

Novel Mutation in the Gene Encoding c-Abl-Binding Protein SH3BP2 Causes Cherubism

Bryan Lo,¹ M. Faiyaz-Ul-Haque,² S. Kennedy,¹ R. Aviv,³ L.-C. Tsui,² and Ahmad S. Teebi^{1*}

¹Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada

²Department of Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada

³Department of Radiology, The Hospital for Sick Children, Toronto, Ontario, Canada

Cherubism is a rare autosomal dominant inherited condition caused by mutations in the c-Abl-binding protein SH3BP2. It is characterized by multiple cystic giant cell lesions of the jaw appearing in early childhood with stabilization and remission after puberty. In the present study, we used direct sequence analysis of the SH3BP2 gene of several individuals from a family with cherubism to search for additional SH3BP2 mutations resulting in cherubism. In affected relatives, we found a previously unreported G to A transition in exon 9 leading to a Gly to Arg substitution at amino acid position 420. G420R has been reported previously with a G to C transversion. To date there have been no disease causing mutations outside exon 9. Therefore, the amino acid sequence from positions 415 to 420 may represent a specific protein domain which, when disrupted, leads to the cherubism phenotype.

© 2003 Wiley-Liss, Inc.

KEY WORDS: cherubism; SH3BP2 mutation hotspot; autosomal dominant; loss of bone in jaws

INTRODUCTION

Cherubism is a rare autosomal dominant inherited condition of the mandible and maxilla manifesting in early childhood [Jones, 1965; Kaugars et al., 1992;

Kozakiewicz et al., 2001]. Jones who described the first case in 1933, suggested the term cherubism as an apt clinical description of the condition [Jones, 1933]. The three sibs he reported all had round cheeks and jaws with a slight upward turning of the eyes to show the white sclera beneath. Their facial appearance, therefore, was reminiscent of the angelic cherubs depicted in Renaissance art [Burland, 1962; Jones, 1965].

The basis for the fullness of cheeks and jaws in cherubism is a non-neoplastic fibrous dysplasia within the mandible and maxilla. The painless expansion typically appears between 2 and 5 years [Jones, 1965; Kaugars et al., 1992]. The non-facial bones are not affected in cherubism and as the condition progresses during childhood there may be interference with tooth development and marked cervical adenopathy [Jones, 1965]. With the onset of puberty the condition begins to stabilize and regress and by mid-20s the facial abnormalities are not usually recognized and residual deformity of the jaws is rare [Jones, 1965; Kaugars et al., 1992].

It is generally recognized that cherubism displays variable expressivity and incomplete penetrance [Riefkohl et al., 1985; Marck and Kudryk, 1992]. In males, the penetrance is close to 100% but in females only 50–75% [Peters, 1979]. Sometimes the condition is so mild that it goes unrecognized and is only diagnosed by radiography. In other cases, the dysplasia is so severe that in addition to extensive bone loss, there is marked deformation resulting in chewing, speech, and swallowing difficulties. In one case, severe orbital involvement led to diplopia [Colombo et al., 2001].

Generally, the mandibular angle, ascending ramus, retromolar region, and posterior maxilla are affected in cherubism [Burland, 1962; Khosla and Korobkin, 1970]. The coronoid can also be involved but the condyles are mostly spared. In most cases, only the mandible is involved and the lesions are bilateral and tend to be symmetric. Histologically, the dysplasia consists of a mononuclear fibroblastic stroma with large numbers of multinucleated giant cells and cyst formation [Southgate et al., 1998]. At the periphery of the lesions there can be newly formed osteoid and bones.

Following the mapping of cherubism to 4p16 [Mangion et al., 1999; Tiziani et al., 1999], mutations resulting in the condition were identified in the SH3BP2

*Correspondence to: Dr. Ahmad S. Teebi, M.D., F.R.C.P.E., Section of Clinical Genetics and Dysmorphology, The Hospital for Sick Children and University of Toronto, Toronto, Canada.
E-mail: ateebi@sickkids.ca

Received 11 September 2002; Accepted 30 January 2003
DOI 10.1002/ajmg.a.20226

gene [Ueki et al., 2001]. All the mutations identified so far are located in exon 9 and result in amino acid substitutions within a 6 amino acid sequence [Ueki et al., 2001]. In this report, we identified a previously unreported G to A transition located in exon 9 in a family with cherubism followed by our craniofacial program. We reviewed the clinical data available for this family and concluded that there is evidence for a mutation hotspot.

CLINICAL REPORT

Pedigree Analysis

The pedigree consists of six individuals in two generations affected with cherubism (Fig. 1). The kindred are of Italian background and there are no other known cases in the family. In generation II, two of five sibs are affected, with one sib being an obligate carrier. In generation III, three of five cousins are affected.

Case History

The first individual of the pedigree to be diagnosed with cherubism is II-6. He was diagnosed at the age of 8 and had surgical excision of his fibrodysplastic lesions at the age of 11. At 41 years of age, he is well and has no health concerns.

The daughter of II-6, III-5, is a 9-year-old girl who developed bilateral cystic lesions of the mandible between 3 and 4 years. On presentation, at the age of 4, there were palpable lesions over the angle of the mandible bilaterally and radiographs showed symmetric multi-cystic lesions. Over the next 3 years, the lesions gradually progressed without producing functional changes to her occlusion and apart from the cherubism she was healthy. At the age of 8, she required surgery to remove two ectopic and impacted permanent canine teeth from her mandible. During the extraction of these teeth, the surrounding soft fibrous tissue in the mandible was curetted and removed. The MRI and CT performed prior to the surgery are shown in Figure 2.

The older sister of II-6, II-3, is currently 51 years old, healthy, and has no clinical history suggestive of cherubism. She has three children, two of which, III-2

and III-3, have cherubism. At the age of 7, III-2 had fibrous dysplastic tissue curetted from her mandible bilaterally. Histopathology confirmed clusters of multinucleated giant cells within a fibrous stroma consistent with the diagnosis of cherubism. At the age of 25, she is well.

At the age of 5, III-3 had a palpable lump in the right angle of the mandible, which gradually over the next 2 years progressed to bilateral lesions of the mandible with minor displacement of several permanent mandibular teeth. By age 8 radiographs began to show resolution and bone refilling, and surgery was never warranted. At the age of 22, she is well.

The brother of II-6, II-5 has a clinical history consistent with cherubism. He currently is 45 years old and had a bone graft to his jaw when he was 17.

Direct DNA sequencing confirmed that in II-3, II-6, III-2, and III-3, one allele of the *SH3BP2* gene contained a G to A transition in exon 9 that predicted a Gly to Arg substitution at amino acid position 420 (Fig. 3).

DISCUSSION

The *SH3BP2* gene was originally identified in mouse by screening a mouse cDNA expression library with a fusion protein containing the Src homology 3 domain (SH3) of the *c-abl* oncogene [Ren et al., 1993]. The human *SH3BP2* homologue has a predicted amino acid sequence 87% identical to the mouse amino acid sequence and there is 81% identity at the DNA level within the coding regions [Bell et al., 1997]. The human *SH3BP2* gene maps to chromosome band 4p16.3 in a region that is syntenic to mouse chromosome 5 [Nasir et al., 1994].

Sequence analysis of the *SH3BP2* gene suggests that it may have a role in signal transduction. The functional domains identified in SH3BP2 include a pleckstrin homology (PH) domain of approximately 100 amino acids that may participate in protein-protein and/or protein-lipid interactions, a SH3 binding domain of 9–10 proline rich amino acids, and a Src homology 2 (SH2) domain, which is a 100 amino acid phosphotyrosine binding pocket. Each of these three domains is predicted to be modular and may mediate the formation and localization of heteromeric protein complexes involved in tyrosine kinase signal transduction pathways [Pawson et al., 2002].

The mutation in the *SH3BP2* gene that we have identified in the present family with cherubism, along with the previously reported mutations causing cherubism, all cluster within exon 9 [Ueki, 2000]. This brings to date 13 families with cherubism in which the causative mutation is a missense mutation found in a stretch of 6 amino acids (415–420). In total there are seven different amino acid substitutions and eight distinct DNA missense mutations. So far, no disease causing mutation outside of this short stretch of DNA in the *SH3BP2* gene has been identified. The ethnic background of the various families is diverse and our pedigree points to a de novo mutation arising in the grandparental generation. Taken together, the evidence points to the existence of a mutation hotspot.

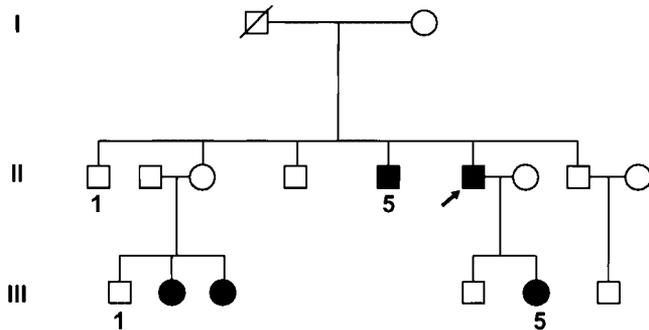


Fig. 1. Pedigree of the family affected with cherubism. Filled symbols identify affected individuals.

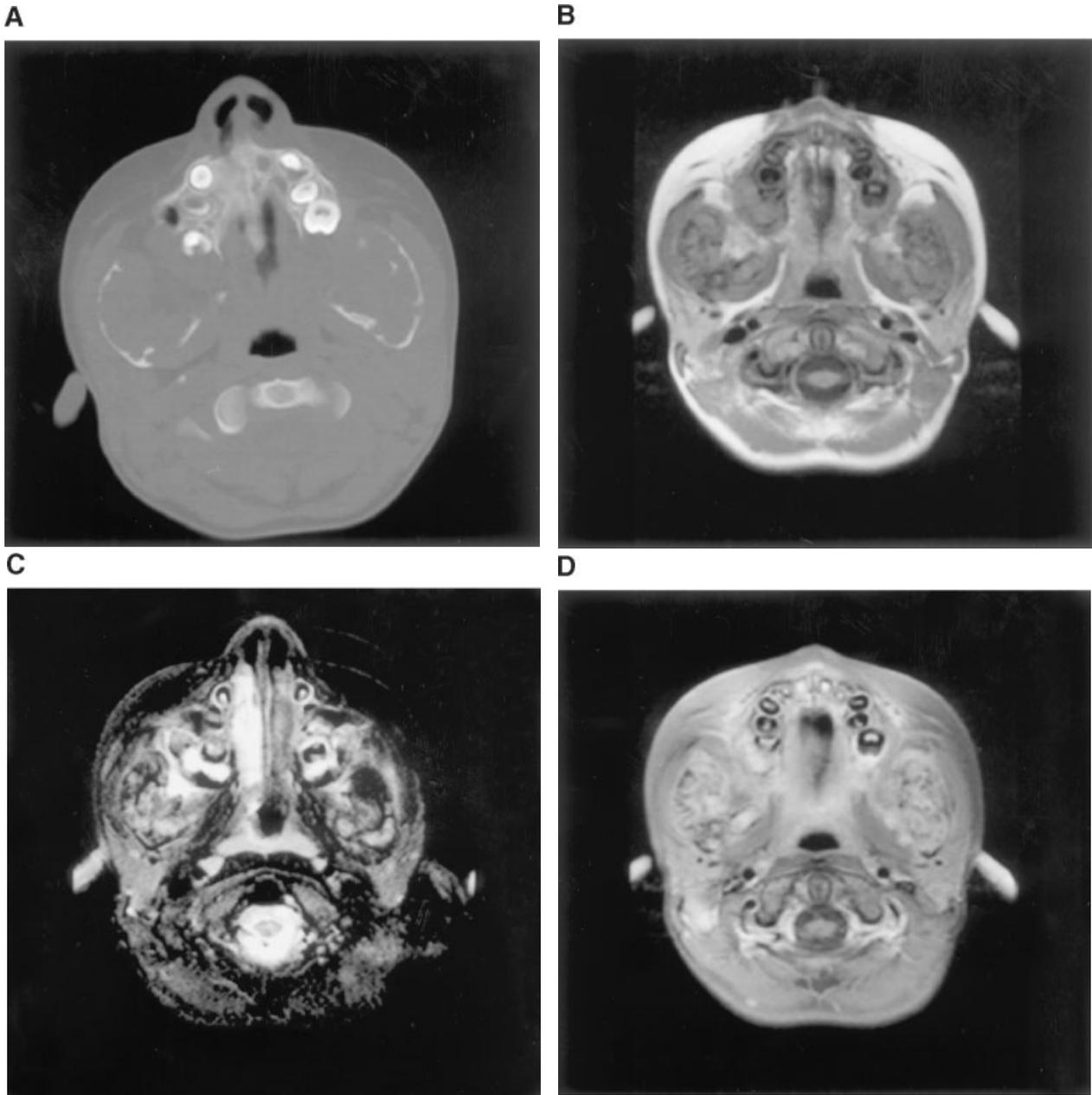


Fig. 2. Axial imaging acquired at the level of the C2 vertebral body: (A) CT bone windows, (B) axial T1-weighting, (C) axial fat saturated T2-weighting, (D) axial fat saturated T1-weighting after gadolinium enhancement. A: There is marked expansion of both mandibular rami by a lytic process with deficiency of the residual cortical outline, which is sclerotic and scalloped. Foci of calcification are present within the left ramus on CT. B–D:

Heterogenous intensity is present within the confines of the bone outline with enhancement after gadolinium. Multiple hypointense foci on fat saturated T1- and T2-weighted images represent calcific deposits and hemosiderin deposition. The process, while being predominantly limited to the ramus, also involves the posterior alveolus of the maxilla, which is eroded and splayed. Small cervical lymph nodes are present.

It has been hypothesized that the mutations in *SH3BP2* causing cherubism are either gain of function mutations or ones that act in a dominant negative manner [Ueki et al., 2001]. The compelling evidence against a haploinsufficiency of *SH3BP2* mechanism is that patients with Wolf–Hirshorn syndrome are deleted for a chromosomal region that includes *SH3BP2* and yet do

not exhibit any cherubism-like phenotype. Moreover, the clustering of missense mutations may well be defining an amino acid domain involved in a critical protein–protein interaction. Since the region of clustering is far away from the SH2 domain and the PH domain, the function of these modular peptide recognition domains is likely not to be affected. It is, therefore,

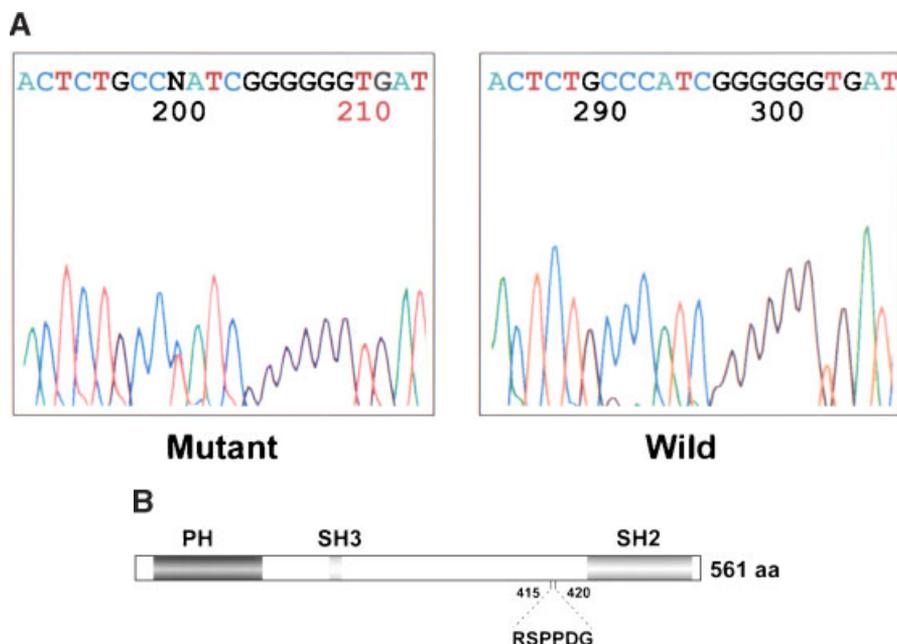


Fig. 3. **A:** Automated sequencing results of the affected male (II-6) showing the heterozygous change (**left panel**) and normal wild type subject (**right panel**). **B:** Schematic diagram of the SH3BP2 protein, showing the position of the three domains; PH, pleckstrin homology domain; SH3, proline rich Src homology 3 binding domain; SH2, Src homology 2 domain and a mutation hotspot at amino acid number 415–420. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tempting to speculate that the amino acids 415–420 participate in another type of protein interaction, that when disrupted, leads to the cherubism phenotype.

ACKNOWLEDGMENTS

The authors are grateful to all members of the family for their participation in this study. The Canadian Genetics Diseases Network (to LCT) sponsored the study.

REFERENCES

- Bell SM, Shaw M, Jou YS, Myers RM, Knowles MA. 1997. Identification and characterization of the human homologue of *SH3BP2*, an SH3 binding domain protein within a common region of deletion at 4p16.3 involved in bladder cancer. *Genomics* 44:163–170.
- Burland JG. 1962. Cherubism: Familial bilateral osseous dysplasia of the jaws. *Oral Surg* 15(Suppl 2):43–68.
- Colombo F, Cursiefen C, Neukam FW, Holbach LM. 2001. Orbital involvement in cherubism. *Ophthalmology* 108:1884–1888.
- Jones WA. 1933. Familial multilocular cystic disease of the jaws. *Am J Cancer* 17:946–950.
- Jones WA. 1965. Cherubism: A thumbnail sketch of its diagnosis and a conservative method of treatment. *Oral Surg* 20:648–653.
- Kaugars GE, Niamtu J III, Svirsky JA. 1992. Cherubism: Diagnosis, treatment, and comparison with central giant cell granulomas and giant cell tumors. *Oral Surg Oral Med Oral Pathol* 73:369–374.
- Khosla VM, Korobkin M. 1970. Cherubism. *Am J Dis Child* 120:458–461.
- Kozakiewicz M, Perczynska-Partyka W, Kobos J. 2001. Cherubism—clinical picture and treatment. *Oral Dis* 7:123–130.
- Mangion J, Rahman N, Edkins S, Barfoot R, Nguyen T, Sigurdsson A, Townend JV, Fitzpatrick DR, Flanagan AM, Stratton MR. 1999. The gene for cherubism maps to chromosome 4p16.3. *Am J Hum Genet* 65:151–157.
- Marck PA, Kudryk WH. 1992. Cherubism. *J Otolaryngol* 21:84–87.
- Nasir J, Lin B, Bucan M, Koizumi T, Nadeau JH, Hayden MR. 1994. The murine homologues of the Huntington disease gene (*Hdh*) and the alpha-adducin gene (*Add1*) map to mouse chromosome 5 within a region of conserved synteny with human chromosome 4p16.3. *Genomics* 22:198–201.
- Pawson T, Raina M, Nash P. 2002. Interaction domains: From simple binding events to complex cellular behavior. *FEBS Lett* 513:2–10.
- Peters WJN. 1979. Cherubism: A study of twenty cases from one family. *Oral Surg* 47:307–311.
- Ren R, Mayer BJ, Cicchetti P, Baltimore D. 1993. Identification of a ten-amino acid proline-rich SH3 binding site. *Science* 259:1157–1161.
- Riefkohl R, Georgiade GS, Georgiade NG. 1985. Cherubism. *Ann Plast Surg* 14:85–90.
- Southgate J, Sarma U, Townend JV, Barron J, Flanagan AM. 1998. Study of the cell biology and biochemistry of cherubism. *J Clin Pathol* 51:831–837.
- Tiziani V, Reichenberger E, Buzzo CL, Niazi S, Fukai N, Stiller M, Peters H, Salzano FM, Raposo do Amaral CM, Olsen BR. 1999. The gene for cherubism maps to chromosome 4p16. *Am J Hum Genet* 65:158–166.
- 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, Reichenberger E. 2001. Mutations in the gene encoding c-Abl-binding protein SH3BP2 cause cherubism. *Nat Genet* 28:125–126.