Acta Med. Nagasaki 63: 49－53

A Mutation in PDGFRB in a Family with Infantile Myofibromatosis

Nobuhiro Ito, MD[1], Satoshi Watanabe, MD, PhD[1,2], Hiroyuki Mishima, DDS, PhD[2], Akira Kinoshita, PhD[2], Masahiko Okada, MD, PhD[1], Hiroyuki Moriuchi, MD, PhD[1], Koh-ichiro Yoshiura, MD, PhD[2]

[1] Department of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

[2] Department of Human Genetics, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan.

Infantile myofibromatosis (IM) is a benign fibrous tumor of infancy and childhood. A genome-wide linkage analysis and whole-exome sequencing were performed on a pedigree of familial cases, and a mutation in exon 12 of the gene for *platelet-derived growth factor receptor beta (PDGFRB)* (NM_002609), c.1681C>T p.R561C was identified. This is the first case in a Japanese pedigree, and we detected the mutation of IM in the family by whole-exome sequencing supported by a genome-wide linkage analysis. A wide spectrum of phenotypes was observed among the affected family members despite all having the same mutation. Recently, an additional mutation on the gene for receptor protein tyrosine phosphatase gamma (PTPRG), an enzyme dephosphorylating PDGFRB, was proposed to explain the full phenotypic penetrance in the affected family members with the *PDGFRB* mutation. However, it is still hypothesized that an additional *PDGFRB* mutation develops to full activation of PDGFRB in cells that have been primed by p.R561C. The pedigree in this study showed a wide spectrum of phenotypes, suggesting that a second hit, such as with other mutations contributing to PDGFRB phosphorylation, would be necessary to induce IM.

ACTA MEDICA NAGASAKIENSIA 63: 49－53, 2019

Key words: autosomal dominant inheritance, infantile myofibromatosis, PDGFRB, whole exome sequencing

---

Introduction

Infantile myofibromatosis (IM) is a benign fibrous tumor of infancy and childhood that was first described by Stout in 1954[1]. IM is usually apparent at birth or develops shortly thereafter, occurring in about 90% of cases before 2 years of age[2]. It is classified into two categories: solitary and multicentric IM. A single lesion arises in the cutaneous or subcutaneous tissues in solitary IM. Soft tissues, including skeletal muscles and bones, are involved in multicentric IM, and visceral locations are involved in 26-35% of cases[2,3]. IM can be either sporadic or familial with autosomal dominant inheritance[4,5]. Many mutations in *platelet-derived growth factor receptor beta (PDGFRB)* have been reported recently in IM patients independently[6-13].

We encountered a Japanese family with IM in which nine members were affected across four generations. Whole-exome sequencing (WES) was performed on this pedigree, and a known causative mutation was identified in exon 12 of the gene for *PDGFRB*, c.1681C>T p.R561C.

Materials and Methods

Subjects

A Japanese family consisting of 37 members across 4 generations included 9 patients with myofibromatosis (Figure 1). The index case developed severe refractory multicentric IM and was treated with vinblastine and methotrexate according to a previous study[14]. III-1 had solitary IM as a congenital splenic tumor. The rest of the affected cases

---

Address correspondence: M. Okada, Department of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki, 852-8501, Japan.

TEL: +81-95-819-7298, FAX: +81-95-819-7301, Email: masahikokada@mac.com

Received August 16, 2019; Accepted August 20, 2019

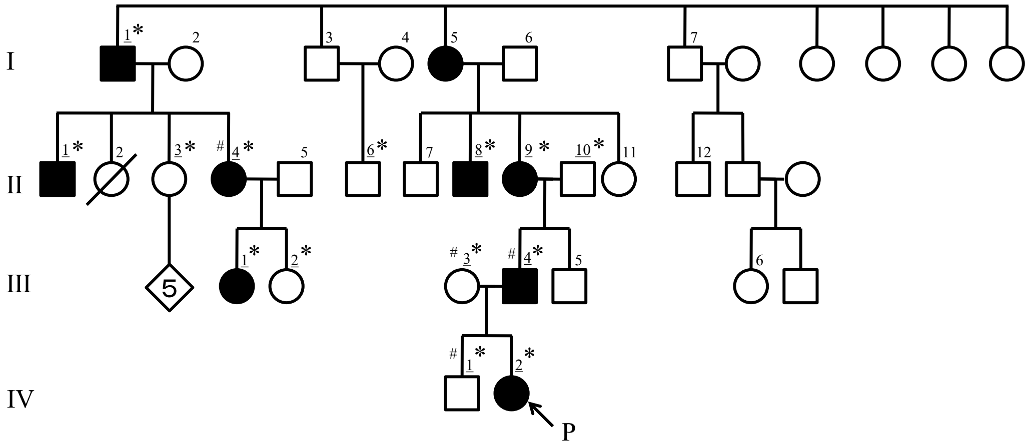

Figure 1. Pedigree chart.
Asterisks and pound symbols indicate family members who underwent linkage analyses and whole-exome sequencing analyses, respectively.

developed recurrent solitary myofibroma. Unaffected members in the pedigree had no symptoms or signs of myofibromatosis or other tumors based on interviews. A total of 14 members participated in this study after providing their written informed consent (Figure 1).

All experimental procedures in this study have been approved by the Institutional Review Board for Ethical, Legal, and Social Issues of Nagasaki University.

DNA extraction and linkage analyses

Blood samples were obtained from all of the participants. DNA extraction was performed by using QIAamp® DNA Mini Kit (Qiagen, Düsseldorf, Germany) according to the manufacturer's protocol. Genotyping data for linkage analyses were obtained using the GeneChip Mapping 10K Xba Assay Kit (Affimetrix, Santa Clara, CA, USA). DNA (250 ng) was processed according to the standard protocol provided by the Affymetrix and the genotype was identified using the Affymetrix GeneChip Genotyping Analysis Software Program (GTYPE) 4.0. Genotype information was converted by in-house Ruby scripts and analyzed by using the MERLIN Software Package version 1.1.2 (Instron, Norwood, MA, USA)[16]. As a dominant model used for the parametric linkage analysis, the disease allele frequency was 0.001 and the penetrances for 0-, 1-, and 2-copies were 0.0001, 1.0, and 1.0, respectively.

WES analyses

WES was performed on four members of the family in this study (marked with '#' on the pedigree chart), consisting of two affected (II-4, III-4) and two unaffected members (III-3, IV-1). Genomic DNA (3 μg) was subjected to exon enrichment using the SureSelect® Human All Exon V4 (Agilent, Santa Clara, CA, USA). The exon enrichment was performed according to the manufacturer's protocol except for the use of KAPA HiFi DNA polymerase. A SOLiD 5500xl system (Life Technologies, Carlsbad, CA, USA) was used for sequencing. Emulsion polymerase chain reaction (PCR) was performed with non-labeled Library PCR Primer 1 (5'-CCACTACGCCTCCGCTTTCCTCTCTATG-3') and biotin-labeled Library PCR Primer 2 (5'-CTGCCCCGGGTTCCTCATTCT-3') using KAPA HiFi DNA polymerase. The emulsion was broken with butanol, and P1-beads coated with clonally amplified DNA were enriched manually using Capture Beads and an enrichment column supplied by Life Technologies. The raw data was analyzed by using the Novoalign CSMPI software package [http://nobocraft.com/], the Picard tools [http://picard.sourceforge.net], the Genome Analysis Toolkit

Table 1.

		II-4	III-3	III-4	IV-1
mean depth		37.66x	45.04x	24.24x	31.72x
coverage	5x	95.6%	97.1%	92.1%	94.7%
	10x	87.5%	91.8%	78.5%	85.4%
	15x	77.1%	84.8%	63.7%	74.1%

(GATK) version 2.4 (Broad Institute of MIT and Harvard, Cambridge, MA, USA)[15,16], and the ANNOVAR software package (University of Pennsylvania, PA, USA)[17].

Results

Linkage analyses

The mean SNP call rate was 98.98%, which was sufficient for further linkage mapping in 14 DNA samples. We therefore proceeded to calculate the LOD scores using these data. The linkage data using the MERLIN software program revealed 3 loci to have LOD scores over 2.00: 5q23.1-31.2, 6p12.3-p22.2 and 19q13.11-q13.13 (Figure 2). The physical positions of these loci in the GRCh37/hg19 reference genome were chr5: 1170282357-140298815, chr6: 24963255-46626353, and chr19: 34334857-38494504, respectively. The LOD score was not high enough to confirm conclusive linkage but was theoretically the highest as far as we used 14 samples in the family.

WES analyses

Nineteen variants, including 15 single nucleotide variants (SNVs) and 4 indels, were identified as candidate mutations for myofibromatosis. Five variants, including 4 SNVs and one indel, were included in the regions identified on the linkage analysis. Finally, two variants completely segregated with affection status in the family. Of the two, PDGFRB, c.1681C>T p.R561C in exon12 of PDGFRB (NM_002609) was deemed likely causative.

Discussion

We identified the causative mutation and gene responsible for familial IM by a WES analysis and linkage analysis. The mutation in exon12 of PDGFRB (NM_002609), c.1681C>T p.R561C, was reported by two groups simultaneously in 2013[6, 7] and another group in the following year[8-13]. Based on the fact that the same mutation was identified by independent groups, it is likely that the amino acid p.561R is important

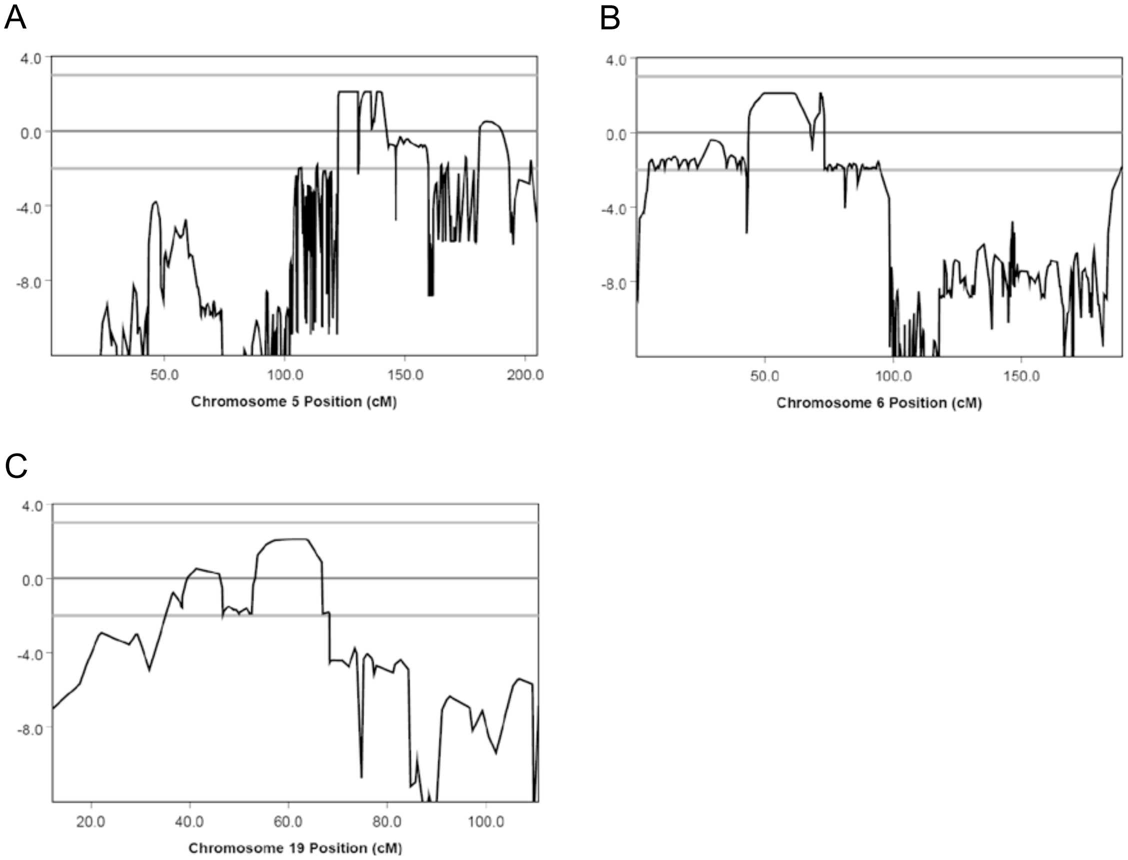

Figure 2. Linkage analysis plot.
Multipoint LOD scores calculated by MERLIN in three chromosomal regions (A: 5q23.1-31.2, B: 6p12.3-p22.2 and C: 19q13.11-q13.13) are shown.

for regulating the kinase signal transduction from PDGFRB. All affected patients of this pedigree had a germline mutation in *PDGFRB*, but their myofibroma lesions were formed in various tissues at various ages. Incomplete penetrance has also been recognized in previously reported families with an autosomal dominant form of IM[8], suggesting that the p.561R mutation alone is not sufficient to cause myofibroma and interaction with some other factors is necessary for tumorigenesis.

PDGFRB mutations have been reported to have a causal influence on other clinical entities. Takenouchi et al. reported two cases of novel overgrowth syndrome that had a mutation in exon 12 of *PDGFRB* c.1751C>G or p.P584R. Pro584 embedded in the zipper or linker peptide segment within the juxtamembrane domain of PDGFRB. One of the two patients also developed myofibroma[18]. Subsequently, they reported the identification of a mutation in *PDGFRB*, c.1696T>C p.Trp566Arg, in two unrelated patients with skeletal overgrowth, further confirming the existence of PDGFRB - related overgrowth syndrome arising from mutations in the juxtamembrane domain of PDGFRB[19]. In addition, Nicolas et al. identified three causative genes of idiopathic basal ganglia calcification (IBGC): *SLC20A2, PDGFRB*, and *PDGFB*; the latter is a nonsense mutation in exon 4 of *PDGFB*, c.439C>T p.Q147X, and loss of function of PDGFB, the main ligand of PDGFRB, has been shown to cause brain calcification through a disruption of the blood-brain barrier integrity in mice[20]. Therefore, it is likely that the loss of signal transduction from PDGFB also leads to basal ganglia calcification in human[21]. A third example is Penttinen syndrome, a distinctive disorder characterized by a prematurely aged appearance with lipoatrophy, epidermal and dermal atrophy, thin hair, underdeveloped cheekbones, and marked acro-osteolysis[21]. Although only sporadic cases have been reported previously, three unrelated patients with Penttinen syndrome had the same *de novo* mutation of PDGFRB c.1994T>C p.V665A. The clinical findings in Penttinen syndrome do not overlap with those of IM, IBGC, and Takenouchi-Kosaki overgrowth syndrome. PDGFRB seems to have multiple functions that vary with tissues.

While the gain of function of PDGFRB greatly contributes to the development of IM[9], the mechanism underlying tumorigenesis remains unknown. Linhares et al[8] recently showed that not only two siblings with IM but also their unaffected mother had the same *PDGFRB* mutation, and that both of the affected children also had a mutation in the gene for receptor protein tyrosine phosphatase gamma (PTPRG), an enzyme dephosphorylating PDGFRB, from their unaffected father. Therefore, the additional PTPRG variation may explain the full phenotypic penetrance in the affected siblings. In addition to *PDGFRB*, mutations in *NOTCH3* and *PTPRG*, both of which are involved in the PDGFR signaling pathway[7,8,12,22], have been reported in autosomal dominant IM[7,8]. However, Arts et al.[9] performed targeted sequencing in 16 cases of solitary myofibroma or multicentric myofibromatosis, and identified *PDGFRB* mutations in 6 out of 8 patients with multicentric myofibromatosis and one 1 of 8 patients with isolated myofibroma. One sporadic case of multicentric myofibromatosis had the same somatic mutation of *PDGFRB* c.1681C>T p.R561C as in our study, accounting for 47% of all alleles, and a second somatic mutation of c.1998C>A p. N666K, accounting for 6% of the reads. Since a receptor with both p.R561C and p.N666K mutations was reported constitutively phosphorylated *in vitro* in transfection experiment[9], it is hypothesized that p.N666K may represent a second hit necessary for achieving the full activation of PDGFRB in cells that have been primed by p.R561C.

Given these findings, we hypothesize that the excess phosphorylation of PDGFRB activates the PDGFRB signal pathway and contributes to tumorigenesis. The affected patients in the present study developed myofibroma in various organs at different times despite having the same mutation with *PDGFRB*. Therefore, there may exist some other variants in addition to the *PDGFRB* mutation responsible for such phenotypic heterogeneity. Although we found no other mutations in the *PTPRG* and *NOTCH3* genes or additional mutations of *PDGFRB* in peripheral blood mononuclear cells, further experiments with tumor cells derived from those patients are required to reveal the mechanisms inducing tumorigenesis of PDGFRB-mutated cells.

Multicentric IM is often refractory to various treatment regimens and life threatening, as in the index case in this study. Establishing a first-line therapy is therefore a matter of great urgency. In this regard, identifying the gene mutations responsible for this disease and better understanding the molecular mechanisms underlying the development of IM will provide us with promising novel therapeutic strategies. PDGF ligands and receptors are oncogenic signaling components that can be activated by various types of genetic alterations in cancer cells. Tyrosine kinase inhibitors, such as imatinib, an ATP analog designed to block the kinase activity of the BCR-ABL oncogene in leukemia, was found to inhibit PDGFRs by binding to the inactive receptor with high affinity. Imatinib has been used successfully to treat gastrointestinal stromal tumors, dermatofibrosarcoma protuberans, and myeloid malignancies associated with PDGFR fusions[10,11]. The mutation identified in *PDGFRB* (NM_002609), c.1681C>T or p.R561C, is situated in the cytoplasmic juxtamembrane domain.

Therefore, imatinib, which can bind to the cytoplasmic kinase domain of PDGFRB, may be effective for treating severe-type IM[23]. Sunitinib and other tyrosine kinase inhibitors have been shown to be promising agents for IM with a p. R561C mutation in PDGFRB in a cell line derived from an IM tumor[24].

In conclusion, we reported the first Japanese pedigree with a p.R561C germ-line mutation in *PDGFRB*. The same mutation has previously been identified in patients with IM of various racial backgrounds. The pedigree in this study showed a wide spectrum of phenotypes, suggesting that a second hit that contributes to PDGFRB phosphorylation would be necessary to induce the development of IM.

Competing Interests

The authors declare no conflict of interest.

References

1. Stout AP. Juvenile fibromatoses. *Cancer.* 1954; **7**: 953-978.
2. Chung EB, Enzinger FM. Infantile myofibromatosis. *Cancer.* 1981; **48**: 1807-1818.
3. Sirvent N, Perrin C, Lacour J-P, Maire G, Attias R, Pedeutour F. Monosomy 9q and trisomy 16q in a case of congenital solitary infantile myofibromatosis. *Virchows Arch.* 2004; **445**: 537-540.
4. Zand DJ, Huff D, Everman D, Russell K, Saitta S, McDonald-McGinn D et al. Autosomal dominant inheritance of infantile myofibromatosis. *Am J Med Genet A*. 2004; **126**: 261-266.
5. Ikediobi NI, Iyengar V, Hwang L, Collins WE, Metry DW. Infantile myofibromatosis: support for autosomal dominant inheritance. *J Am Acad Dermatol*. 2003; **49**:148-150.
6. Cheung YH, Gayden T, Campeau PM et al. A recurrent PDGFRB mutation causes familial infantile myofibromatosis. *Am J Hum Genet*. 2013; **92**: 996-1000.
7. Martignetti JA, Tian L, Li D, Ramirez MC, Camacho-Vanegas O, Camacho SC et al. Mutations in PDGFRB cause autosomal-dominant infantile myofibromatosis. *Am J Hum Genet*. 2013; **92**: 1001-1007.
8. Linhares ND, Freire MCM, Cardenas RGCCL, Bahia M, Puzenat E, Aubin F et al. Modulation of expressivity in PDGFRB-related infantile myofibromatosis: a role for PTPRG? *Genet Mol Res*. 2014; **13**: 6287-6292.
9. Arts FA, Sciot R, Brichard B, Renard M, de Rocca Serra A, Dachy G et al. PDGFRB gain-of-function mutations in sporadic infantile myofibromatosis. *Hum Mol Genet*. 2017; **26**: 1801-1810.
10. Mudry P, Slaby O, Neradil J, Soukalova J, Melicharkova K, Rohleder O et al. Case report: rapid and durable response to PDGFR targeted therapy in a child with refractory multiple infantile myofibromatosis and a heterozygous germline mutation of the PDGFRB gene. *BMC Cancer*. 2017; **17**: 119-125.
11. Murray N, Hanna B, Grraf N, Fu H, Mylène V, Campeau PM et al. The spectrum of infantile myofibromatosis includes both non-penetrance and adult recurrence. *Eur J Med Genet*. 2017; **60**: 353-358.
12. Lepelletier C, Al-Sarraj Y, Bodemer C, Shaath H, Fraitag S, Kambouris M et al. Heterozygous PDGFRB mutation in a three-generation family with autosomal dominant infantile myofibromatosis. *Acta Derm Venereol*. 2017; **97**: 858-859.
13. Pond D, Arts FA, Mendelsohn NJ, Demoulin JB, Scharer G, Messinger Y. A patient with germ-line gain-of-function PDGFRB p.N666H mutation and marked clinical response to imatinib. *Genet Med*. 2018; **20**: 142-150.
14. Azzam R, Abboud M, Muwakkit S, Khoury N, Saab R. First-line therapy of generalized infantile myofibromatosis with low-dose vinblastine and methotrexate. *Pediatr Blood Cancer*. 2009; **52**: 308.
15. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010; **20**: 1297-1303. doi:10.1101/gr.107524.110.
16. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C et al. A Framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet*. 2011; **43**: 391-398. doi:10.1038/ng.806.
17. Wang K, Li M, Hakonarson H. ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010; **38**: e164. doi:10.1093/nar/gkq603.
18. Takenouchi T, Yamaguchi Y, Tanikawa A, Kosaki R, Okano H, Kosaki K. Novel overgrowth syndrome phenotype due to recurrent de novo PDGFRB mutation. *J Pediatr*. 2015; **166**: 483-486.
19. Minatogawa M, Takenouchi T, Tsuyusaki Y, Iwasaki F, Uehara T, Kurosawa K, Kosaki K, Curry CJ. Expansion of the phenotype of Kosaki overgrowth syndrome. *Am J Med Genet A*. 2017; **173**: 2422-2427.
20. Nicolas G, Jacquin A, Thauvin-Robinet C, Rovelet-Lecrux A, Rouaud O, Pottier C et al. A de novo nonsense PDGFB mutation causing idiopathic basal ganglia calcification with laryngeal dystonia. *Eur J Hum Genet*. 2014; **22**: 1236-1238.
21. Johnston JJ, Sanchez-Contreras MY, Keppler-Noreuil KM, Sapp J, Crenshaw M, Finch NA et al. A point mutation in PDGFRB causes autosomal-dominant Penttinen syndrome. *Am J Hum Genet*. 2015; **97**: 465-474.
22. Demoulin JB, Essaghir A. PDGF receptor signaling networks in normal and cancer cells. *Cytokine Growth F R*. 2014; **25**: 273-283.
23. Apperley JF, Gardembas M, Melo JV, Russell-Jones R, Bain BJ, Baxter EJ et al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. *N Engl J Med*. 2002; **347**: 481-487.
24. Sramek M, Neradil J, Macigova P, Mudry P, Polaskova K, Slaby O et al. Effects of sunitinib and other kinase inhibitors on cells harboring a PDGFRB mutation associated with infantile myofibromatosis. *Int J Mol Sci*. 2018; **19**: 2599; doi:10.3390/ijms19092599