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A germline *PDGFRB* splice site variant associated with infantile myofibromatosis and resistance to imatinib

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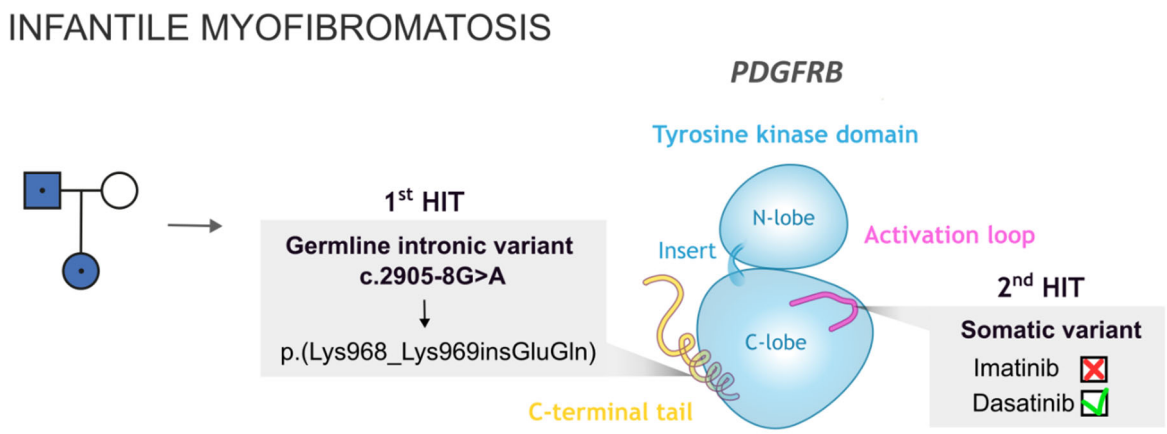
ABSTRACT



Purpose

Infantile myofibromatosis is characterized by the development of myofibroblastic tumors in young children. In most cases, the disease is caused by somatic gain-of-function variants in platelet-derived growth factor receptor beta *(PDGFRB)*. Here, we report a novel germline intronic *PDGFRB* variant, c.2905-8G>A, in six unrelated infants with multifocal myofibromatosis and their relatives.

Methods

We performed constitutional and tumor DNA and RNA sequencing to identify novel variants, which were subsequently characterized in cellular assays.

Results

All patients had multiple skin nodules, four had bone lesions and two had aggressive disease with bowel obstruction. The c.2905-8G>A substitution creates an alternative acceptor splice site in intron 21, inserting two codons in the *PDGFRB* transcript. Functional studies revealed that the splice change induced a partial loss of function, contrasting with previously-described

variants. In four tumor samples, we identified a second somatic hit at position Asp850 in *PDGFRB* exon 18, triggering constitutive receptor activation and resistance to imatinib. In addition to vinblastine and methotrexate, two patients had received imatinib without objective response. One of them switched to dasatinib with concomitant improvement.

Conclusion

This splice-site *PDGFRB* variant favors the development of myofibroma featuring an acquired oncogenic variant in the same gene and resistance to targeted therapy.

KEYWORDS

Drug resistance, tyrosine kinase inhibitor, targeted therapy, cancer predisposition, receptor tyrosine kinase

Introduction

Infantile myofibromatosis (IMF) is a rare disease characterized by the development of myofibromas in the skin, muscles, bones and internal organs during the first months of life [1,2]. These benign soft-tissue tumors often regress spontaneously. The disease severity depends on the number of lesions and their location. Compression of internal organs, intestinal occlusion and airway obstruction may cause significant morbidity and mortality. Treatments include surgery and vinblastine/methotrexate-based chemotherapy for severe cases [1].

Myofibroma is classified as a perivascular tumor, which expresses pericyte biomarkers, such as smooth muscle actin and NG2. Accordingly, genetic analysis by multiple laboratories, including ours, revealed that the most frequent gene alteration in IMF occurs in platelet-derived growth factor receptor beta (PDGFRβ, encoded by the *PDGFRB* gene, HGNC:8804), a receptor tyrosine kinase that controls the migration and proliferation of vascular smooth muscle cells and pericytes [3-6]. We demonstrated that *PDGFRB* variants lead to the constitutive receptor activation in the absence of ligands, PDGF-BB and -DD [4]. Most variants occur in exons 12 and 14, which encode the juxtamembrane domain and the kinase domain N-lobe, respectively [5]. About half of the tumors harbor two variants in the same *PDGFRB* allele. PDGFRβ can be targeted by tyrosine kinase inhibitors (TKI), such as imatinib, which has been tested in a limited number of patients with encouraging results [1,7-10].

In the multicentric form of the disease, different lesions of the same individual sometimes carry different *PDGFRB* somatic variants, suggesting a predisposing factor [4]. Although most IMF cases are sporadic, a dominantly inherited form of the disease was also reported (MIM: 228550) [11,12]. Most families carry the pathogenic NM_002609.3:c.1681C>T p.(Arg561Cys) germline *PDGFRB* variant in exon 12, leading to a weak gain of function [1,13]. Lesions in these patients harbor a second somatic hit in *PDGFRB*, such as c.1998C>A p.(Asn666Lys) in exon 14, which fully activates the receptor in tumor cells [3-5]. Other germline variants in *PDGFRB* and *NOTCH3* (HGNC:7883) were reported in isolated families [1,12,14]. *PDGFRB* gain-of-function variants are also associated with Kosaki overgrowth syndrome (MIM: 616592) and Penttinen syndrome (MIM: 601812) [6,15,16]. These variants produce a stronger constitutive activation of the receptor, causing skin, blood vessel and bone alterations [13,17]. Some of these patients also develop myofibroma. Nevertheless, the constitutional genetic factors that predispose to infantile myofibromatosis remain incompletely understood.

In the present study, we describe a novel germline intronic *PDGFRB* variant in six unrelated infants with IMF, including three with aggressive disease. The variant induced alternative splicing of the *PDGFRB* transcript, resulting in the in-frame insertion of two residues and a partial loss of function. In tumors, it was associated with a second somatic hit in *PDGFRB* exon 18, which constitutively activated the receptor and induced resistance to imatinib.

Materials and methods

Study design

The study aimed at collecting samples and clinical data from patients with infantile myofibromatosis and was approved by the institutional ethical review board of UCLouvain (reference B403201732634) [5]. Only patients carrying the variant of interest are reported here. One de-identified sample and associated data had been provided by the PALGA biobank and previously published [5,18]. Informed consent was obtained from the other patients or their legal representatives.

Reagents and antibodies

Recombinant human platelet-derived growth factor (PDGF)-BB was purchased from PeproTech (Cranbury, NJ, USA), while imatinib and dasatinib were obtained from LC Laboratories (Woburn, MA, USA). The anti-phosphotyrosine antibody (pY99, #sc-7020) was obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Antibodies against PDGFRβ (#3169), phospho-S473-AKT (#4060), AKT (#9272), phospho-Y783-PLCγ1 (#2821), PLCγ1 (#2822), phospho-Y705-STAT3 (#9145), and STAT3 (#12640) were purchased from Cell Signaling Technology (Danvers, MA, USA). The anti-β-actin antibody (#A5441) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

The coding sequence of human wild-type (WT) *PDGFRB* (NM_002609.3) was cloned into pEF/myc/cyto and pcDNA3 plasmids (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Variants were introduced via site-directed mutagenesis in the *PDGFRB* WT sequence using the QuickChange II XL kit from Agilent (Santa Clara, CA, USA). The inserts of all the

constructs were validated by Sanger sequencing (Eurofins). Sanger sequences were aligned to the GRCh38 genome build for analysis.

Cell culture and luciferase reporter assay

Porcine aortic endothelial (PAE), MCF7, 10T1/2, and HEK 293T cells were cultured in Dulbecco's modified Eagle medium (Gibco, Life Technologies, Grand Island, NY, USA), supplemented with 10% fetal calf serum, 50 U/ml penicillin, and 50 µg/ml streptomycin.

For luciferase reporter assays, MCF7 or 10T1/2 cells (50000/well) were seeded in 24-well plates. After 24 h, cells were co-transfected with three plasmids: either a *PDGFRB* variant or a control (250 ng), pEF1-βGal (250 ng), and pSRE-Luc (250 ng) using TurboFect Transfection Reagent (Thermo Fisher Scientific). After 4 h, PDGF-BB or inhibitors (imatinib or dasatinib) were added to the appropriate wells at the indicated concentrations. Cells were lysed 24 h after transfection, followed by luciferase activity measurement as previously described [19]. The luminescence intensity was normalized against the β-galactosidase activity used as internal control.

Minigene assay

Intron 21 with the c.2905-8G>A variant was inserted in the *PDGFRB* coding sequence cloned in pcDNA3 (GenScript, Piscataway, NJ, USA). The corresponding WT sequence was generated through site-directed mutagenesis. HEK 293T cells (5.10^5 cells/well) were transfected in 6 well-plates using the calcium phosphate method, as described [20]. RNA extraction was performed using the NucleoSpin RNA kit (Macheray-Nagel, Düren, Germany), which includes a digestion step to remove contaminating DNA. Reverse transcription PCR (RT-PCR) was carried out with 1 µg of RNA, M-MLV reverse transcriptase (Thermo Fisher Scientific) and oligo(dT) primers (Promega, Madison, WI, USA) in a final reaction volume of 20 µL. The PCR was conducted using the following primer sequences: forward 5'-CTGGGAGATCTTCACCTTGGG-3' and reverse 5'-CGTCAGCAACCTCGGGTTT-3', along with Taq DNA polymerase obtained from Thermo Fisher Scientific. Subsequently, the PCR products were analyzed by agarose gel electrophoresis in the

presence of SYBR green. Purification of PCR products was carried out using the Wizard® SV Gel and PCR Clean-Up System kit from Promega. Sanger sequencing was performed by Eurofins.

Stable transfection and western blot analysis

PAE cells were seeded in 6-well plates at 5.10^5 cells/well. The following day, cells were transfected with *PDGFRB* WT, variant or a control cloned in the pEF/myc/cyto vector. Cells were cultured in the presence of 2.5 mg/mL of G418 (Sigma-Aldrich) for one week. PDGFRβ-expressing cells were sorted by flow cytometry using anti-PDGFRβ (AH 17.2) monoclonal antibody [21], and a secondary antibody conjugated to phycoerythrin (#715-116-150, Jackson Immunoresearch, West Grove, PA, USA).

For western blot analysis, PAE cells stably expressing PDGFRβ were seeded in T25 flasks. Cells were washed with PBS the next day, starved for 6 h in serum-free medium, and stimulated with PDGF-BB (25 ng/mL) for 15 minutes at 37°C. Reactions were stopped by placing cells on ice, followed by cell lysis and preparation of lysates as described [19]. Membranes were visualized using the SuperSignal West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific). Membrane reprobing was performed after stripping in 0.4 M NaOH for 7.5 minutes.

Results

Description of patients

A two-month-old girl (**patient 1**) was admitted to hospital with clinical signs of protein-losing enteropathy without apparent cause. Rectal biopsies showed abnormalities, consisting of a fibroblastoid proliferation with wider separation of mucosal glands than normal (Supplementary Figure 1). The overall architecture remained intact. The fibroblastoid cells were arranged in small irregular bundles without cytological atypia and were compatible with a diagnosis of visceral localization of infantile myofibromatosis. At the same time, skin biopsies of the right hip lump were taken, showing a similar image of irregular bundles of spindle cells in the dermis, immunoreactive to smooth muscle actin, but not to desmin, further supporting the abovementioned diagnosis of infantile myofibromatosis. In addition, whole body MRI revealed asymptomatic bone and lung lesions. Analyses of the *PDGFRB* gene by Sanger sequencing of all coding exons and intron-exon boundaries revealed a germline heterozygous c.2905-8G>A variant of uncertain significance, which was located in the acceptor splice site of intron 21 and was absent from the gnomAD genome database. No variant was detected in *NOTCH3*. Tumor samples were not available for sequencing. She received methotrexate and vinblastine for one year. After four years, she remained stable with inactive residual lesions in the lungs and skin.

The younger brother of patient 1 was born at term after an uneventful pregnancy. At the age of two weeks, the parents noticed a painless lesion on the lower back. Whole body MRI evaluation revealed a T2 hyperintense lesion in the erector spinae muscle, presumed to be a myofibroma. Another T2 hyperintense lesion was seen in the spleen. DNA sequence analysis revealed that he carried the c.2905-8G>A heterozygous variant. No biopsies were taken of the suspected lesions. At most recent follow-up at the age of four months, the patient was growing and developing well. Repeated abdominal ultrasound evaluations did not show growth of the suspected lesions, or occurrence of new lesions.

The father of the patient had a mild form of infantile myofibromatosis: two lesions were surgically removed in childhood (upper arm and shoulder), and one small lesion is still present on the left ear. His retrieved pathology report confirmed a diagnosis of infantile myofibromatosis and he was shown to be heterozygous for the c.2905-8G>A variant.

We next searched for additional patients with infantile myofibromatosis carrying the same variant. Five cases were identified in different medical centers. Their clinical features and genetic data are summarized in Table 1 and Figure 1A.

Patient 2 is a male born at 33 weeks of gestation. Prenatal ultrasound had revealed fetal hypoechoic lungs and dilated loops of bowel. Following delivery, he required immediate resuscitation and intubation. He was transferred to the neonatal intensive care unit. The detailed course of the disease is provided as supplementary information. He had multiple subcutaneous nodules, lung opacities, lytic bone lesions and abdominal masses associated with intestinal obstruction, requiring surgery. Pathological analysis of small bowel biopsies was consistent with myofibromatosis. Genetic testing yielded a somatic *PDGFRB* c.2549A>T p.(Asp850Val) variant in exon 18 in addition to the germline heterozygous c.2905-8G>A variant, which was inherited from his asymptomatic mother. His treatment included methotrexate and vinblastine, initiated at day 10, and imatinib (6 mg/kg/day) initiated at day 48, based on previous reports showing effectiveness in IMF and after approval by the institutional review board. In a context of treatment failure with continued abdominal obstruction and high respiratory needs, imatinib was replaced by dasatinib (60 mg/m^2/day) at day 135 of life. After one month on dasatinib and chemotherapy, he was weaned off his respiratory support to high flow nasal cannula, his abdominal lesions and obstruction began to resolve, and his condition overall continued to improve. However, after 7 months of life, he developed severe pulmonary hypertension, with multiple potentially contributing factors, including dasatinib toxicity [22,23]. Pulmonary hypertension resolved after dasatinib discontinuation at day of life 232 (after three months of treatment). The patient's condition kept improving and, 7 months after stopping dasatinib, he received his final doses of IV chemotherapy. There have been no signs of disease progression, relapse, or new sites of disease since that time. He was completely weaned off home oxygen and, at last follow up, he was growing and developing well at 26 months of age.

Patient 3 was diagnosed with multifocal infantile myofibromatosis at the age of five months. She had multiple bone lesions with a left mandibular tumefaction, right parietal and left frontal sites, which had been developing since the age of one month with minimal functional impact. Her parents and two brothers (six and three years old, respectively) were healthy. No familial history of cancer was reported. A spontaneous reduction of the left mandibular mass

was observed. Management consisted of regular clinical monitoring every six weeks without treatment. Genetic analysis of the tumor revealed the splice site variant c.2905-8G>A in *PDGFRB*, with no second hit identified. This variant was subsequently detected in a normal blood DNA sample and heterozygosity was confirmed. She remained in complete remission 3.4 years after diagnosis.

Patient 4 had a lesion on the left hand, which was discovered a few weeks after birth, without functional impact. Histology revealed a proliferation of spindle-shaped cells with no sign of malignancy, suggesting a tumor of the myofibromatosis spectrum. Further workup revealed another lesion on the inferior border of the right gluteal head, as well as lesions in the skull. The evolution was clinically favorable with spontaneous regression without surgery or chemotherapy. Molecular analysis of the first nodule revealed the presence of c.2905-8G>A and an additional c.2546_2548del p.(Arg849_Asp850delinsHis) variant in *PDGFRB* exon 18. The intronic variant was confirmed as heterozygous germline. His father was 32 years old and had no particular medical history. His mother, aged 25, had a cervical paraganglioma *in situ*, in a context of familial predisposition linked to a constitutional pathogenic variant in the *SDHD* gene which was not transmitted to the patient. He had a 5-year-old brother who had undergone surgery in the neonatal period for a cervical lesion labelled as hamartoma, and who was being managed for local recurrence.

By reanalyzing our previously published data regarding 69 patients with myofibroma, including 19 with multicentric infantile myofibromatosis, we found the c.2905-8G>A variant in a tumor sample from **patient 5**, a male newborn with multicentric infantile myofibromatosis who had been included in our previous study following the identification of a *PDGFRB* c.2548G>T p.(Asp850Tyr) somatic variant [patient #50 in reference [5]]. The c.2905-8G>A variant was present in 54% of the reads obtained from the tumor sample, suggesting a germline modification. Normal DNA was not available from this archived case. Information regarding the evolution of his disease or his familial history was lacking.

Finally, we identified the same intronic variant in a recently reported male newborn with severe infantile myofibromatosis (**patient 6**), who was successfully treated with surgery and chemotherapy (vinblastine and methotrexate for 6 months) [24]. He received imatinib for one month. In addition to the germline variant c.2905-8G>A, which was also present in the father but not in the mother, sequencing of a tumor sample had revealed a *PDGFRB* p.(Asp850Val)

somatic variant [24]. Altogether, contrasting with previous IMF sequencing reports, the four somatic variants identified in our patient series were all located in exon 18.

The c.2905-8G>A variant affects PDGFRB splicing

The c.2905-8G>A variant was predicted to introduce a new acceptor splice site in intron 21 (Figure 1B). Alternative splicing using this new site would introduce six nucleotides in the *PDGFRB* transcript. To test this prediction, RNA was extracted from a frozen tumor sample from patient 3 and was analyzed by Illumina RNA sequencing. Figure 1C shows that about one in four reads contained the expected 6-nucleotide insertion between exons 21 and 22. To further demonstrate that c.2905-8G>A alone was sufficient to induce a change of splicing, we developed a minigene approach. Intron 21 was inserted into the *PDGFRB* cDNA cloned into an expression vector. This minigene was transiently transfected in human HEK 293T cells, which do not express endogenous *PDGFRB*. After RNA extraction, a 431 bp fragment spanning the junction between exons 21 and 22 was amplified by RT-PCR, followed by Sanger sequencing. Intron 21 was correctly spliced in cells transfected with the minigene (Figure 1D). Transfection of a construct including the variant intron resulted in the insertion of the expected 6 nucleotides, as demonstrated by Sanger sequencing (Figure 1D, right panel).

Functional characterization of the c.2905-8G>A variant receptor

The insertion of the GAGCAG sequence within the *PDGFRB* open reading frame results in the in-frame insertion of two amino-acid residues at the end of the kinase domain: p.(Lys968_Lys969insGluGln). We will refer to the variant receptor protein as ins969EQ. We first tested the impact of this variant on the receptor signaling, using a sensitive luciferase reporter assay [4]. In contrast to p.(Arg561Cys), the most common germline *PDGFRB* variant associated with IMF, ins969EQ did not induce any constitutive activation (Figure 2A). Instead, the variant response to stimulation by PDGF-B was much reduced, suggesting a partial loss of function. To further analyze the receptor activation, we introduced the variant receptor into porcine aortic endothelial cells, a classical model to study PDGF receptor signaling [25]. Western blot analysis revealed that the variant was expressed at a normal level, but was much less phosphorylated on tyrosines upon stimulation (Figure 2B). Phosphorylation of signaling

mediators such as phospholipase C γ (PLCγ) and AKT was also decreased, confirming the partial loss of function. Finally, we monitored the ligand-induced degradation of the receptor in the presence of cycloheximide. The variant had a slower degradation kinetics compared to the wild type (Supplementary Figure 2).

Interaction between ins969EQ and exon 18 somatic variants

In four patients, a second somatic hit was detected in *PDGFRB* exon 18, changing codon Asp850 in the activation loop of the kinase domain. Combined variants in *PDGFRB* on the same allele are frequent in IMF [4]. The p.(Asp850Val) variant was previously reported in myofibroma and activates the receptor constitutively [4]. According to a prediction by AlphaFold, this loop was located opposite to the alpha helix modified by the ins969EQ variant, indicating that these two segments did not make any direct contact (Figure 3) [26].

We next studied the effect of the exon 18 variants combined with ins969EQ in a luciferase reporter assay. First, our results showed that each exon 18 variant, namely p.(Asp850Val), p.(Asp850Tyr) and p.(Arg849_Asp850delinsHis), activated PDGFRβ constitutively when transfected alone in the 10T1/2 pericytic cell line (Figure 4A). Double variants were similarly activated, indicating that the exon 18 variants overcame the loss of function associated with ins969EQ. We also combined ins969EQ with other variants that are known to activate the receptor in myofibroma, namely c.1696T>C p.(Trp566Arg) (in the juxtamembrane domain encoded by exon 12) and p.(Asn666Lys) (in the kinase domain N-lobe) [4]. Surprisingly, these variants lost their constitutive activity when combined with ins969EQ (Figure 4A). Taken together, these results showed that the negative effect ins969EQ was counteracted by an exon 18 variant, but not with oncogenic variants located in the two main variant hotspots for IMF.

We had previously shown that the p.(Asp850Val) variant confers resistance to the tyrosine kinase inhibitor imatinib but remains sensitive to dasatinib with a published IC50 of 100 nM [4,13]. Similarly, p.(Asp850Tyr) and p.(Arg849_Asp850delinsHis) variants were only weakly inhibited by imatinib, but remained sensitive to dasatinib (Figure 4B-C). Imatinib was active against p.(Trp566Arg) and p.(Asn666Lys) receptors, used as positive controls. The combination with ins969EQ did not affect the sensitivity to TKI significantly.

Discussion

We identified a new heterozygous germline intronic *PDGFRB* c.2905-8G>A variant in eight patients with infantile myofibromatosis from six unrelated families. At the time of diagnosis, this variant was absent from human genome databases, including gnomAD. It was subsequently reported as a variant of uncertain significance in ClinVar. The c.2905-8G>A variant was also present in three healthy relatives, suggesting incomplete penetrance, which was previously observed in families with IMF [1,14]. Our data suggest that this may be the second most common germline pathogenic variant associated with infantile myofibromatosis, after c.1681C>T p.(Arg561Cys) in exon 12, which was identified in at least 15 families [1,11,12]. The other IMF-associated germline variants were reported only once. One patient of the present study was part of a previously published series of 19 IMF cases, suggesting a frequency of 5% [5]. Three other patients were identified in two genetic centers that have analyzed a total of 15 IMF cases since the introduction of *PDGFRB* testing (frequency of 20%, subject to multiple recruitment biases). Because of its intronic location, c.2905-8G>A was initially ignored in the molecular genetic report of several of our patients. Hence, we recommend to systematically include this variant in the genetic analysis of IMF cases.

We demonstrated that the c.2905-8G>A variant creates a functional alternative acceptor splice site in intron 21, leading to the observed insertion of six nucleotides in the *PDGFRB* messenger RNA, and the predicted addition of two residues in frame at the end of the kinase domain of the protein. Unlike other IMF-associated variants, the ins969EQ receptor did not show any constitutive activation, but a markedly reduced response to ligand stimulation, in several transfected cell lines, suggesting a partial loss of function.

The p.(Arg561Cys) germline variant is consistently associated with a second somatic hit in *PDGFRB*, most often p.(Asn666Lys) in exon 14 [5]. In our patients, the second hit was recurrently located at codon 850 in exon 18, which accounts for only 12% of *PDGFRB* variants associated with IMF [5]. We showed that exon 18 variants overcame the loss of function induced by ins969EQ, in contrast to exon 12 and 14 variants, suggesting positive selection. The constitutive activation of the double variant in our assays suggests that it is oncogenic and likely to cause myofibroma development in our patients. However, the exact mechanism whereby c.2905-8G>A favors a second hit and triggers myofibromatosis in patients remains to be clarified.

We demonstrated that Asp850 variants are fully resistant to imatinib in cellular assays, as opposed to the vast majority of *PDGFRB* variant receptors previously reported in infantile myofibromatosis and myeloid neoplasms [5,6]. In our patient series, based on previous reports suggesting imatinib efficacy in IMF [7,8,10], two patients with severe IMF had received imatinib without objective response. Patient 2 subsequently received dasatinib while continuing chemotherapy and supportive care, with significant improvement. Although this observation is encouraging, future studies are needed to confirm dasatinib efficiency. Our data suggested that Asp850 variants were sensitive to that TKI, at clinically relevant concentrations. Dasatinib was chosen based on its demonstrated safety profile and approved use in pediatric leukemia. Nevertheless, the administration of dasatinib was associated with pulmonary hypertension, which resolved upon withdrawal. Even though multiple other parameters could have favored pulmonary hypertension in this case, including lung alterations, this side effect should be carefully evaluated when administering dasatinib in IMF cases, especially in very young patients. Other TKI might be considered in the treatment of IMF in the future, such as avapritinib, which was recently approved for gastrointestinal stromal tumors carrying a *PDGFRA* (HGNC:8803) p.(Asp842Val) variant, which corresponds to *PDGFRB* p.(Asp850Val) [27].

Importantly, none of the patients had signs of Kosaki or Penttinen syndrome, which are associated with germline gain-of-function *PDGFRB* variants [15,16]. Features that appear later in life, such as blood vessel malformation or corneal alteration, were not systematically evaluated [1,28,29].

In conclusion, we demonstrated that the intronic c.2905-8G>A variant is strongly associated with familial infantile myofibromatosis, with a significant impact on disease treatment and genetic counselling. This is a unique example of a non-oncogenic germline variant that favors the occurrence of a secondary driver variant conferring resistance to targeted therapy.

Data Availability

We submitted the novel variants of *PDGFRB* to ClinVar with the accession numbers SCV005039001, SCV005039002 and SCV005046489. The remaining data of this study can be obtained upon request addressed to the corresponding author.

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Author Contributions

Conceptualization: B.B., M.F., S.S., J.M.P., J.B.D.; Data curation and Formal analysis B.B., M.F., C.L., A.M.; Funding acquisition: J.B.D.; Investigation: B.B., M.F., C.L., T.L., J.B., S.S., D.K. I.D.L., U.R.K., V.M.C., D.O., B.Br., R.D.K., J.M.P., J.B.D.; Methodology: B.B., M.F., J.M.P., J.B.D.; Resources: J.B., S.S., D.K., I.D.L., U.R.K., V.M.C., D.O., B.Br., R.D.K., J.M.P., J.B.D.; Supervision: J.M.P., J.B.D.; Writing - original draft: J.B.D.; and Writing - review & editing: all authors.

Ethics Declaration

This study was reviewed and approved by the *Comité d'éthique hospitalo-facultaire*, (UCLouvain, reference B403201732634). One de-identified sample was provided by the PALGA biobank [18] and had been reported in a prior publication [5]. Written informed consent was obtained from all other participants or their legal representatives. This research adheres to the principles set out in the Declaration of Helsinki.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary Figure 1 shows histological examination of patient biopsies.

Supplementary Figure 2 shows that the ins969EQ variant affects the receptor protein stability.

Supplementary Table 1 shows ACMG classification of *PDGFRB* variants and associated scores.

Supplementary data provides a detailed description of patient 2.

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FIGURES

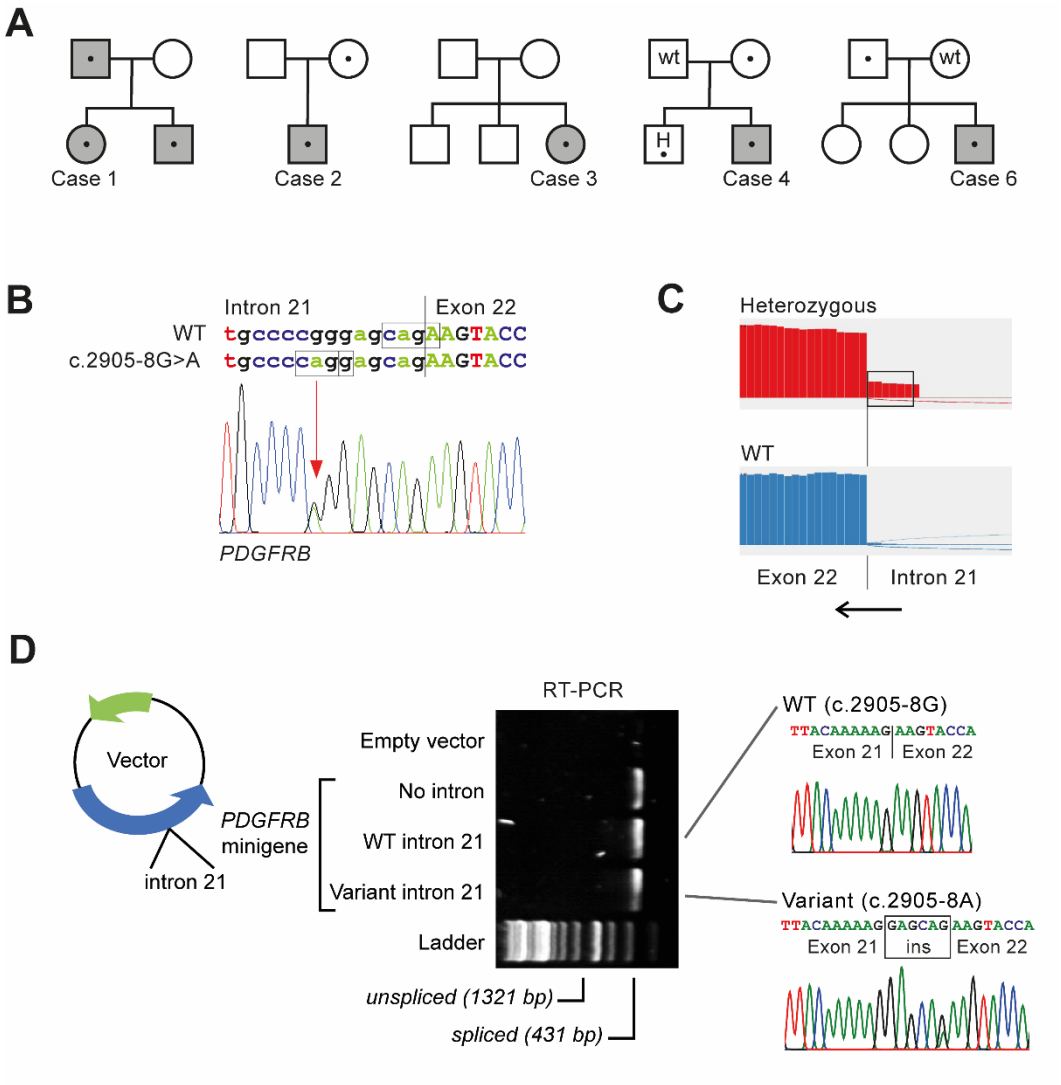

Figure 1. A *PDGFRB* splice site variant in familial infantile myofibromatosis

A. Pedigrees of IMF families. Patients with myofibroma are represented by grey symbols. Black dots indicate *PDGFRB* c.2905-8G>A variant heterozygotes, while "wt" denotes a patient who did not carry the variant. Other patients could not be sequenced. No information is available regarding case 5. H: patient diagnosed with a hamartoma.

B. Example of Sanger sequencing results after PCR amplification of a genomic DNA fragment spanning *PDGFRB* intron 21 and exon 22. The c.2905-8G>A variant creates a new acceptor splice site in intron 21. The red arrow points to the heterozygous variant nucleotide, and the rectangles emphasize the acceptor splice site.

C. RNA was extracted from a tumor sample from case 3 and sequenced. Alignment of the reads on the human genome (*PDGFRB* is located on the negative strand of chromosome 5), showed an insertion of six nucleotides at the junction between intron 21 and exon 22 (upper panel). The additional nucleotide shown on the figure belongs to exon 21. The lower panel shows the same region in a control sample (blue).

D. Schematic representation of the minigene constructs. Intron 21 was inserted in the *PDGFRB* cDNA cloned into the pcDNA3 vector. HEK293T cells were transfected with the empty vector, a vector containing the *PDGFRB* cDNA without intron, with wild-type intron 21 or mutated intron 21. Twenty-four hours after transfection, total RNA was extracted, converted to cDNA and amplified by PCR using oligonucleotides located in exons. PCR products were analyzed in an agarose gel (with a GeneRuler 1 kb DNA ladder from Thermo Fisher Scientific), purified and sequenced by the Sanger method, revealing a 6-nucleotide insertion in the transcript produced from the variant minigene.

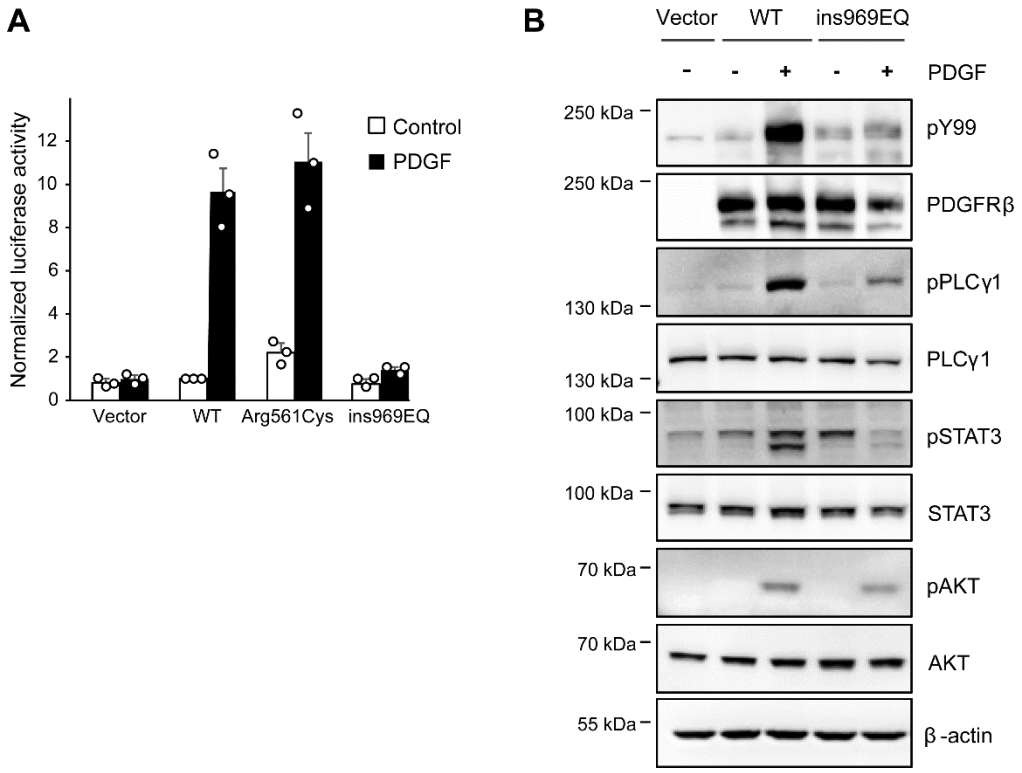

Figure 2. PDGFRβ ins969EQ variant shows a decreased response to PDGF stimulation

A. MCF7 cells were co-transfected with plasmids encoding PDGFRβ (WT or variant), a control β-galactosidase reporter, and a serum-responsive luciferase reporter. Cells were treated with 25 ng/mL PDGF-BB for 24 h. Values were normalized to the WT condition. The mean of three independent experiments is shown with SEM.

B. Western blot analysis was conducted on PAE cells stably transfected with PDGFRβ WT, ins969EQ, or an empty vector. Following a 6-hour starvation period, cells were stimulated for 15 minutes. Immunoblotting of total cell lysates was performed using indicated antibodies. One representative experiment out of three is shown.

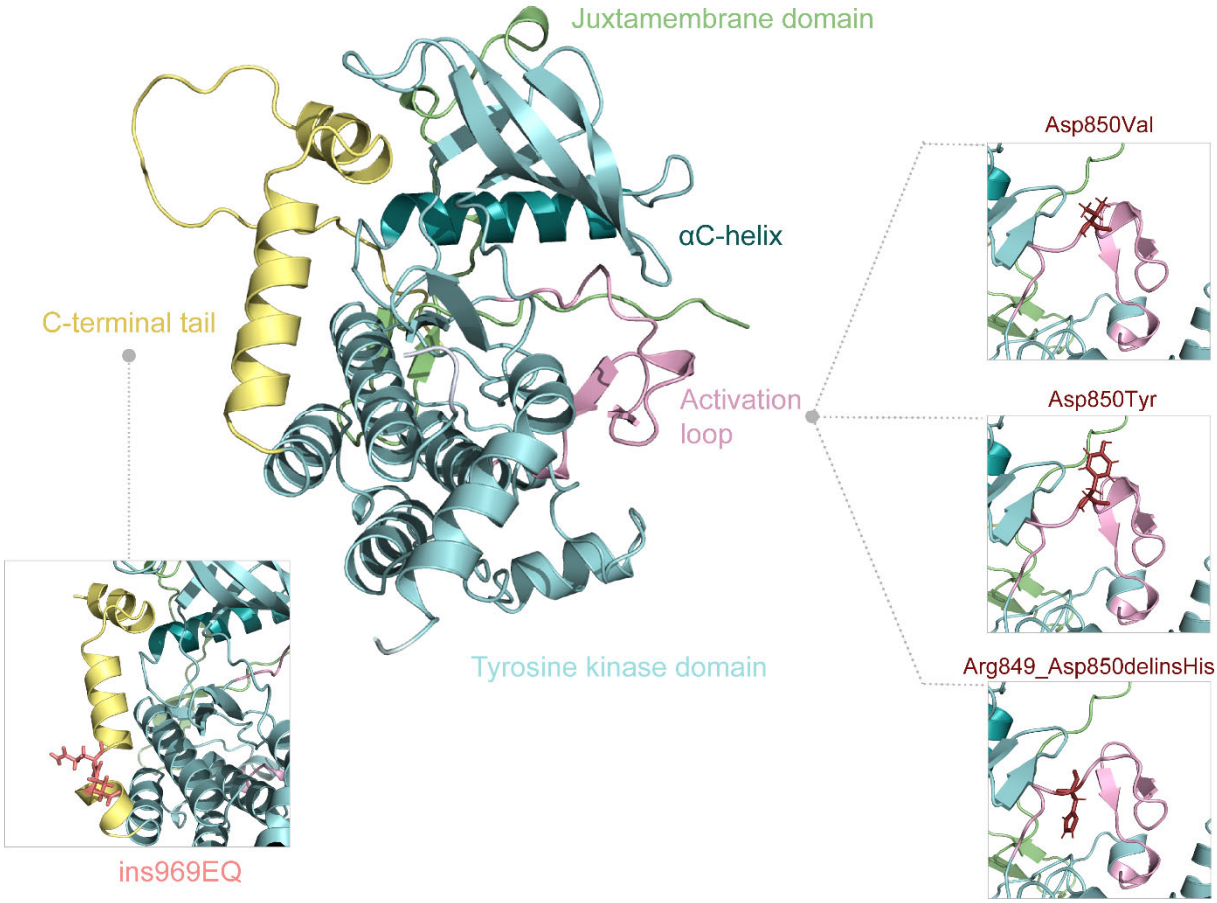

Figure 3. Position of the variants in the PDGFRβ tyrosine kinase domain structure.

AlphaFold v2.2.2-generated models displaying the PDGFRβ intracellular domain, highlighting the variants ins969EQ, p.(Arg849_Asp850delinsHis), p.(Asp850Val), and p.(Asp850Tyr). Structures were visualized using PyMol. The unstructured kinase insert and C-terminal region were removed.

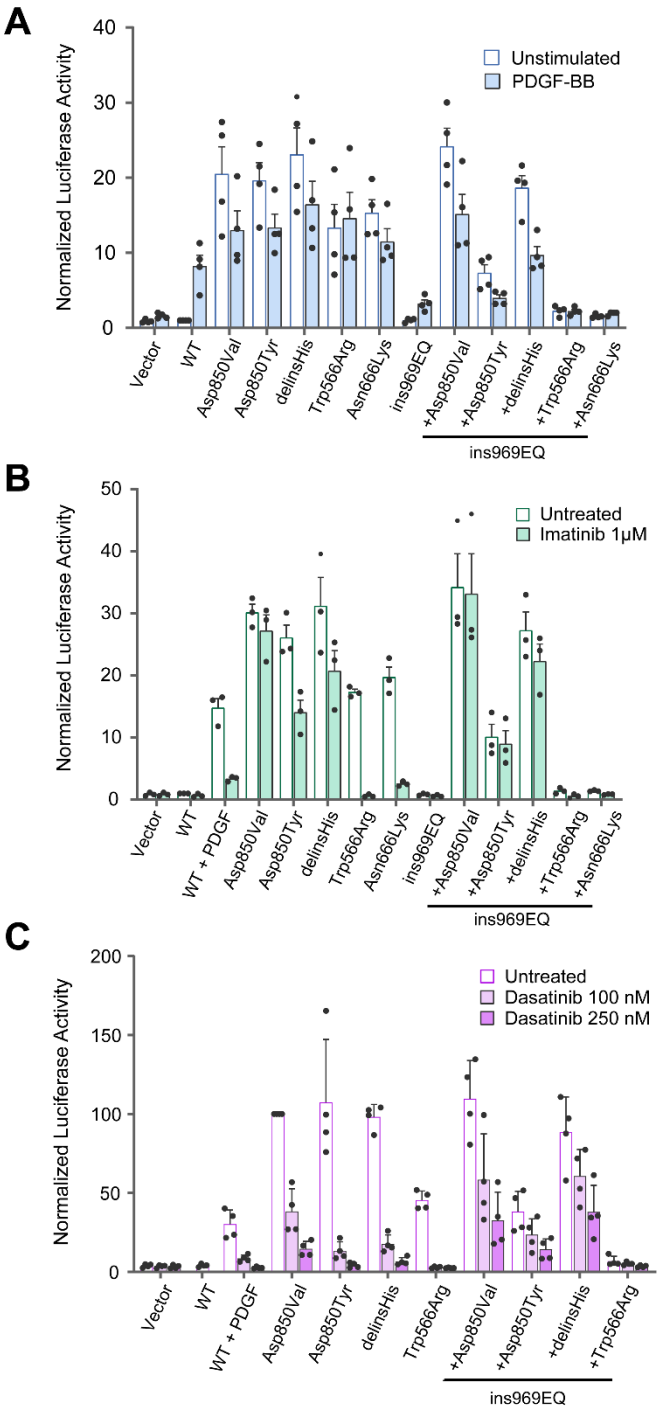

Figure 4. Impact of ins969EQ and exon 18 variants on sensitivity to tyrosine kinase inhibitors.

10T1/2 cells were co-transfected with an empty vector, *PDGFRB* WT or variant, along with a serum-responsive luciferase reporter. Double variants were inserted *in cis*. Cells were treated with 25 ng/mL PDGF-BB (A), imatinib (B), or dasatinib (C) at the indicated concentrations. Values were normalized to WT condition. Histograms depict mean from four (A and C) or three (B) independent experiments with SEM. DelinsHis refers to p.(Arg849_Asp850delinsHis).