SHORT REPORT

De novo *GLI3* mutation in acrocallosal syndrome: broadening the phenotypic spectrum of *GLI3* defects and overlap with murine models

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Acrocallosal syndrome (ACS) is characterised by postaxial polydactyly, hallux duplication, macrocephaly, and absence of the corpus callosum, usually with severe developmental delay. The condition overlaps with Greig cephalopolysyndactyly syndrome (GCPS), an autosomal dominant disorder that results from mutations in the *GLI3* gene. Here we report a child with agenesis of the corpus callosum and severe retardation, both cardinal features of ACS and rare in GCPS, who has a mutation in *GLI3*.
Since others have excluded *GLI3* in ACS, we suggest that ACS may represent a heterogeneous group of disorders that, in some cases, may result from a mutation in *GLI3* and represent a severe, allelic form of GCPS. The finding is important for counselling families with suspected ACS.

Acrocallosal syndrome (ACS) is an uncommon disorder, first described in 1979 by Schinzel.[1] Features include postaxial polydactyly, hallux duplication, macrocephaly, and absence of the corpus callosum, usually with severe developmental delay. Although patients usually share the core features, many other associated abnormalities have been described. Initially thought to be an autosomal dominant condition, subsequent reports of consanguinity, as well as of affected ACS sibs born to unaffected parents, have suggested autosomal recessive inheritance. The similarity between ACS and Greig cephalopolysyndactyly syndrome (GCPS) has been highlighted by several authors.[2] GCPS is an autosomal dominant disorder and one of five syndromes known to result from mutations in the *GLI3* gene.[3–5] Clinical features include pre- and postaxial polydactyly with cutaneous syndactyly in the hands and preaxial polydactyly in the feet. Craniofacial abnormalities, which are generally mild, include hypertelorism, a high forehead, and craniosynostosis. In contrast to ACS, the corpus callosum is usually present with normal intellectual development, although there are two case reports of GCPS associated with absence of the corpus callosum.[6] Suggestions that the two conditions may be allelic have not been supported by linkage analysis; in one familial case of ACS exclusion of the region of chromosome 7 around *GLI3* has been reported.[7] It is likely, however, that ACS is genetically heterogeneous and phenotypic overlap with GCPS suggests *GLI3* as a candidate gene. We report a child with agenesis of the corpus callosum and severe retardation, both cardinal features of ACS and rare in GCPS, who has a mutation in *GLI3*.

CASE REPORT

The proband, now 3 years old, was the first child born to healthy, unrelated parents. He was delivered at term by elective caesarean section after fetal abnormalities had been detected on antenatal ultrasound screening. He was in good condition at birth and weighed 4240 g (90th-97th centile) with an OFC of 39.5 cm (>97th centile). He had bilateral cleft lip and palate, a large anterior fontanelle extending down his forehead, overriding coronal sutures, and small ears with uplifted lobes (fig 1A). Hypertelorism was confirmed by measurement and cranial MR showed agenesis of the corpus callosum. His hands showed bilateral postaxial nubbins, a broad thumb on the right hand, and a partially duplicated thumb on the left. He also had 2/3 partial cutaneous syndactyly on the right and 3/4 partial cutaneous syndactyly on the left. There was a single flexor crease on the index finger of the right hand. The nails appeared normal. The feet displayed bilateral duplication of the big toe and 2/3, 3/4, and 4/5 cutaneous syndactyly (fig 1B). Other abnormalities noted at birth included exomphalos, repaired shortly after birth, secundum ASD, and widely spaced nipples. Chromosomal analysis was normal and a diagnosis of acrocallosal syndrome was made. Ocular abnormalities have since been detected including nystagmus, optic nerve hypoplasia, and a divergent strabismus. Recent imaging showed an extra interparietal bone and suture (fig 1C). Development has been delayed. He sat unsupported at 8 months and walked independently at 21 months. On the Griffiths Development Scales at a chronological age of 56 months his mental age was 21 months with delays in all subscales.

In view of the phenotypic overlap between ACS and GCPS, we screened the proband for mutations in the *GLI3* gene. DNA from the affected child, his parents, and from 50 controls was analysed by PCR, followed by single stranded conformational polymorphism (SSCP) and heteroduplex analysis. Primers covered the entire coding sequence of *GLI3*. SSCP analysis showed a variant band for our patient that was not present in either parent or in 100 control chromosomes. Sequence analysis showed a heterozygous missense mutation in exon 15, a G to C substitution at nucleotide position 2800 resulting in a proline replacing an alanine at amino acid 934 of the GLI3 protein (fig 2A). Since this sequence change is not present in normal control chromosomes and represents a de novo mutation, this is strong evidence that it is pathogenic. To our knowledge this is the first mutation identified in a patient with the major features of ACS and adds it to the increasing list of phenotypes associated with *GLI3* mutations.

DISCUSSION

Reports to date document five missense mutations in the *GLI3* gene, four in GCPS patients and one in a postaxial polydactyly type A/B patient.[3–5] The mutation reported here is 3′ to those previously reported and is located in the middle of domain 5, an evolutionarily highly conserved region (fig 2C). The amino acid sequence within the fifth conserved domain of GLI3 shows high preservation across species for the GLI1, GLI2, and GLI3 proteins. Furthermore, the alanine at position 934 is conserved in all cases (fig 2D). This supports the suggestion

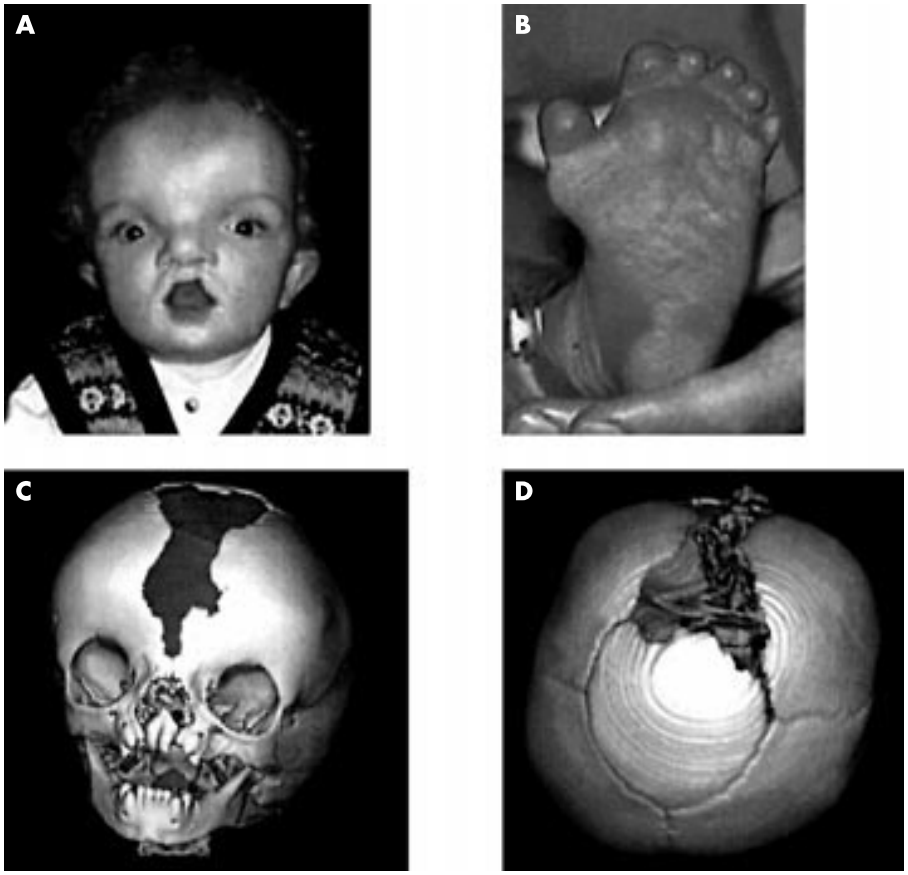

Figure 1 Clinical photographs of the patient with acrocallosal syndrome. (A) Severe hypertelorism with frontal bossing. Note repaired bilateral cleft lip. (B) Left foot soon after birth. There is insertional polydactyly with 2/3 and 3/4 syndactyly. (C, D) MRI cranial reconstruction showing extra bony plate within anterior fontanelle; this is reminiscent of the interfrontal bone seen in the murine *Xt* (extra toes) *Gli3* mutant.

that both this residue and domain 5 are highly functionally significant to these proteins.

Previous case reports have shown that the phenotype associated with visible and submicroscopic 7p13 deletion is of GCPS associated with psychomotor retardation.[8 9] However, the craniofacial manifestations seen in this patient are considerably more severe. It is possible that this represents the action of genetic modifiers or that certain phenotypic manifestations which are dosage sensitive do not always manifest in the haploinsufficient state. However, an alternative explanation of our findings is that the A934P mutation may be acting in a dominant negative fashion whose mechanism differs from those seen in *GLI3* mutations described to date.

There is considerable phenotypic overlap between the ACS and GCPS. Both have similar patterns of polydactyly of the

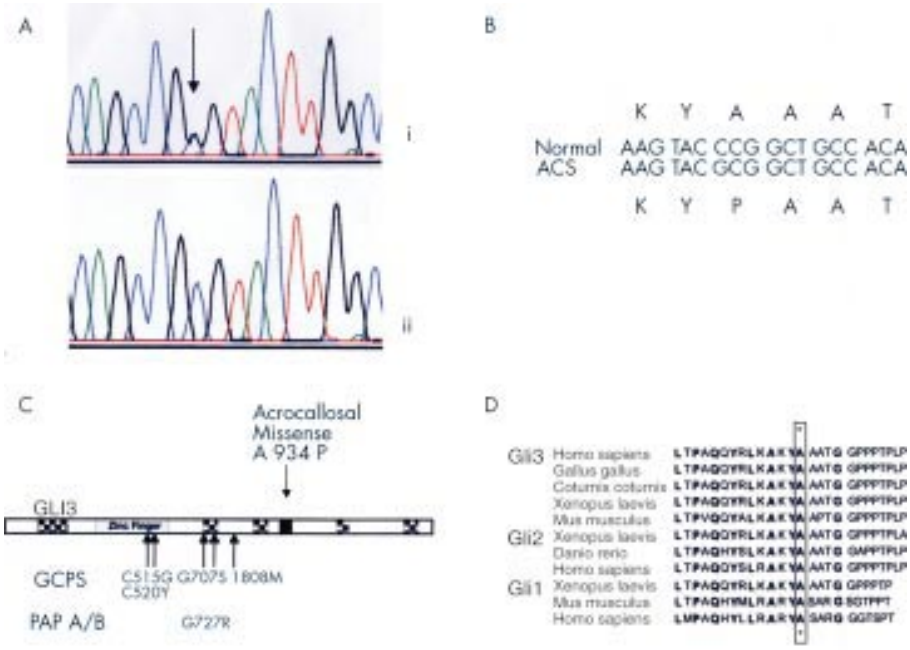

Figure 2 Molecular analysis of the *GLI3* gene. (A) Antisense sequence of *GLI3* coding sequence around the mutation. (i) Heterozygous sequencing of the patient; G-C substitution of nucleotide 2800 is arrowed. (ii) Normal control. (B) *Gli3* sequence, in sense direction, showing mutation position and amino acid substitution. (C) Position of missense mutations within the *GLI3* gene. (D) Evolutionary conservation of *Gli* gene family within fifth conserved domain of *Gli3* including A934.

hands and feet and macrocephaly. Features observed in our patient which usually distinguish ACS from GCPS are agenesis of the corpus callosum and severe mental retardation. However, these features are occasionally reported in GCPS, emphasising the phenotypic and likely genotypic heterogeneity of both syndromes. The identification of this *GLI3* mutation adds weight to the suggestion that GCPS and ACS can represent different manifestations of the same disorder. This is further supported by the similarity of the ACS phenotype in our patient to the *Xt* mouse mutant, which results from a defect in *Gli3*. The identification of an extra interparietal bone within the anterior fontanelle of our ACS patient, a finding described by others,[10] is reminiscent of findings in the mouse model for GCPS (the *Xt* mutant) in which cranial development is disturbed also with the development of an extra interfrontal bone (fig 1C). Since others have excluded *GLI3* in ACS, we suggest that ACS may represent a heterogeneous group of disorders. In some cases the phenotype results from a mutation in *GLI3* and represents a severe, allelic form of GCPS. This indicates that mutations at different sites within *GLI3* may confer a different prognosis in terms of both physical health and intellectual impairment. The finding is important for counselling of families with suspected ACS born to unaffected parents; recurrence risks for those found to carry de novo heterozygous defects involving *GLI3* will not be 25% (that is, those of autosomal recessive inheritance) and will be considerably lower.

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