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Corneal Infantile Myofibromatosis Caused by Novel Activating Imatinib-Responsive Variants in *PDGFRB*

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Purpose: To investigate the genetic cause, clinical characteristics, and potential therapeutic targets of infantile corneal myofibromatosis.

Design: Case series with genetic and functional *in vitro* analyses.

Participants: Four individuals from 2 unrelated families with clinical signs of corneal myofibromatosis were investigated.

Methods: Exome-based panel sequencing for platelet-derived growth factor receptor beta gene (*PDGFRB*) and notch homolog protein 3 gene (*NOTCH3*) was performed in the respective index patients. One clinically affected member of each family was tested for the pathogenic variant detected in the respective index by Sanger sequencing. Immunohistochemical staining on excised corneal tissue was conducted. Functional analysis of the individual *PDGFRB* variants was performed *in vitro* by luciferase reporter assays on transfected porcine aortic endothelial cells using tyrosine kinase inhibitors. Protein expression analysis of mutated PDGFRB was analyzed by Western blot.

Main Outcome Measures: Sequencing data, immunohistochemical stainings, functional analysis of *PDGFRB* variants, and protein expression analysis.

Results: We identified 2 novel, heterozygous gain-of-function variants in *PDGFRB* in 4 individuals from 2 unrelated families with corneal myofibromatosis. Immunohistochemistry demonstrated positivity for alpha-smooth muscle actin and β-catenin, a low proliferation rate in Ki-67 (< 5%), marginal positivity for Desmin, and negative staining for Caldesmon and CD34. In all patients, recurrence of disease occurred after corneal surgery. When transfected in cultured cells, the *PDGFRB* variants conferred a constitutive activity to the receptor in the absence of its ligand and were sensitive to the tyrosine kinase inhibitor imatinib. The variants can both be classified as likely pathogenic regarding the American College of Medical Genetics and Genomics classification criteria.

Conclusions: We describe 4 cases of corneal myofibromatosis caused by novel *PDGFRB* variants with autosomal dominant transmission. Imatinib sensitivity *in vitro* suggests perspectives for targeted therapy preventing recurrences in the future.

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Infantile myofibromatosis (Online Mendelian Inheritance in Man [OMIM] #228550) is a rare tumor of mesenchymal origin occurring with a prevalence of 1:150 000 life births.[1] Characteristically, the fibrous tumor presents at birth or in early childhood.[2] The tumorous lesions are most commonly located in the head and neck region and can present on the skin, soft tissue, bone, and visceral organs. Rare cases of ocular and periocular tissue involvement have been described including lesions of the sclera,[3] as well as extraocular lesions of the eyelid skin, periorbital soft tissue, and orbital bones.[4] Skin lesions are typically diagnosed early in life as they present as painless, subcutaneous, nodular, and blueish skin alterations with slight skin discoloration due to vascularization.[5] If left untreated, the skin lesions can spontaneously resolve and leave a mark of skin indentation. Visceral involvement is associated with a poor prognosis of the disease. The localized form can occur at different sites. Corneal myofibromas have been described in individuals with neurofibromatosis type 2.[6] Myofibromateous lesions characteristically show positive stainings for Vimentin and alpha-smooth muscle actin (α-SMA),[7] whereas they are negative for the vascular endothelial marker CD34,[8] the melanin marker S-100,[9] and have a low Ki-67 proliferation index.[10]

Infantile myofibromatosis is an autosomal dominant genetic disease caused by heterozygous pathogenic variants in the platelet-derived growth factor receptor beta gene

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(*PDGFRB*) and the neurogenic locus notch homolog protein 3 gene (*NOTCH3*).[7] Variable disease manifestations within a family and incomplete penetrance have also been described.[7]

PDGFRB encodes for the PDGFRβ protein, which is a class III receptor-tyrosine kinase serving as a cell-surface receptor for homodimeric PDGFB and PDGFD ligands.[11] It is composed of a glycosylated extracellular domain of 5 immunoglobin-like modules functioning as the ligand-binding site, a hydrophobic helical transmembrane domain, and an intracellular domain consisting of a juxtamembrane (JM) and a kinase domain that orchestrates signal transduction within the cell.[11] Platelet-derived growth factor receptor beta is mainly expressed by cells of mesenchymal origin, e.g., vascular smooth muscle cells, fibroblasts, and pericytes. During embryonic development, it is crucial for blood vessel formation, and kidney development, amongst others. In a physiologic adult state, PDGF is involved in wound healing and soft-tissue homeostasis.[12] The kinase activity of PDGFRβ is controlled by the JM domain, the activation loop, and the C-terminal tail. Ligand binding to the receptor results in dimerization and conformational changes which destabilize the inhibitory function and increase both the kinase activity and trigger autophosphorylation.[13]

Germline pathogenic variants in *PDGFRB* have also been described in Penttinen type of premature aging syndrome (OMIM #601812)[14] and Kosaki overgrowth syndrome (OMIM #616592).[15]

In cases of *PDGFRB* activating spectrum disorder (PASV), multicentric infantile myofibromatosis can co-occur with aneurysm formation or vascular dysplasia (e.g., aneurysms of cerebral, coronary, and renal arteries), overgrowth, skeletal abnormalities (e.g., scoliosis, acro-osteolysis, and osteopenia with fracture tendency), skin atrophy, cardiac (e.g., masses and hypertrophy), brain abnormalities (e.g., intracranial myofibromas, cerebellar malformations, and white matter disease), and psychiatric disorders.[16] Wenger et al have recently characterized the disease spectrum according to a phenotype-genotype analysis into PASV1 and PASV2. There are overlapping features in both groups. The less severe group of PASV1 characteristically involves multicentric myofibromas in infancy and risk for vascular dysplasia and aneurysm formation. The more severe PASV2 involves progressive disease with multisystem disorders including premature aging, vascular malformations including aneurysms (fusiform, less likely saccular), leukoencephalopathy, and other abnormalities of bone, brain, and connective tissue. Ocular involvement in the form of anterior and posterior synechiae has also been described.[16]

In vitro assays have objectified an increased PDGF receptor tyrosine kinase activity due to gain-of-function (GOF)-mutations in *PDGFRB* and reduction in the kinase activity by the tyrosine kinase inhibitors imatinib[17] and sunitinib.[18] Imatinib was first identified as a small-molecule inhibitor of the protein tyrosine kinase BCR-ABL, which constitutes of a fusion of ABL1 located on chromosome 9 and BCR on chromosome 22 by a reciprocal translocation. The so-called Philadelphia Chromosome with constitutive activation of the ABL kinase domain was identified as a hallmark of chronic myeloid leukemia and is also present in patients with acute lymphocytic leukemia.[19] Imatinib has quickly advanced to a safe, efficient, and frontline therapy for chronic myeloid leukemia. In addition, imatinib is also a potent inhibitor of the platelet-derived growth factor receptors (PDGFRα and PDGFRβ) and the stem cell receptor tyrosine kinase and effectively inhibits the tyrosine kinase activity.[20] It has shown effective clinical response in human cancers except for brain cancer and is administered systemically for the treatment of multicentric myofibromatosis and gastrointestinal stromal tumor.[16] However, imatinib resistance has been described in mutations of the activation loop of PDGFR alpha through inhibition of imatinib binding.[13,21]

In this paper, we have characterized 4 individuals with rare corneal myofibromatosis. Immunohistochemical tissue examination, genetic testing, functional characterization of the identified variants, and ophthalmologic disease control and monitoring for > 9 years give new insights into this rare ophthalmic disease. We present 2 novel activating imatinib-responsive variants in *PDGFRB*. Our findings open new avenues for a topical administration of the tyrosine kinase inhibitor for the treatment of ocular myofibromatosis and the prevention of recurrences.

Methods

Patient Consent

Informed written consent of individual F1-III-13 and F2-II-2, who is also the legal guardian of individual III-4, was obtained before genetic testing was performed. The research in this study adhered to the tenets of the Declaration of Helsinki. The local ethics committee ruled that approval was not required for this study (sign 23-1181-retro).

Histomorphometric Analysis

The excised corneal tissue of F1-III-13 and F2-III-4 was characterized histomorphologically. The specimens were fixed in 4% formaldehyde, dehydrated, embedded in paraffin, and cut on a microtome. Four μm sections were stained with Hematoxylin–eosin and subjected to immunohistochemical assessment staining with the following antibodies: α-SMAc (Dako/CE, 3E6, Mouse, 1:100), Beta-catenin (mouse antibody, Dako/CE, clone β-Catenin-1, dilution 1:600), Desmin (mouse antibody, Dako/CE, clone D33, dilution 1:500), Caldesmon (Zeta Corp, clone ZM79, dilution 1:400), Ki-67 (rabbit antibody, Cellmarque/CE, clone SP6, dilution 1:100), and CD34 (mouse antibody, Cellmarque/CE, clone QB End10, dilution 1:700). Histology was evaluated by 3 experienced pathologists (A.M.S., C.V.O., and R.B.).

Polymerase Chain Reaction and Sanger Sequencing

Ethylenediaminetetraacetic acid blood was drawn from the patients and DNA was extracted. In individual F1-III-13, exon 12 and the flanking exon-intron boundaries of *PDGFRB* (NM_002609.4) were amplified using oligonucleotide primers (sequences available upon request) and sequenced on a 3500 Genetic Analyzer (Applied Biosystems). In individual F2-II-2, Sanger Sequencing of *PDGFRB* exon 14 was performed to test for the pathologic variant found in his son F2-III-4. The Sanger Sequencing data were evaluated with the Sequence Pilot (SeqPilot, software version 4.4.0, JSI Medical Systems) program.

Next Generation Sequencing

In individual F2-III-4, whole exome sequencing with phenotype-based targeted analysis of *PDGFRB* and *NOTCH3* was performed. Libraries were prepared with the Agilent SureSelect XT HS library prep kit (Agilent Technologies). Hybrid-capture enrichment of the target region was done using the Agilent SureSelect All Exon v7 and sequenced on an Illumina *NextSeq* 500 platform (Illumina). The overall average coverage depth was 99x, and 97.4% of target regions were covered at > 10x. Coverage depth > 10x for *PDGFRB* target regions was 99.6%, and for *NOTCH3* target regions it was 94.5%.

Bioinformatic Filtering of Variants

The next-generation sequences were aligned to the human genome (GRCh37/hg19) using the QIAGEN CLC Biomedical Workbench (QIAGEN). Variants were annotated, filtered and evaluated using in-house developed software. The pathogenic potential of individual candidate variants was evaluated according to internal laboratory standards following the guidelines of the American College of Medical Genetics and Genomics (ACMG),[22] Association for Clinical Genomic Science,[23] and Clinical Genome Resource.[24] The in silico prediction tool rare exome variant ensemble learner (REVEL) was used for the evaluation of the pathogenicity of the variant.[25]

HA-PDGFRB Mutated Constructs

Mutations were introduced in *PDGFRB* cDNA (NM_002609.4) cloned in the pEF-myc-cyto vector (Invitrogen) by site-directed mutagenesis using Quikchange XLII (Agilent Technologies) as described.[26] Sequences were verified by Sanger sequencing (Eurofins).

Cell Transfection

Subconfluent HEK293T cells were transfected with 3 µg of PDGFRB constructs using the calcium phosphate method in 6-well plates. The next day, cells were lysed and 30 µg of proteins were loaded on Novex 4% to 12% Tris-Glycine Gel (Thermo Fisher Scientific) and transferred on polyvinylidene difluoride membrane using Power Blotter (Thermo Fisher Scientific). Nonspecific sites were blocked in Tris-buffered saline with 5% milk and 0.1% Tween 20. Proteins of interest were revealed with specific primary antibodies (anti-PDGFRβ [Cell signaling,] and anti-β-actin (Merck]), Horseradish peroxidase-labelled secondary antibodies, and Supersignal West Pico plus chemiluminescent substrate (Thermo Fisher Scientific). Images were captured using a Fusion Solo S Western Blot imager (Vilber).

Luciferase Reporter Assay

Porcine aortic endothelial cells were seeded at 50 000 cells per well in 24-well plates and incubated overnight at 37°C. Cells were transfected with 250 ng of each plasmid in duplicate: pEF-myc-cyto-PDGFRB constructs, pGRR5-Luc, and β-galactosidase using Turbofect reagent (Thermo Fisher Scientific). After 4 hours of incubation at 37°C, cells were washed 3 times with phosphate-buffered saline and starved in 1 ml of medium without serum in presence of dimer of the B chain of human PDGF at 25 ng/ml and imatinib 1 µM as indicated. The day after, cells were lysed in 100 µL of Passive lysis buffer 1X for 20 minutes under agitation. Luciferase activity was revealed with luciferase assay reagent (Promega) and luminescence was measured during 5 seconds using a Glomax Discover microplate reader (Promega). The β-galactosidase activity was quantified in presence of its substrate o-nitrophenyl-β-D-galactopyranoside by reading absorbance at 405 nm, as described.[26] Normalized luciferase activities were obtained by dividing luminescence values by β-galactosidase activities and using the wild-type receptor as a reference. For the statistical evaluation, we have used Excel (Microsoft Corporation).

Results

Case Report

The male individual III-13 from Family 1 (F1-III-13) is the firstborn child to nonconsanguineous parents. The initial diagnosis of infantile myofibromatosis type 1 (OMIM #228550) was made following the excision of multiple small dermal myofibromas and a mandibular myofibroma. He was born with skin nodules at the right lower leg and left thigh and hypopigmented skin areas of 0.5 × 0.5 cm located at the right forehead and the left scapula. Sonography of the nodules depicted a well-bordered, hypoechogenic mass with no evidence of vascularization. At the age of 10 weeks, both lower extremity nodules were excised and a histopathologic evaluation was performed. Macroscopically, the tumor excised from the right lower leg showed a clear boundary and a capsule. Microscopic examination of the lesion showed myofibroblastic cells arranged in bundles and trains with many vessels. The second lesion excised from the left thigh was also described to have a capsule and a circumscribed border. A hemangiopericytomatous pattern was seen with a continuous fibrous pattern in the silver stain. Large slit-like vessels, partially with fibrin deposition, and central pseudocyst formation were also observed.

A sonographic examination of the abdominal organs at 7.5 months of age showed no evidence of visceral abnormalities. At the age of 9 years, a dental orthopantomogram examination showed an osseous myofibroma at the left mandible as an incidental finding. The hypodense, oval-shaped cystic mandibular lesion with thinning of the cortical bone is shown in Figure 1 (yellow ring). Surgical resection followed and histology confirmed a manifestation of myofibromatosis and no evidence of malignancy. The patient reported that he had previous whole-body magnetic resonance images in childhood or adolescence which showed no abnormalities of the internal organs or brain.

At the age of 14 years, the first ocular manifestation was noticed. At the age of 18 years, he visited our ophthalmologic department with a circumscribed corneal opacification with superficial vascularization on the left eye, imaged by slit-lamp photography (Fig 2). Slit-lamp-OCT (SL-OCT) imaging revealed the 3-dimensional extension and the nonhomogenous structure of the lesion (Fig 2A.2), the largest diameter being 900 µm. The lesion was removed by lamellar keratectomy with intraoperative mitomycin C. Postoperative slit-lamp photography (Fig 2A.3) and SL-OCT (Fig 2A.4) showed the complete removal of the lesion. At 18 weeks after surgery, a recurrence of the myofibromateous lesion was observed and documented with slit-lamp and SL-OCT images (Fig 2A.5, A.6). The patient was tall (199 cm) and had a prominent forehead, similar to his father's, as well as scoliosis.

The family history revealed a first-degree maternal cousin (F1-III-15) with a history of a similar corneal

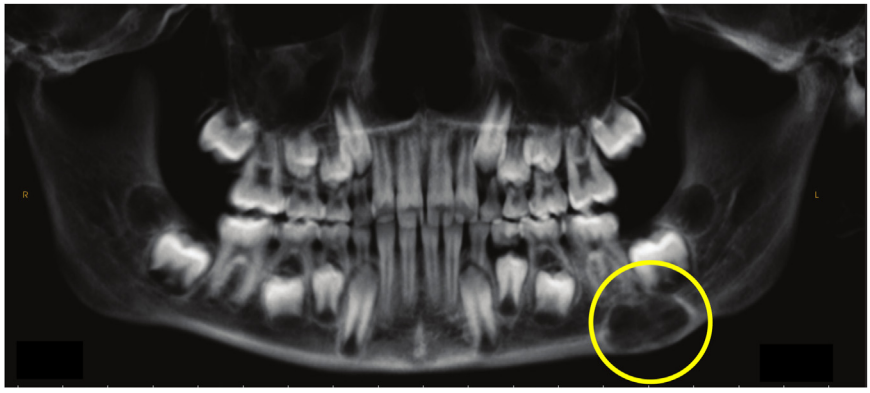

Figure 1. Osseous myofibroma lesion at the left mandible of patient 1. At 9 years of age, individual F1-III-13 was diagnosed with the incidental finding of a myofibroma at the left mandible. The cone-beam computed tomography imaging shows a hypodense, oval-shaped cystic lesion of the left mandible with thinning of the cortical bone (yellow ring).

pathology, for which he had undergone corneal transplantation at an external hospital. The mother (Fig 3A, F1-II-6, hatched area), the patient's maternal grandmother (Fig 3A, F1-I-4, hatched area), and the above-mentioned sister of the mother (Fig 3A, F1-II-7, hatched area) are said to have had neoplasms in the eye in the sense of a pterygium or pterygium-like lesion. As no surgical excision and hence no histologic tissue evaluation has been carried out, it is unknown whether the lesion described as "pterygia" could have been corneal and/or conjunctival myofibromateous lesions. No other maternal or paternal family members showed signs of corneal abnormalities.

The male individual III-4 of family 2 (F2-III-4) is the second-born child to nonconsanguineous parents. At the age of 5 weeks, the parents observed a nodule located at the left eyebrow which was not surgically excised but shrunk in size, leaving behind a skin indentation. At the age of 11 years, he presented to our ophthalmic department with a corneal lesion similar to the one observed in F1-III-13, causing photophobia and glare sensitivity. Macroscopically, a circumscribed corneal opacification was observed on the left eye and imaged by slit-lamp photography (Fig 2B.1). Slit-lamp-OCT depicted an inhomogeneous lesion spanning 870 μm at the largest diameter (Fig 2B.2). It was continuously monitored and at the age of 13, a lamellar keratectomy with intraoperative mitomycin C was performed to excise the tumor. The specimen was sent for (immuno)histopathologic evaluation. In the postoperative slit-lamp photography (Fig 2B.3) and SL-OCT (Fig 2B.4), the corneal lesion was absent. At 14.5 weeks after primary surgical excision, recurrence of disease was noted. A repeat lamellar keratectomy was performed. Three months after surgery, disease recurrence was noted. The clinical findings shown on slit lamp (Fig 2B.5) and SL-OCT images (Fig 2B.6) were photographed 2 years and 2 months after the second surgical removal.

The patient's father, individual F2-II-2, first noted a lesion of his left eye at the age of 40 years. At a visit to an ophthalmologist at the age of 42 years, a corneal opacification was noted nasally on the right eye (Fig 2C.1) and a nasal pterygium-like lesion on the left eye (Fig 2C.2). After 2 years, the lesions were mostly stable (Fig 2C.3, C.4), continuously so on the right eye (Fig 2C.5). Due to increasing discomfort and compromised vision on the left eye, surgical excision of the left-sided pterygium-like lesion followed at the age of 51 years. Recurrence of disease was noted only 3 months after surgery and is shown on the slit lamp image (Fig 2C.6) and Casia 2 (Fig 2C.7) 19 months after surgery.

The disease onset in the described cases of corneal myofibroma ranges between the age of 11, 14, and 40 years, respectively. In all affected individuals, tumor recurrence was noted after surgical excision.

In all affected individuals, there was no history of previous surgery, inflammation, or trauma to the eye before the development of the corneal lesions. Furthermore, there was no evidence of Penttinen syndrome.

Histological Analysis of Corneal Specimen Yielded the Diagnosis of Corneal Myofibromatosis

Histological analysis of corneal tissue led to the diagnosis of corneal myofibromatosis (Fig 4). Hematoxylin–eosin staining revealed spindle-shaped cells (Fig 4A, E). Immunohistochemical staining in both patients demonstrated positivity for α-SMA (Fig 4B, F), and β-catenin, a low proliferation rate in Ki-67 $< 5\%$ (Fig 4C, G), and marginal positivity for Desmin (Fig 4D, H). In individual F1-III-13, immunohistochemical staining showed extensive positivity for β-catenin and negative staining for Caldesmon, and in individual F2-III-4, negative staining for CD34.

Genetic Analysis

After histologic confirmation of myofibromatosis in individuals F1-III-13 and F2-III-4, molecular genetic analyses were initiated for both patients. Individual F2-II-2 was clinically affected, treated at an external hospital, and ultimately received corneal transplantation. He underwent genetic testing, allowing for segregation analysis of the variant within family 2.

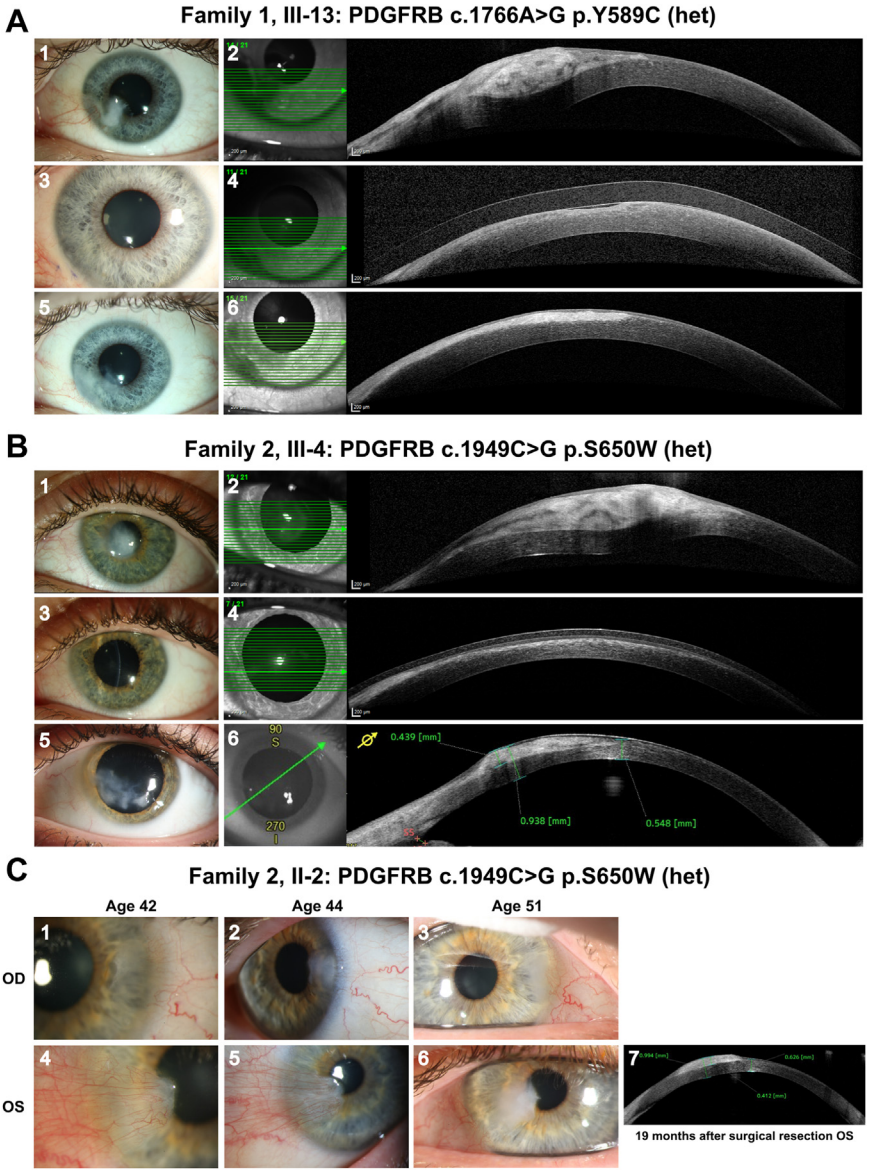

Figure 2. Pre- and postoperative clinical presentation of corneal myofibroma of individuals F1-III-13 and F2-III-4. **A.1,** Pre-operative slit-lamp photography of individual F1-III-13 depicts a white corneal tumor of the left eye with superficial vascularization. **A.2,** In the preoperative slit-lamp-OCT (SL-OCT), the extension and structural composition of the tumor can be acknowledged. **A.3,** Postoperative slit-lamp photography after lamellar keratectomy shows a clear cornea and (**A.4**) the postoperative SL-OCT proves the absence of the tumor. Note early postoperative bandage contact lens on top of cornea. **A.5,** Recurrence of disease noted 18 months after the surgical excision is shown on slit lamp and (**A.6**) SL-OCT images. **B.1,** Preoperative slit-lamp photography of individual F2-III-4 shows a white lesion at the corneal center. **B.2,** The preoperative SL-OCT shows the non-homogenous structure and extension of the tumor. **B.3,** Post-operative slit-lamp photography after lamellar keratectomy shows a clear cornea. A contact lens for surface protection is present (as in D, too). **B.4,** In the post-operative SL-OCT, the tumor was absent. **B.5,** Two years and 2 months after the second surgical excision of the lesion, recurrence of disease was noted on the slit lamp and (**B.6**) SL-OCT. **C.1,** Individual F2-II-2 with bilateral lesions of the cornea (right eye [OD], **C.1**–**C.3**) and of both conjunctiva and cornea (left eye [OS]; **C.4**–**C.7**). **C.1**–**C.3,** The corneal lesion of the right eye seems stable over time. **C.4,** Pterygium-like lesion of the left eye with growth of corneal tissue onto the cornea (**C.5**) which was mostly stable over a time course of 2 years. **C.6,** Three months after the surgical excision of the left eye in February 2022, recurrence of disease with a corneal lesion was noted. It is depicted 1 year and 7 months after surgery on slit lamp imaging and (**C.7**) Casia 2 anterior segment OCT imaging. PDGFRB = platelet-derived growth factor receptor beta.

Individual F1-III-15, the maternal cousin to our patient F1-III-13, had previously undergone exome-based panel sequencing for *PDGFRB* and *NOTCH3* due to suspected infantile myofibromatosis, corneal myofibromas, and a positive family history for cutaneous myofibromatosis. The heterozygous variant *PDGFRB* c.1766A>G (p.Tyr589Cys) was identified and confirmed by Sanger Sequencing as depicted in the Sequence Pilot (SeqPilot) screenshot (Fig 3A,

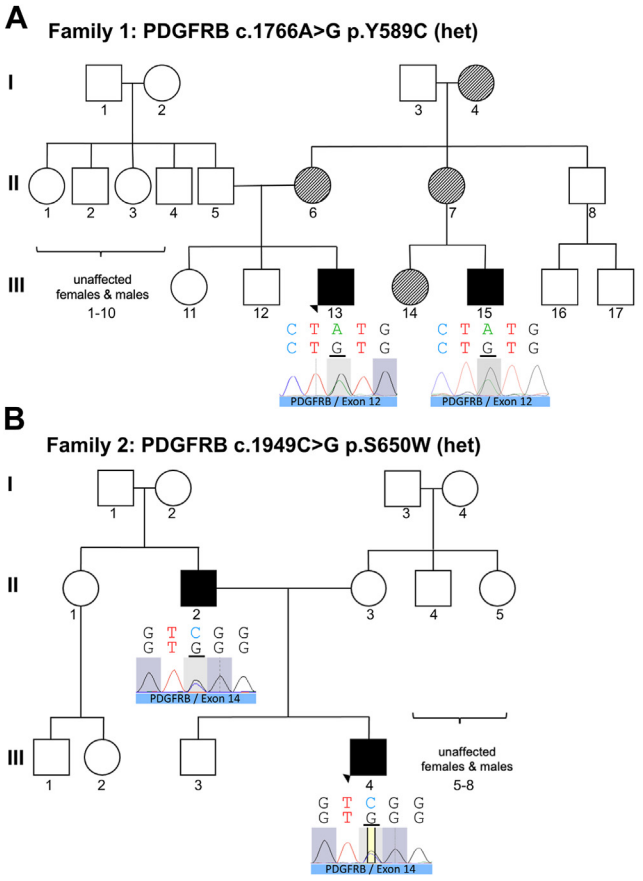

Figure 3. Family pedigrees and sequencing data of patient 1 and 2. **A,** Pedigree of family 1 with individuals F1-III-13 (black arrowhead) and F1-III-15 with pathogenic platelet-derived growth factor receptor beta gene (*PDGFRB*) variants. Sanger sequencing screenshots by Sequence Pilot (SeqPilot) of the affected individuals show *PDGFRB* c.1766A>G (p.Y589C) in exon 12 of *PDGFRB*. Clinically affected but not genetically tested female family members of the maternal side (F1-I-4, F1-II-6, F1-II-7, and F1-III-14) with a "pterygium"-like phenotype, a lesion affecting the conjunctiva and cornea, are highlighted in oblique stripes. **B,** Pedigree of family 2 with affected individuals F2-II-2 and F2-III-4. Exome-based panel sequencing of *PDGFRB* revealed the variant c.1949C>G (p.S650W), which was confirmed by Sanger sequencing of *PDGFRB* exon 14.

III-15), whereas a wild-type sequence for *NOTCH3* was detected. The *PDGFRB* variant could initially only be classified as a variant of unclear significance.

For F1-III-13 (Fig 3A, black arrowhead), targeted testing with Sanger Sequencing for the familial heterozygous variant c.1766A>G (p.Tyr589Cys) in *PDGFRB* was performed. We could confirm the above-mentioned *PDGFRB* variant in exon 12 as shown in the Sanger Sequencing SeqPilot screenshot (Fig 3A, F1-III-13). Through cosegregation in the family, the variant can be reclassified as likely pathogenic, applying the following adjusted ACMG criteria: The variant co-segregates within the family (PP1). It has not been found in control populations (dbSNP, ExAC, or the 1000 Genomes Project). There was no evidence in the database that the variant can also be found in nonaffected individuals (PM2_supporting). In silico prediction tools classify the variant as pathogenic (PP3). The patient phenotype is highly specific for the disease and the diagnosis of a corneal myofibroma has been confirmed by histopathology (PP4_moderate). The p.Tyr589Cys variant is located in the JM domain of PDGFRB, which is a mutation hotspot regarding the phenotype of infantile myofibromatosis (PM1).[22]

Individual III-4 from family 2 (Fig 3B, F2-III-4, black arrowhead) and his father (F2-II-2) are affected by corneal myofibromateous lesions (Fig 3B, C). As no previous genetic testing was performed within this family, we opted for exome-based panel sequencing of individual III-4 from family 2 for *PDGFRB* and *NOTCH3*, which revealed the heterozygous variant *PDGFRB* c.1949C>G (p.Ser650Trp, Fig 3D).

The father of this patient, individual F2-II-2, is clinically affected and was tested by Sanger sequencing for the respective variant in *PDGFRB* exon 14. He was found to also carry the variant (Fig 2B). This confirms the autosomal dominant transmission of the disease in the described families.

The variant could be classified as likely pathogenic using these ACMG criteria: the variant was not found in the Exome Variant Server, the ExaC-Browser, the gnomAD-Browser or dbSNP database, providing evidence that this variant is not detected in unaffected individuals (PM2_supporting). The variant is located in the PDGFRB kinase domain and also corresponds to a mutation hotspot related to the infantile myofibromatosis phenotype (PM1).[22] The in silico prediction tool REVEL classifies the variants as pathogenic (PP3) with a REVEL score of 0.868 [*PDGFRB* c.1766A>G (p.Tyr589Cys)] and 0.902 [*PDGFRB* c.1949C>G (p.Ser650Trp)]. The patient phenotype is highly specific for the disease and the myofibromateous entity of the corneal lesion was confirmed histopathologically (PP4_moderate).

Neither of the 2 pathogenic variants have been described in the literature before. Both could at first be classified as likely pathogenic and finally reclassified as pathogenic, taking into account the well-established functional studies supporting a damaging effect of the variants shown in this study (PS3).

The further sequencing data for both genes were unremarkable (data not shown).

Functional Characterization of PDGFRB Variants

Residues Y589 and S650 are located in the JM and kinase domains of the receptor, respectively (Fig 5A), which are 2 hotspots for *PDGFRB* mutations associated with infantile myofibromatosis.[27] We measured the activity of both receptor mutants in a luciferase reporter assay that is sensitive to signaling via signal transducer and activator of transcription. The constitutively activated p.P584R germline variant was used as a positive control.[17] Figure 5B shows that the 2 mutants were significantly activated in the absence of ligand, compared to the unstimulated wild-type receptor (p.Y589C vs. wild-type $P = 0.012$; p.S650W vs. wild-type $P = 0.031$). This was not due to a change in receptor expression, as shown by western blot (Fig 5C). These results indicated that the new variants reported here confer a GOF, in agreement with previous findings on infantile myofibromatosis.[27] We next

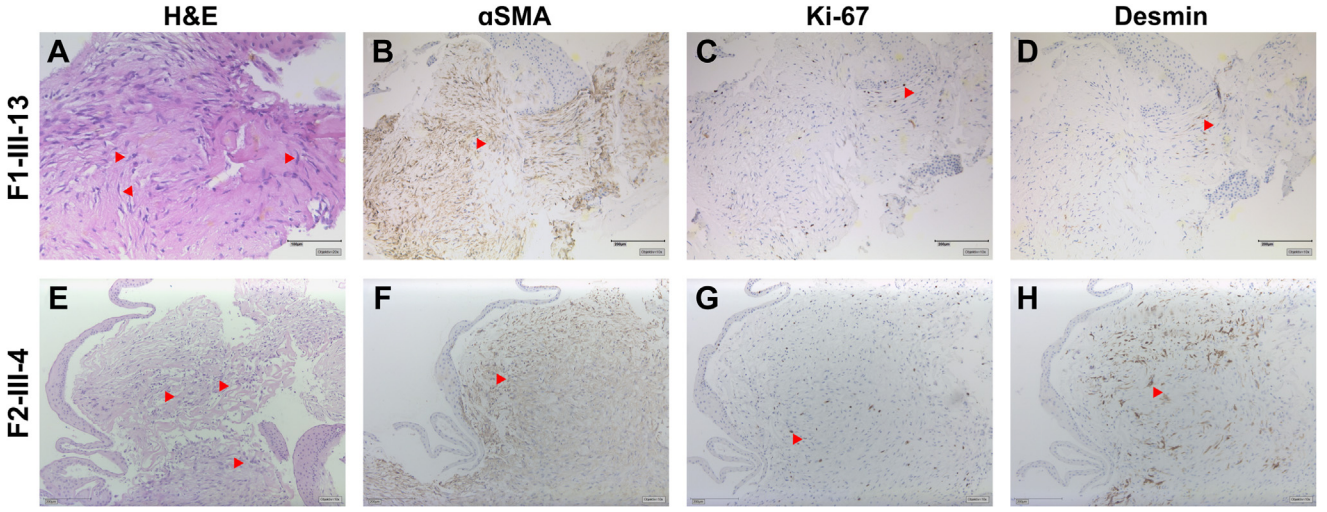

Figure 4. Histopathologic evaluation of resected tumors of individual F1-III-13 and F2-III-4. **A, E,** Hematoxylin–eosin (H&E) staining at 20x (**A**) and 10x (**E**) magnification, respectively, shows spindle-shaped cells. **B, F,** Immunohistochemical staining (10x magnification) demonstrates a positive staining for alpha smooth muscle actin (α-SMA) in both specimens. **C, G,** Ki-67 staining shows a low proliferation rate of < 5%. **D, H,** Desmin staining was marginally positive (see red arrow heads).

evaluated whether imatinib, which is a potent PDGFRβ kinase inhibitor, was active against the mutant receptors. As shown in Figure 5B, this compound completely blocked the activation of the different receptor mutants at a clinically relevant concentration of 1 μM compared with untreated (p.P594R $P = 0.019$; p.Y589C $P = 0.002$; S650W $P = 0.023$).

Interestingly, an activating somatic p.Y589D variant had been reported before in an isolated subcutaneous myofibroma.[27] A few variants at position S650 are also mentioned in the Catalogue of Somatic Mutations in Cancer, such as p.S650L, but have not been characterized. We speculated that the substitution of serine 650 by a bulky aromatic amino acid such as tryptophan may prevent the inhibition

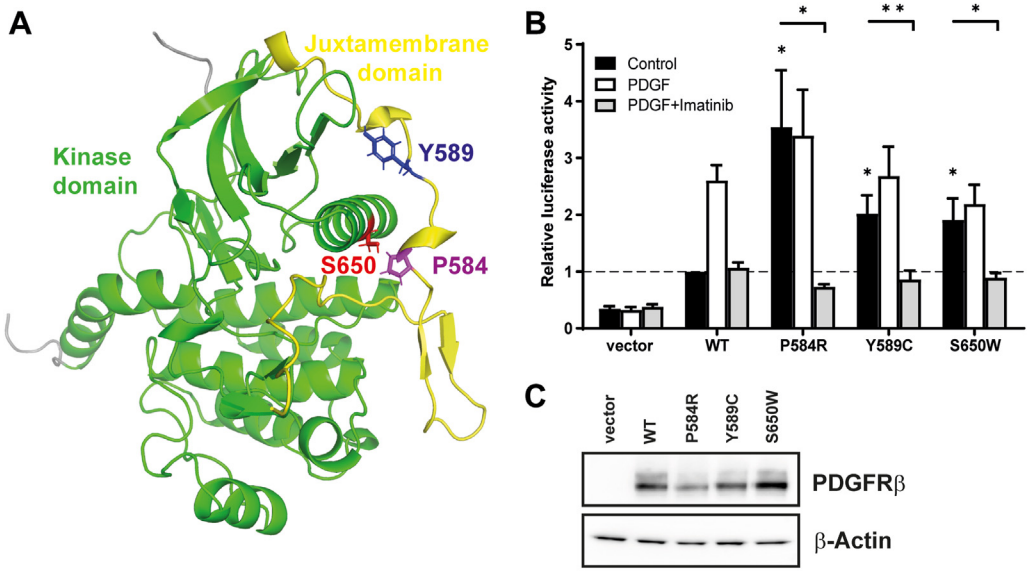

Figure 5. Characterization of platelet-derived growth factor receptor beta (PDGFRB) variants. **A,** Localization of PDGFRB variants in the kinase domain structure. The structure is based on the highly conserved PDGFR alpha intracellular part (Protein Data Bank reference 5K5X) and was visualized using Pymol. The juxtamembrane domain is represented in yellow (with tyr589 in blue) and the tyrosine kinase domain in green (with ser650 in red). **B,** Platelet-derived growth factor receptor beta variant p.Y589C and p.S650W are constitutively active and sensitive to imatinib. Luciferase reporter assays were performed in porcine aortic endothelial cells after transient transfection with the indicated receptors. Cells were left untreated (black bars), stimulated with platelet-derived growth factor (PDGF; white bars) or treated with both PDGF and imatinib (grey bars). The constitutively activated p.P584R variant was used as a positive control. The mean of 6 independent experiments is shown with standard error of the mean (Student t test; $*P \leq 0.05$; $**P \leq 0.01$). **C,** Expression of PDGFRB variants in transfected HEK293T cells. Lysates of transiently transfected cells were analyzed by western blot using anti-PDGFRB and anti-β-actin antibodies. WT = wild-type.

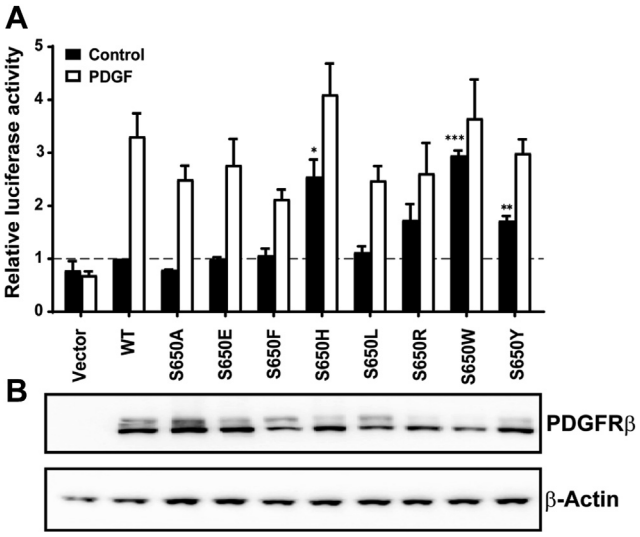

Figure 6. Substitution of serine 650 by histidine or tyrosine also activates platelet-derived growth factor receptor beta (PDGFRB). **A,** Serine 650 was mutated in the indicated amino-acid. Luciferase reporter assays were performed in porcine aortic endothelial cells after transient transfection with the indicated receptors. Cells were stimulated by platelet-derived growth factor (PDGF) as positive control (white bars). The mean of 3 independent experiments is shown with standard error of the mean (Student t test; $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$). **B,** Expression of PDGFRB variants in transfected HEK293T cells. Lysates of transiently transfected cells were analyzed by western blot using anti-PDGFRB and anti-β-actin antibodies. WT = wild-type.

of the kinase domain by the JM domain (Fig 5A). To test this hypothesis, we introduced different amino acids at that position and tested the activity of the corresponding mutant receptors. We observed that substituting serine 650 with an alanine (p.S650A) did not change the receptor activity, suggesting that the presence of a serine at that position is not essential (Fig 6A). By contrast, the amino acid change to tryptophan resulted in strong constitutive PDGFRβ activity (p.S650W $P = 0.001$) compared with the wild-type receptor. Two aromatic amino acids, histidine (p.S650H $P = 0.02$) and tyrosine (p.S650Y $P = 0.007$) also induced a constitutive PDGFRβ activity compared with the wild-type receptor. Other residues, including phenylalanine, did not confer a gain of function. This was not due to higher expression of the receptor mutant, as shown by Western blot. Interestingly, the mutant p.S650W identified here shows high constitutive receptor activity but is less expressed compared to the wild type (Fig 6B). None of these changes affected the receptor response to its ligand PDGF-B (Fig 6A).

Discussion

We describe 4 cases of human corneal myofibromatosis and performed genetic characterization of patients with ocular manifestations of myofibromatosis. We found 2 novel GOF variants in *PDGFRB* and evaluated the rescue effect of the tyrosine kinase inhibitor imatinib *in vitro*.

In the literature, 1 case of solitary scleral myofibroma[3] and 1 case of solitary conjunctival myofibroma[28] have been described so far.

The disease onset in the described corneal myofibroma cases ranges between the age of 11, 14, and 40 years, respectively. In all affected individuals, tumor recurrence was noted after corneal surgical excision. We describe a follow-up period of 3 years and 1 month in individual F1-III-13, 9 years in individual F2-II-2, and 3 years and 5 months in individual F2-III-4.

Differential diagnoses that need to be considered include corneal keloid[29] and Salzmann's nodular degeneration.[30] Corneal keloids can occur as solitary nodules or involve the entire stroma with a tendency to slow progression. Ocular injury, e.g., perforating injury and ocular inflammations (e.g., childhood keratitis, corneal ulcers, and purulent ophthalmia neonatorum) are discussed as potential etiological factors.[29] Bilateral manifestations are regularly described[31] and observed in Lowe's syndrome, amongst others.[32] Similar to corneal myofibromas, immunohistochemistry is positive for α-SMA and Vimentin but negative for Desmin. Salzmann's nodular degeneration characteristically involves nodular thickening, i.e., subepithelial fibrosis, at multiple sites of the corneal surface.[30] It has been hypothesized that chronic epithelial wound repair and remodeling mechanisms with upregulated matrix metalloproteases could be causative of the disease development.[33] In the individual F2-II-2, a pterygium-like structure was present on the left eye. As there was no tissue available for histologic examantion, we cannot rule out conjunctival myofibroma or a mixture of pterygium and corneal myofibroma.

The phenotype of our individuals can be distinguished from the clinical picture of ocular pterygium-digital keloid dysplasia (OPDKD). Our patients did not show any abnormalities of the fingers as described for OPDKD.[34] Additionally, myofibromatosis was not reported in OPDKD cases. Ocular pterygium and *PDGFRB* mutations were also observed in patients with the premature aging syndrome named Penttinen syndrome.[35] The clinical features of Penttinen syndrome are not present in our patients. The reasons why different GOF *PDGFRB* variants cause different diseases remain poorly understood.

To our knowledge, this is the first time that the underlying genetic variants in patients with ocular involvement of myofibromatosis have been deciphered and characterized.

At the time of ocular manifestation, our patients had multicentric myofibromatosis without known visceral involvement in tissues of mesenchymal origin. Potential exogenous risk factors we have discussed with the patients include ultraviolet-light exposure, dry eye disease, and ocular manipulation e.g., by contact lenses or eye rubbing.

The individuals F1-III-13 and F1-III-15 carry the heterozygous pathogenic variant *PDGFRB* c.1766A>G (p.Tyr589Cys) in exon 12. Functionally, the amino acid substitution in the JM domain leads to a loss of the inhibitory function of the domain and a constitutive activation of the receptor kinase activity.

The heterozygous pathogenic variant *PDGFRB* c.1949C>G (p.Ser650Trp) identified in individuals F2-II-2

and F2-III-4 has not yet been described in the literature. The variant segregates within the family. Further genetic testing of more family members was not possible due to missing consent for predictive genetic testing.

Both variants were first classified as likely pathogenic (ACMG Class 4) and could finally be reclassified as pathogenic (ACMG Class 5), taking into account the functional studies supporting a damaging effect of the variants shown in this study.

It is well established that mutations in the JM domain of PDGFRB (and other class III receptors tyrosine kinases such as PDGFR alpha, stem cell receptor tyrosine kinase, and FMS-like tyrosine kinase 3) alleviate the inhibition of the kinase domain, leading to constitutive activation.[11] The p.Ser650Trp variant may work similarly, by decreasing the interaction between the JM and the tyrosine kinase domain, favoring activation of the receptor. This was previously only described in the JM, not in the tyrosine kinase domain, which makes p.Ser650Trp a new type of mutation from a biochemical standpoint. Our analysis further suggests that the substitution of S650 by other bulky aromatic amino acids could activate the receptor (Fig 6). However, the other substitutions are less likely to be found in human pathologies because they require 2 nucleotide changes instead of 1 in the here-described case.

Due to the small number of patients with myofibromatosis described, the spectrum of possible symptoms is not yet fully known. To date, the incidence of serious complications, mild disease courses, and undiagnosed or lifelong asymptomatic cases is unknown.[16] In PDGFRB-activating variant spectrum disorder, it should be assumed that the disease can also exhibit a certain dynamic and that new symptoms can occur even in later stages of life. As screening for individuals with PDGFRB-activating variant spectrum disorder, Wenger et al.[16] recommend magnetic resonance angiography, cardiac echocardiography, and magnetic resonance imaging of the brain. The intervals at which these examinations are to be carried out have not been specified.

There are several case reports of a therapeutic response of myofibromas to treatment with the tyrosine kinase inhibitors imatinib and sunitinib.[18] The *in vitro* sensitivity of imatinib in GOF-variants observed in our patients opens a discussion of imatinib as a potential targeted therapy to prevent and treat recurrences. Systemic and multicentric manifestations of myofibromatosis and OPDKD are treated systemically by imatinib.[16,36]

The local administration of imatinib mesylate eye drops recently tested in a small randomized, double-blind, placebo-controlled study has been discussed as a safe and well-tolerated treatment option for dry eye disease.[37]

Imatinib mesylate has been identified as a potent inhibitor of the Discoidin domain receptor 1,[37] which belongs to the group of receptor tyrosine kinases and the family of nonintegrin collagen receptors. Discoidin domain receptor 1 has been shown to affect inflammation, tumor progression, fibrosis, and atherosclerosis.[38]

We believe that imatinib mesylate eye drops could offer a potentially effective and safe targeted therapy for patients with PDGFRB-activating variants and corneal myofibromatosis. However, the application form of imatinib mesylate or other tyrosine kinase inhibitors as eye drops has not been approved to date. Further investigations are needed to assess the effect of topical imatinib mesylate eye drops on the development of corneal myofibromas.

In conclusion, we present 4 individuals from 2 unrelated families with the rare phenotype of corneal myofibromatosis and describe the *in vitro* responsiveness of imatinib, inhibiting the constitutively active PDGF receptor in the PDGFRB-activating mutations. Our study highlights the incentive to formulate imatinib mesylate eye drops for the treatment of corneal myofibromas that are insufficiently treated by surgical resection only.

Web Resources

Catalogue of Somatic Mutations in Cancer (COSMIC): https://cancer.sanger.ac.uk.
Exome Aggregation Consortium Browser (ExAC): http://exac.broadinstitute.org/.
Genome Aggregation Database browser (gnomAD): http://gnomad.broadinstitute.org/.
Human Gene Mutation Database (HGMD): http://www.hgmd.org/.
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim.
Rare Exome Variant Ensemble Learner (REVEL): https://sites.google.com/site/revelgenomics/.

Data Availability

There are restrictions to the availability of the dataset due to the personal data of genetic testing, which is not made publicly available.

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Footnotes and Disclosures

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HUMAN SUBJECTS: Human subjects were included in this study. Informed written consent of individual F1-III-13 and F2-II-2, who is also the legal guardian of individual III-4, was obtained before genetic testing was performed. Due to the nature of the study, institutional review board approval was not required. All research adhered to the tenets of the Declaration of Helsinki. No animal subjects were used in this study.

Author Contributions:

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Data collection: Howaldt, Lenglez, Velmans, Schultheis, Kohlhase, Vokuhl, Büttner, Netzer, Demoulin, Cursiefen

Analysis and interpretation: Howaldt, Lenglez, Velmans, Schultheis, Clahsen, Matthaei, Kohlhase, Vokuhl, Büttner, Netzer, Demoulin, Cursiefen

Obtained funding: N/A

Overall responsibility: Howaldt, Cursiefen

Abbreviation and Acronym:
ACMG = American College of Medical Genetics and Genomics; **α-SMA** = alpha-smooth muscle actin; **GOF** = gain-of-function; **JM** = juxtamembrane; ***NOTCH3*** = notch homolog protein 3 gene; **OMIM** = Online Mendelian Inheritance in Man; **OPDKD** = ocular pterygium-digital keloid dysplasia; **PASV** = platelet-derived growth factor receptor beta activating spectrum disorder; **PDGFRB** = platelet-derived growth factor receptor beta; **REVEL** = rare exome variant ensemble learner; **SL-OCT** = slit-lamp-OCT.

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Infantile myofibromatosis, PDGFRB, Imatinib, Corneal myofibroma, Receptor tyrosine kinase inhibitor.

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