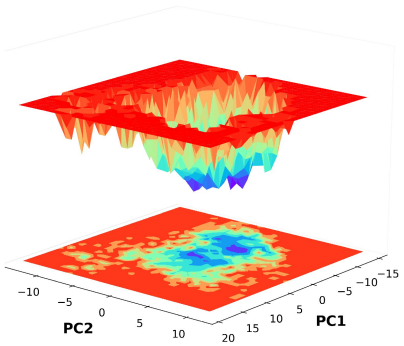

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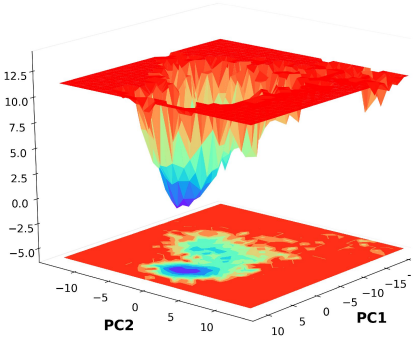

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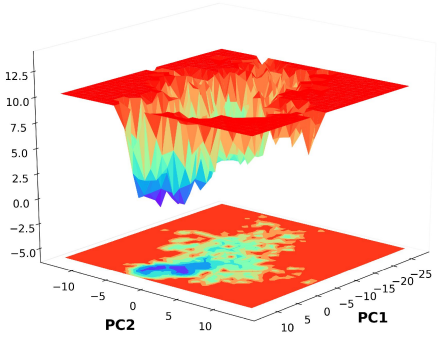

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