**SH3BP2 Mutations in Cherubism Lead to Increased NFAT Activation**

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**Introduction:** The NFATc1 isoform (also termed NFAT2) is the master transcription factor for osteoclastogenesis and has been shown to play a critical role in osteoclast development. Wild type SH3BP2 increases activation of nuclear factor of activated T cells (NFAT) and cherubism (OMIM 118400) is a rare autosomal dominant disorder with incomplete penetrance characterized by extensive pathological bone remodeling within the mandible or maxilla due to heterozygous germline mutations in the gene that encodes SH3BP2. In cherubism there is intense bone resorption, and there are numerous giant cells that express the osteoclast marker tartrate-resistant acid phosphatase (TRAP). All SH3BP2 mutations described thus far in patients with cherubism occur within a six amino acid region encoded by exon 9. Two observations make it unlikely that these mutations result in a loss of function. First, SH3BP2 has been localized to chromosome 4p16.3, a region that is frequently deleted in patients with Wolf-Hirschhorn syndrome. Despite haploinsufficiency of SH3BP2, patients with Wolf-Hirschhorn syndrome have distinctive facial and developmental features that show no resemblance to those of cherubism. Secondly, the discrete clustering of the missense mutations in cherubism within a six amino acid domain of the protein suggests that these mutations affect a specific function of the SH3BP2 protein. We evaluated the effect of wild type and mutant SH3BP2 on NFAT activity.

**Materials and Methods:** The protocol was approved by the institutional review board and written consent was obtained from the parents. We evaluated a patient with cherubism and his family (5 family members total). Genomic DNA was isolated directly from peripheral blood leukocytes by standard techniques. Exon 9 of the SH3BP2 gene was amplified from ten nanogram samples of genomic DNA and the PCR products were isolated and sequenced by Thermosequenase terminator cycle sequencing. All mutations were introduced into wild type SH3BP2 by overlap extension-PCR. The normal or mutant vectors were transiently transfected into Jurkat TAg cells in the presence of a 3X NFAT-luciferase reporter gene plus cDNA’s encoding either wild type or mutant forms of SH3BP2. The inset is an immunoblot of whole cell lysates from co-transfected Jurkat TAg cells (above the bar graph) after sequential incubation of the same membrane to antisera against SH3BP2 and β-actin.

**Results:** We identified a novel guanine to adenine transition that predicts the non-conservative replacement of aspartic acid (GAT) with asparagine (AAT) at codon 419 (D419N) of SH3BP2 in a patient with typical features of cherubism (Figure 1). This mutation is within the six amino acid domain in exon 9 in which all previously described mutations in patients with cherubism have been identified. Molecular genetic analysis of the proband’s parents and two siblings demonstrated that the father and both sibs were carriers of the D419N allele but lacked phenotypic features of cherubism, indicating incomplete penetrance of the mutation (Figure 1). Moreover, the clinically unaffected male sibling represents the first male with an SH3BP2 mutation who lacks clinical evidence of cherubism. We transiently co-expressed cDNA’s encoding wild type or four different mutant forms of SH3BP2 plus NFAT-luciferase reporter gene and luciferase activity was evaluated by luminometer using the dual luciferase protocol. Whole cell lysates were analyzed by immunoblot and enhanced chemiluminescence (ECL).

**Discussion:** Our studies show that SH3BP2 mutations in cherubism behave as activators of NFATc1/c2 action. However it remains unclear how SH3BP2 mutations cause the giant cell tumors in cherubism, or why the phenotype regresses after puberty. Moreover, we found evidence for incomplete penetrance of the SH3BP2D419N mutation, which suggests that other as yet unknown genes and/or environmental factors may modulate the effect(s) of this mutation on the development of cherubism. Further genotype-phenotype associations in extended cherubism pedigrees may disclose the basis for the variability in the bone lesions and may provide important new insights into the molecular biology of bone resorption.


**Figure 1** Sequence analysis of exon 9 of the SH3BP2 gene and family pedigree. The guanine to adenine transition is in one of two alleles in lanes 2, 3, and 4. The arrow in lane 4 indicates the proband’s mutation. The SH3BP2D419N mutation is represented by the filled in upper left quadrant. The cherubism phenotype is represented by the filled in lower right quadrant. The proband is indicted by the arrow.