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ORIGINAL ARTICLE

Novel mutations in the *SH3BP2* gene associated with sporadic central giant cell lesions and cherubism

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Central giant cell lesion (CGCL) is a reactive bone lesion that occurs mainly in the mandible, characterized by the multinucleated osteoclast-like giant cells in a background of oval to spindle-shaped mononuclear cells. The etiology is unknown and occurs more commonly in young adults. Cherubism, a rare disease found predominantly in females has histologic characteristics indistinguishable from those of CGCL and is caused by mutations mostly present in exon 9 of the *SH3BP2* gene. In this study, we investigated four cases of CGCL and one case of cherubism. DNA was extracted from peripheral blood and tumor tissue and all coding and flanking regions of the *SH3BP2* amplified by PCR and directly sequenced to identify underlying mutations. Two novel mutations were found; a heterozygous missense mutation c.1442A>T (Q481L) in exon 11 in one sporadic case of CGCL and a heterozygous germline and tumor tissue missense mutation c.320C>T (T107M) in exon 4 in one patient with cherubism. These findings open a new window to investigate the possible relationship between the pathogenesis of the cherubism and CGCL.

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Introduction

The central giant cell lesion (CGCL) was first described by Jaffe (1953) as a giant-cell reparative granuloma of the jaw bones. CGCL is a reactive intra-osseous lesion resulting from a local reparative reaction of unknown etiology. It occurs mainly in the mandible and the age of most patients ranges from 10 to 25 years (de Lange *et al*, 2007c). Radiographic findings are diverse, varying from small unilocular lesions to large multilocular lesions with displacement of teeth, root resorption, and cortical perforation (Horner, 1989). Histologically, CGCL is characterized by the presence of multinucleated osteoclast-like giant cells in a background of oval to spindle-shaped mononuclear cells (Neville *et al*, 2002). Cherubism (MIM 118400) is an autosomal dominant inherited syndrome characterized by excessive bone degradation of the upper and lower jaws followed by development of fibrous tissue masses, which causes a characteristic facial swelling (Ueki *et al*, 2001). The gene responsible for cherubism is located on chromosome 4p16.3 and named *SH3BP2* (Mangion *et al*, 1999). To date, all mutations of the *SH3BP2* identified in familial cherubism are present in exon nine and affect three amino acids within a conserved six amino acid sequence (RSPPDG), the SH3-binding domain (Hatani and Sada, 2008). Recently, a mutation in the *SH3BP2* gene was described in a case of non-familial cherubism (Imai *et al*, 2003) and also in one aggressive case of sporadic cherubism (Carvalho *et al*, 2008). Although the etiology of CGCL is unknown, the similar histologic features of this lesion with cherubism suggest that both conditions share the same genetic alteration. Therefore, we investigated all coding and flanking sequences of the *SH3BP2* gene in patients with CGCL.

Material and methods

In this report, we studied frozen samples from four cases of CGCL and one case of cherubism, collected between 1998 and 2004 and stored at −80°C. The criteria for CGCL diagnosis is described elsewhere (Neville *et al*, 2002; de Lange *et al*, 2007c). All lesions were located in the midline or posterior region of the mandible. The University Ethics Committee approved the work. After an informed written consent signed by all patients, fragments of the lesions were obtained during surgical

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removal procedures. A piece of the fragment was fixed in 10% formalin buffer and paraffin-embedded tissue blocks were used for routine histologic staining to confirm the clinical diagnosis. All five lesions studied showed typical histologic features of CGCL described in the literature (Neville *et al*, 2002; de Lange *et al*, 2007c). Another piece of the specimen sample was immediately stored at −80°C for subsequent molecular analyzes. In addition, peripheral blood was collected from all subjects. The CGCL was considered aggressive when the lesion was larger than 4 cm in size and the cortical bone was damaged or expanded and teeth displaced (Chuong *et al*, 1986).

One case was referred to the School of Dentistry as bilateral CGCL and her clinical chart did not report cherub face or familial cases of cherubism. After an intra oral examination, a discreet expansion of the mandible was noted. Radiographic findings showed bilateral multilocular lesions of the mandible with tooth displacement. Although no cherub face was seen, our diagnostic hypothesis was that of cherubism with low expressivity. Therefore, in addition to clinical and biochemical data, DNA of tumor and blood cells was extracted and the *SH3BP2* gene investigated.

All patients were unrelated and underwent detailed hematologic and biochemical evaluation, including parathyroid hormone (PTH) levels, alkaline phosphatase activity, serum calcium and phosphorus concentrations. All these parameters were within normal ranges.

Genomic DNA was extracted from lesions and peripheral blood using the GenomicPrep kit according to the manufacturer's instructions (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). All coding and flanking regions of the *SH3PB2* gene were investigated using previously described PCR primers and reaction conditions (Imai *et al*, 2003). Briefly, PCR reactions were performed in a final volume of 50 μl containing 200 ng of template DNA, 200 μM dNTPs, 10 pM of each primer and 1.25 U of a proofreading Taq polymerase (Platinum® *Taq* DNA Polymerase High Fidelity; Invitrogen, Carlsbad, CA, USA). Thirty-five cycles of amplification were performed in a PTC-100-60 thermocycler (MJ Research, Watertown, MA, USA) with the appropriate parameters. The amplified products were analyzed by electrophoresis on a 6.5% polyacrylamide gel followed by silver stain. DNA bands corresponding to expected size were purified using the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Piscataway, NJ, USA). DNA sequencing reactions were carried out using the BigDye Terminator kit in a DNA ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). All experiments were performed in duplicate with both strands and from different amplification products to lessen artificial artifacts. Numbering of nucleotide and amino acid refers to the complete cDNA sequence of *SH3BP2*. In order to exclude single nucleotide polymorphisms (SNPs), we sequenced DNA from 50 unrelated control subjects with the same ethnic background. This group was comprised of patients without history of CGCL or Cherubism. The NCBI SNP database was also checked to rule out polymorphisms.

Results

To verify the presence of mutations of the *SH3BP2* gene in CGCL and cherubism patients, all 13 coding and flanking regions were sequenced from PCR products from tumor DNA as well as peripheral blood DNA. Clinical and radiological features as well as the molecular alterations found in the *SH3BP2* gene are shown in Table 1.

The patient with cherubism (#1) presented a germline heterozygous substitution of cytosine to thymine c.320C > T (T107M) in exon 4 (Figure 1a). This mutation leads to an exchange of the amino acid threonine to methionine in the N-terminal pleckstrin domain. The presence of this germline mutation led us to screen the patient's close relatives. Besides her parents, no other relatives were available for clinical assay. Oral mucosal cells were collected from them, DNA extracted and sequencing of the *SH3BP2* gene performed.

The mother was shown to carry the same heterozygous mutation c.320C > T found in the propositus (data not shown). To predict whether the molecular alterations present in both patients would affect protein function, the algorithm SORTING INTOLERANT FROM TOLERANT (SIFT) (http://blocks.fhcrc.org/sift/SIFT.html) was used.

Analysis of the *SH3BP2* gene in patient with non-aggressive CGCL (#2) showed the presence of a novel heterozygous mutation c.1442A > T (Q481L) in exon 11 (Figure 1b), leading to an exchange of amino acid Glutamine to Leucine in the C-terminal SH2 domain. No germline mutation was found in this patient.

Table 1 Clinical data, radiological features and mutations detected in patients with cherubism (#1) and central giant cell lesion (#2 to #5)

Case no.	*Age (years)*	*Sex*	*Aggressiveness*	*X-ray findings*	*Mutation (germline)*	*Mutation (tumor)*
1	12	F	Yes	Large destructive multilocular radiolucent lesions with tooth displacement	c.320C > T	c.320C > T
2	41	F	No	Small unilocular radiolucent lesion	Wild type	c.1442A > T
3	46	M	Yes	Large destructive multilocular radiolucent lesion	Wild type	Wild type
4	11	M	Yes	Large destructive multilocular radiolucent lesion	Wild type	Wild type
5	19	M	No	Multilocular radiolucent lesion	Wild type	Wild type

Nucleotides are numbered according to the ENST00000356331.

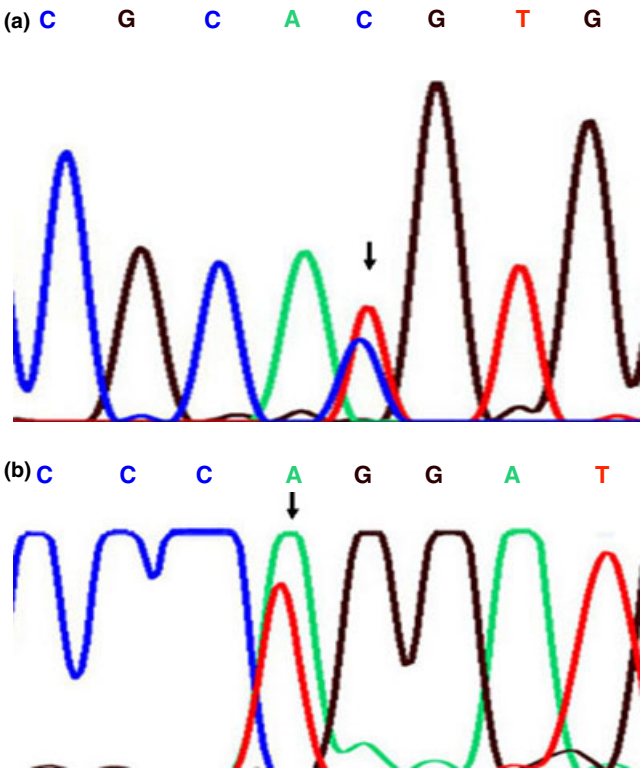

Figure 1 Chromatogram sequence data of the propositus #1 showing the heterozygous c.320C > T mutation (**a**); Chromatogram sequence data of the propositus #2 showing the heterozygous c.1442A > T mutation (**b**)

Discussion

A number of conditions can present with lesions that histologically are indistinguishable from CGCL of the jaws, including giant cell tumors, aneurysmal bone cyst, brown tumors of hyperparathyroidism, cherubism and other inherited syndromes associated with genetic mutations such as Noonan-like/multiple giant cell lesion syndrome, Neurofibromatosis type 1 and Ramon syndrome (Pina-Neto *et al*, 1986; van Damme and Mooren, 1994; Lee *et al*, 2005).

The gene *SH3BP2* that codes for the adapter protein SH3BP2 was identified in 2001 on chromosome 4p16.3 (Mangion *et al*, 1999; Tiziani *et al*, 1999; Ueki *et al*, 2001). A mutation in exon 9 of the *SH3BP2* gene was described in a case of non-familial cherubism (Imai *et al*, 2003). Subsequently, *SH3BP2* gene molecular analysis of exon 9 in patients with sporadic central or peripheral giant cells lesions have not demonstrated the presence of mutations (de Lange *et al*, 2007b; Lietman *et al*, 2007; Idowu *et al*, 2008). In this report, we analyzed all coding and flanking regions of the *SH3BP2* gene and found two novel mutations of *SH3BP2*, one associated with a sporadic case of CGCL and one in a patient with cherubism.

Three functional domains of *SH3BP2* have been described; the N-terminal pleckstrin homology domain (PH), a 10-amino acid SH3 binding site and C-terminal SH2 domain (Ren *et al*, 1993). Patient #2 presented a heterozygous mutation c.1442A > T (Q481L) in exon 11, a SH2 domain of the *SH3BP2*. This mutation was present only in tumor cells. The Src homology 2 domain (SH2) is involved in recognition of phosphorylated tyrosine (pTyr) and SH2 domains typically bind pTyr-containing ligands via two surface pockets allowing proteins with SH2 domains to localize to tyrosine phosphorylated sites (Ingley, 2008). Analysis of the protein function based on sequence homology and the physical properties of amino acids using the SIFT software program shows that the Q481L alteration in the SH2 domain affects protein function, possibly arresting SH3BP2 to localize tyrosine phosphorylated sites. To date, functional studies of *SH3BP2* mutations have not been performed. But their association with disease, location in areas of the molecule that are highly conserved between species (http://www.ensembl.org/Homo_sapiens/searchview?species=;idx=;q=sh3bp2) and most important, their absence in normal controls, suggest that they are pathogenic.

Lietman *et al* (2007) studied exon 9 of *SH3BP2* gene in 10 giant cell tumors of bone and nine giant cell reparative granulomas and did not find any molecular alteration. In addition, they showed that SH3BP2 transcript and protein as well as NFATc1 protein were abundantly expressed in CGCL. They also found a smaller transcript in the RNA from one giant cell lesion and speculated that a mutation outside exon 9 could be responsible for this truncated protein. Idowu *et al* (2008) did not find mutations in exon 9 of the *SH3BP2* gene in CGCL of the jaw and neither did de Lange *et al* (2007b). It is important to emphasize that these authors limited to exon 9 their search for *SH3BP2* gene mutations.

At this study, we found a novel germline mutation in exon 4 (c.320C > T) in one young female patient presenting with cherubism with low expressivity. This mutation led to a substitution of threonine to methionine in the N-terminal pleckstrin domain. PH domain is only found in eukaryotes and is the eleventh most populous domain family in the human genome (Lander *et al*, 2001). PH domains is only found in cellular signaling proteins such as serine/threonine kinase, tyrosine kinases, regulators of G-proteins, endocytotic GTPases, adaptors, as well as cytoskeleton associated molecules and in lipid associated enzymes (Ingley and Hemmings, 1994; Lemmon, 2004).

Our patient with cherubism fulfilled some clinical and radiographic criteria used in its diagnosis such as bilateral swelling of the jaw with expansion or thinness of the cortical bone, bilateral multilocular radiolucencies, tooth displacement and root resorption (de Lange *et al*, 2007a). Cherubism shows 100% penetrance in males and 50–70% in females (von Wowern, 2000; Horton, 2002; Marx and Stern, 2002) and the expressivity is wide, ranging from bilateral non-expansible to highly destructive lesions (von Wowern, 2000). Because of incomplete penetrance of cherubism in females and to its variability of expressivity, some cases of cherubism may be under-diagnosed. Recently, we reported a novel mutation in the *SH3BP2* gene outside exon 9 in one aggressive case of cherubism (Carvalho *et al*, 2008).

Given that all but one previous report of mutations in the *SH3BP2* have looked only in exon 9, additional studies analyzing the entire gene and trying to correlate genotype–phenotype features in individuals with cherubism will help to establish the basis for the disease's phenotypic variability.

Up to now, it has been suggested that the *SH3BP2* gene plays a role in regulating increased osteoblast and osteoclast activities (Ueki *et al*, 2007). Furthermore, it is proposed that mutations in the *SH3BP2* gene could cause pathologic activation of osteoclasts, probably by dysfunction of the *SH3BP2* gene in the regulatory pathway of osteoclastogenesis (Miah *et al*, 2004; Hyckel *et al*, 2005). In this process, *SH3BP2* has an influence on the regulation of the PTH receptor and PTH-related protein (PTHrP), interacting with the chaperone protein 14-3-3 (Foucault *et al*, 2003), which was recently described as a regulatory protein of type I PTH/PTHrP receptor (Tazawa *et al*, 2003).

Lietman *et al* (2006) showed that heterozygotic mutations in exon 9 of the *SH3BP2* led to increased NFAT (nuclear factor of activated T-cells), an osteoclastogenic mediator, indicating that the cherubism results from gain of function mutations in *SH3BP2*. The NFATc1 isoform (also termed NFAT2) has been proposed as an important master transcription factor for osteoclastogenesis (Foucault *et al*, 2003a,b). Dysfunction of SH3BP2 protein by point mutations causes pathologic activation of osteoclasts in cherubism, suggesting that endogenous SH3BP2 has a regulatory role in the development and/or activation of osteoclasts in jaw bones (Miah *et al*, 2004). The maturation of osteoclasts requires osteoblasts and stromal cells releasing macrophage colony-stimulating factor and the receptor for activation of nuclear factor κB ligand (RANKL) that are essential to promote osteoclastogenesis (Roodman, 1999).

In this study, novel *SH3BP2* gene mutations in sporadic CGCL and in one case of cherubism were demonstrated. Considering the diverse clinical behavior of cherubism it is possible that some non-familial cases of cherubism are undistinguishable from sporadic cases of CGCL. Further sequencing of all coding as well as flanking and promoter regions of the *SH3BP2* gene in CGCL and non-familial cherubism cases with variable expressivity and penetrance are necessary to provide important insights into molecular mechanisms associated with these lesions.

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Author contributions

VM Carvalho, PF Perdigão, L De Marco and RS Gomez contributed in study concepts, study design, definition of intellectual content and in manuscript preparation and editing. VM Carvalho, PF Perdigão, FR Amaral and PEA Souza contributed in data acquisition.

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