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Abstract

Cherubism is a rare developmental lesion of the jaw that is generally inherited as an autosomal dominant trait. Recent studies have revealed point mutations in the SH3BP2 gene in cherubism patients. In this study, we examined a 6-year-old Korean boy and his family. We found a Pro418Arg mutation in the SH3BP2 gene of the patient and his mother. A father and his 30-month-old younger brother had no mutations. Immunohistochemically, the multinucleated giant cells proved positive for CD68 and tartrate-resistant acid phosphatase (TRAP). Numerous spindle-shaped stromal cells expressed a ligand for receptor activator of nuclear factor kB (RANKL), but not in multinucleated giant cells. These results provide evidence that RANKL plays a critical role in the differentiation of osteoclast precursor cells to multinucleated giant cells in cherubism. Additionally, genetic analysis may be a useful method for differentiation of cherubism.

KEYWORDS: cherubism, SH3BP2, CD68, TRAP, RANKL

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Case Report

Investigation of the SH3BP2 Gene Mutation in Cherubism

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Cherubism is a rare developmental lesion of the jaw that is generally inherited as an autosomal dominant trait. Recent studies have revealed point mutations in the SH3BP2 gene in cherubism patients. In this study, we examined a 6-year-old Korean boy and his family. We found a Pro418Arg mutation in the SH3BP2 gene of the patient and his mother. A father and his 30-month-old younger brother had no mutations. Immunohistochemically, the multinucleated giant cells proved positive for CD68 and tartrate-resistant acid phosphatase (TRAP). Numerous spindle-shaped stromal cells expressed a ligand for receptor activator of nuclear factor kB (RANKL), but not in multinucleated giant cells. These results provide evidence that RANKL plays a critical role in the differentiation of osteoclast precursor cells to multinucleated giant cells in cherubism. Additionally, genetic analysis may be a useful method for differentiation of cherubism.

Key words: cherubism, SH3BP2, CD68, TRAP, RANKL

Cherubism is a rare developmental lesion of the jaw that is generally inherited as an autosomal dominant trait. It is characterized by a proliferation of fibrous tissue and a large number of giant cells. Cherubism was first described as 'familial multilocular cystic lesion of the jaw' by Jones in 1933 [1]. It affects all 4 quadrants of the jaw that cause a characteristic round and symmetrically full face in most patients. Cherubism is usually found in children by 5 years of age, and the affected mandible and maxilla begin to swell. The clinical alterations typically progress until puberty, then stabilize and slowly regress [2, 3].

In the latest genetic research, the gene responsible for cherubism was discovered. It is mapped to chromosome 4p16.3 and named SH3BP2 (SH3-binding protein) [2]. Point mutations in the SH3BP2 gene were demonstrated in 12 of 15 families by Ueki *et al.* in 2001 [4]. All the mutations identified so far are located in exon 9 and result in amino acid substitutions within a 6-amino acid sequence from position 415 to 420 [2, 4-7]. However, recently, Carvalho *et al.* described a novel mutation in exon 3 of the SH3BP2 in an aggressive case of cherubism [8]. In addition, SH3BP2 is rarely mutated in exon 9 in giant cell lesions outside cherubism, which means that cherubism is a separate entity [9, 10]. And recently, Ueki *et al.* have reported that SH3BP2 is a critical regulator of myeloid cell responses to M-CSF and RANKL stimulation using a mouse model, and have suggested why the SH3BP2 missense mutation causes bone loss and inflammation in the patient [11].

In this article, we investigated mutational analysis of the SH3BP2 gene, including CD68, tartrate-resis-

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tant acid phosphatase (TRAP), and a ligand for receptor activator of nuclear factor kB (RANKL) immunohistochemistry in a Korean child with cherubism.

Case Report

A 6-year-old Korean boy was referred to our clinic because of his symmetrical painless swelling of mandible and maxilla. At first, his parents noticed the full, round lower contour of his face. Physical and mental development was normal. No exophthalmos was found and his visual activity was normal. Family history was absent, and no other family members had characteristic features clinically.

Oral examination disclosed no gross abnormalities except fusion of the central and lateral incisor on the right side of the mandible. Panoramic radiography demonstrated multiple multilocular cystic spaces in the bilateral mandible and maxilla. The mandibular lesions were extended to the angles and ascending ramus, but the condyles were not affected. Three-dimensional (3D) CT imaging revealed bilateral multilocular cystic lesions in the maxilla and mandible that had a soap bubble appearance (Fig. 1).

A microscopic examination of the incisional biopsy specimen revealed numerous multinucleated giant cells together with spindle-shaped fibroblastic cells within a fine fibrillar collagenous stroma. Multinucleated giant cells were scattered randomly throughout the cellular and fibrous tissue (Fig. 2A).

To elucidate the origin of the multinucleated giant cells, we performed immunohistochemical analysis. CD68 (clone PG-M1, Transduction Labaratories, Lexinton, KY, USA), TRAP (clone 26E5, Biogenex, San Ramon, CA, USA) and RANKL (clone 70525, R&D System, Minneapolis, MN, USA) antibody were used as the primary antibodies. The sections were stained using the avidin-biotin complex (ABC) technique with monoclonal antibodies. Antigen retrieval was performed using a microwave oven for 20 min (CD68, TRAP) or autoclave for 5 min (TRAP) before exposure to the primary antibody. The peroxidase reaction was developed using diaminobentine. A Meyer's hematoxylin was used as a counterstain. As a result, the multinucleated giant cells in the lesion proved positive for CD68 marker (Fig. 2B). Some of the mononuclear cells of the lesion also exhibited a mild immunoreactivity. In addition, TRAP activity was also consistently detected in the multinucleated giant cells. They contained brownish intracytoplasmic granules (Fig. 2C). Numerous spindle-shaped stromal cells expressed a ligand for RANKL antibody, but not in multinucleated giant cells (Fig. 2D).

Blood samples were obtained from the patient and his younger brother, his parents with their written informed consent for DNA analysis. Genomic DNA was extracted from blood using an AccuPrep® Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). We analyzed exon 9 because the mutation hotspot is located in this region [4]. We used the polymerase chain reaction (PCR) to amplify exon 9 of the SH3BP2 gene. Primers for the PCR experiments were designed based on the SH3BP2 gene sequence. Samples were prepared in a final volume of 50 μl that contained sense (5'-AGGGGAGCAGAGGGTGG-3') and antisense (5'-GGGACACAGAAGCAGGAAG-3') primers and Taq polymerase (TaKaRa, Japan) under a standard buffer condition. The amplification conditions of the fragments were as follows: after an initial denaturation at 94℃ for 5 min, samples were amplified by 35 cycles at 94℃ for 30 sec, 60℃ for 30 sec, 72℃ for 1 min, followed by a final extension at 72℃ for 10 min, and hold at 4℃. PCR products were electrophoresed in a 1.5% (w/v) agarose gel, visualized under UV light with ethidium bromide staining and purified using an AccuPrep® PCR Purification Kit (Bioneer, Daejeon, Korea). Isolated PCR products were sequenced on an Applied Biosystems 310 Genetic Analyzer (Applied Biosystems Inc., Foster

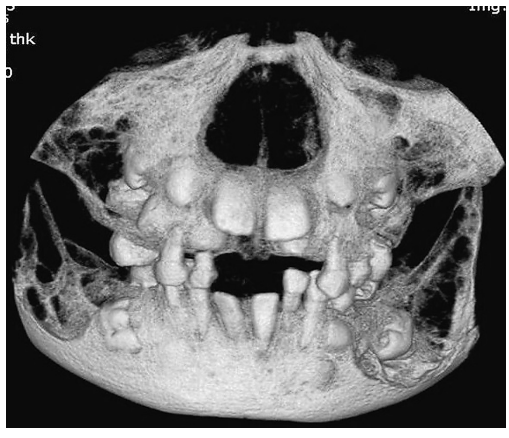

Fig. 1 The three-dimensional CT image shows bilateral multilocular cystic lesions in the maxilla and mandible.

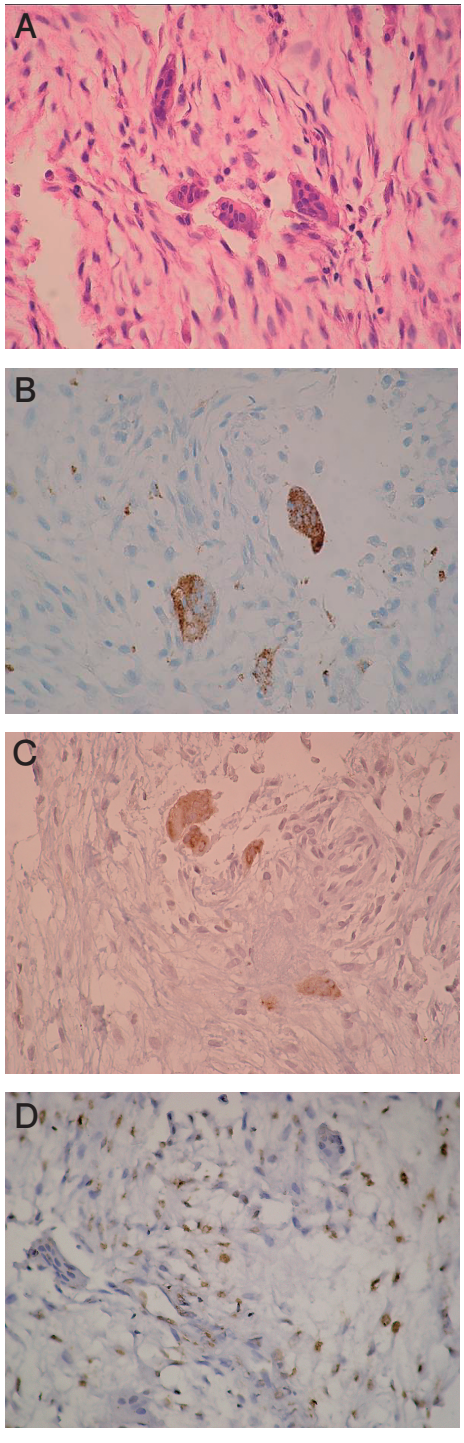

Fig. 2 Microscopic findings showed numerous multinucleated giant cells together with spindle-shaped fibroblastic cells within a collagenous stroma (A). Multinucleated giant cells proved positive for both CD68 (B) and TRAP (C). Numerous spindle-shaped stromal cells expressed a ligand for RANKL antibody, but not in multinucleated giant cells (D).

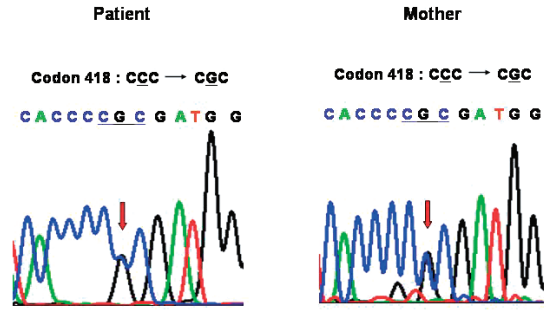

Fig. 3 DNA sequencing chromatogram of the patient and his mother shows a missense mutation at codon 418 (CCC->CGC), creating an amino acid change from proline to arginine. The arrow indicates the missense mutation.

City, CA, USA). After sequencing of genomic DNA, we found a missense mutation Pro418Arg in exon 9 of the SH3BP2 gene of the patient and that of his mother (Fig. 3A, B). His father and younger brother did not have this mutation.

Discussion

Though the presence of numerous small vessels with large endothelial cells and perivascular cuffings has previously been reported, only a small number of blood vessels were found in the present case and there was no hemosiderin deposition. These results made it difficult to make a diagnosis of cherubism. Recent genetic advances have been made in relation to cherubism with the identification of the gene SH3BP2 [2, 5]. SH3BP2 was initially identified as a protein that can bind to c-Abl via its SH3-binding domain. It is expressed in a number of tissues and considered to be a proliferative signal-transducing protein [4]. SH3BP2 has 13 exons, but all mutations of SH3BP2 reported as yet in patients with cherubism occur within a 6-amino acid region (amino acid 415-420) encoded by exon 9 [2, 4-7]. Recent study has revealed that there are no mutations in the SH3BP2 gene in patients with aggressive central giant cell granuloma [9, 10]. These results indicate that cherubism is a distinct entity, and we can use this genetic analysis for specific diagnosis of cherubism.

The mutation as described here (Pro418Arg) is a well-known mutation hotspot at amino acid numbers 415 to 420, allowing us to clearly diagnose the patient with cherubism, not central giant cell granuloma. To our knowledge, this is the second proven SH3BP2 gene mutation case of cherubism in Asian populations [5]. Though both cases had this point mutation, the patient in the present study showed clinical features of cherubism, but his mother didn't show the phenotype. The reason we assumed a diagnosis of cherubism is that the penetrance of cherubism is higher in men (100%) than women (50–70%) [2, 3, 5].

Exactly how mutations in SH3BP2 lead to cherubism is not yet known. The mutation of the gene may interfere with signaling. SH3BP2 binds to a variety of cytosolic proteins, including LAT, Syk, and 14–3–3, and it is an integral component of a signaling pathway that activates NFAT (nuclear factor of activated T cell) [12, 13]. A recent study has demonstrated that this mutation increase NFAT activity [14]. NFAT has been proposed to be the master transcription factor for osteoclastogenesis, and experimental evident supports a critical role for the protein in osteoclast development [10, 14]. We therefore believe that this mutation in our patient may reinforce the signaling pathways that lead to the development and activation of the osteoclast.

There have been some indications that multinucleated giant cells have an osteoclastic nature [4, 5]. According to a recent study of the mouse model of cherubism by Ueki *et al.* [14], the SH3BP2 mutation is regarded to cause increased myeloid cell responses to M-CSF and RANKL stimulation, and that these cells form macrophages with high levels of TNF-α and large osteoclasts. Similarly, in this study, these multinucleated cells stained positive for CD68 and TRAP. Numerous spindle-shaped stromal cells expressed a ligand for RANKL, but not in multinucleated giant cells. Based on these findings and those of others, we suggest that multinucleated giant cells in the lesion may be formed from monocyte/macrophage precursors. These precursor cells may differentiate into osteoclasts under the influence of RANKL-expressing stromal cells, so they are believed to be osteoclastic in nature.

In summary, we detected the point mutation Pro418Arg in the SH3BP2 gene in our patient and demonstrated that genetic analysis is a useful method in the identification of cherubism. Further investigations are required to determine how this mutation leads to cherubism.

References

1. Jones WA: Familial multilocular cystic disease of the jaws. Am J Cancer (1933) 17: 946–-950.
2. Mangion J, Rahman N, Edkins S, Barfoot R, Nguyen T, Sigurdsson A, Townend JV, Fitzpatrick DR, Flanagan AM, Stratton MR: The gene for cherubism maps to chromosome 4p16.3. Am J Hum Genet (1999) 65: 151–157.
3. Meng XM, Yu SF and Yu GY: Clinicopathologic study of 24 cases of cherubism. Int J Oral Maxillofac Surg (2005) 34: 350–356.
4. Ueki Y, Tiziani V, Santanna C, Fukai N, Maulik C, Garfinkle J Ninomiya C, doAmaral C, Peters H, Habal M, Rhee-Morris L, Doss JB, Kreiborg S, Olsen BR and Reichenberger E: Mutations in the gene encoding c-Abl-binding protein SH3BP2 cause cherubism. Nat Genet (2001) 28: 125–126.
5. Imai Y, Kanno K, Moriya T, Kayano S, Seino H, Matsubara Y and Yamada A: A missense mutation in the SH3BP2 gene on chromosome 4p16.3 found in a case of nonfamilial cherubism. Cleft Palate Craniofac J (2003) 40: 632–638.
6. de Lange J, van Maarle MC, van den Akker HP and Redeker EJ: A new mutation in the SH3BP2 gene showing reduced penetrance in a family affected with cherubism. Oral Surg Oral Med Oral Pathol Oral Radiol Endod (2007) 103: 378–381.
7. Li CY and Yu SF: A novel mutation in the SH3BP2 gene causes cherubism:case report. BMC Med Genet (2006) 7: 84–87.
8. Carvalho VM, Perdigao PF, Pimenta FJ, de Souza PE, Gomez RS and De Marco L: A novel mutation of the SH3BP2 gene in an aggressive case of cherubism. Oral Oncol (2007) Mar 14; [Epub ahead of print].
9. Idowu BD, Thomas G, Frow R, Diss TC and Flanagan AM: Mutations in SH3BP2, the cherubism gene, were not detected in central or peripheral giant cell tumours of the jaw. Br J Oral Maxillofac Surg (2007) Jun 1; [Epub ahead of print].
10. Lietman SA, Prescott NL, Hicks DG, Westra WH and Levine MA: SH3BP2 is rarely mutated in exon 9 in giant cell lesions outside cherubism. Clin Orthop Relat Res (2007) 459: 22–27.
11. Ueki Y, Lin CY, Senoo M, Ebihara T, Agata N, Onji M, Saheki Y, Kawai T, Mukherjee PM, Reichenberger E and Olsen BR: Increased myeloid cell responses to M-CSF and RANKL cause bone loss and inflammation in SH3BP2 "cherubism: mice. Cell (2007) 128: 71–83.
12. Miah SM, Hatani T, Qu X, Yamamura H and Sada K: Point mutations of 3BP2 identified in human- inherited disease cherubism result in the loss of function. Genes Cells (2004) 9: 993–1004.
13. Foucault I, Liu YC, Bernard A and Deckert M: The Chaperone protein 14–3–3 interacts with 3BP2/SH3BP2 and regulates its adaptor function. J Biol Chem (2003) 278: 7146–7153.
14. Lietman SA, Kalinchinko N, Deng X, Kohanski R and Levine MA: Identification of a novel mutation of SH3BP2 in cherubism and demonstration that SH3BP2 mutations lead to increased NFAT activation. Hum Mutat (2006) 27: 717–718.