Normal erythrocyte membrane Gsa bioactivity in two unrelated patients with acrodysostosis

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Abstract
Shortening of the tubular bones of the hands and feet with cone shaped epiphyses is known as peripheral dysostosis and is common to several syndromes including acrodysostosis and Albright's hereditary osteodystrophy (AHO). The underlying defect in AHO is known to be a reduction in bioactivity of the α subunit of the signal transducing protein, Gs, and heterozygous deactivated mutations have been shown in the Gsa gene. Because of additional overlapping clinical and radiological features it has been suggested that acrodysostosis and AHO represent poles of a single diagnostic spectrum. We have measured Gsa bioactivity in two unrelated patients with a clinical diagnosis of acrodysostosis and found both to be normal. Mutation analysis of the Gsa gene showed no sequence variation in 12 of the 13 exons examined. These results indicate that, at least in a proportion of patients with acrodysostosis, the condition is aetologically distinct from AHO.

Keywords: acrodysostosis; pseudopseudohypoparathyroidism; Gsa; Albright's hereditary osteodystrophy.

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Shortening of the tubular bones of the hands and feet with cone shaped epiphyses but without other major skeletal abnormalities was called peripheral dysostosis by Brailsford1 in 1948. Between 1963 and 1971, several authors described a distinctive syndrome characterised by severe generalised peripheral dysostosis associated with nasal hypoplasia and mental retardation. The names PNM and acrodysostosis were suggested, the latter being more widely used.2,3 Additional findings included short stature (often of prenatal onset), relative first ray hyperplasia in the feet, loss of caudal widening of vertebrointerpedicular distances, advanced skeletal maturation, increased mandibular angle, recurrent otitis media and hearing loss, but no biochemical or endocrine disturbance.4

Similar skeletal changes may occur in Albright's hereditary osteodystrophy (AHO) where they are frequently associated with short stature and developmental delay.5 The biochemical defect underlying AHO is a reduction in activity of the α subunit of a signal transducing G protein, Gs, which stimulates intracellular adenyl cyclase in response to various peptide hormones.6 Several heterozygous deactivating mutations in the Gsa gene on chromosome 20q13 have now been described.7 Patients with AHO are divided into two distinct phenotypes known as pseudohypoparathyroidism (PHP) and pseudopseudohypoparathyroidism (PPHP) on the basis of endocrine findings. In those with PHP, resistance can be shown to various hormones which are transduced by Gsa, most notably PTH. In contrast, no endocrine resistance is found in PPHP despite the equivalent reduction in Gsa bioactivity. Ectopic ossification, the basis of which is unknown, occurs frequently but not always in both forms of AHO.

Although recent molecular advances have proved the value of delineating conditions on the basis of consistently observed combinations of clinical features, in some instances closely overlapping conditions have turned out to have a common aetiology. As a result of the phenotypic overlap between acrodysostosis and PPHP, some authors have considered them part of a single disease spectrum.8 9 10 To address this question we have measured Gsa bioactivity and screened for mutations in the Gsa gene in two unrelated children with clinical features of acrodysostosis.

Case reports and methods

Case 1
This is the third of four children born to healthy, unrelated parents. Intrauterine growth retardation was detected prenatally and she was delivered at 38 weeks' gestation by caesarian section, weighing 2020 g (<3rd centile) with a head circumference of 32 cm (3rd-10th centile). There were no particular neonatal problems. Growth has continued below the 3rd centile while head circumference has remained on the 10th. Striking midfacial hypoplasia with a flattened nose, epicanthic folds, and small hands were noted early on. She has had recurrent upper respiratory infections and otitis media associated with febrile convulsions on two occasions. Developmental milestones were delayed with sitting at 11 months, crawling at 15 months, and walking at 2 years. Investigations for delayed speech showed bilateral glaucoma requiring grommets while audiograms indicated a possible additional high frequency hearing loss. Ophthalmological examination at 2 years of age was normal and investigations including renal, liver, and bone biochemical profiles, thyroid function, chromosomes, and urinary amino and organic acids have been normal. Examination at 4 years confirmed persisting midfacial hypoplasia with marked flattening of the nasal bridge and a small antverted nose with a short columella (fig
1A), striking generalised bony shortening in
the hands with medial deviation of the left
middle finger at the PIP joint, and small broad
feet with relatively long halluces bilaterally.
Hand x rays showed generalised shortening of
the tubular bones associated with cone shaped
ephyses (fig 1B).

All three sibs and both parents are of normal
stature with no evidence of peripheral dysosto-
sis or nasal hypoplasia.

CASE 2
This is the only child of healthy, unrelated par-
ents and was delivered at 33 weeks' gestation
by emergency caesarian section because of
intrauterine growth retardation with oligohy-
dramnios and breech presentation. At birth his
weight was 1740 g (<3rd centile), length 44.5
cm (<3rd centile), and head circumference
30.1 cm (<3rd centile). He was noted to have a
flat midface and short digits at birth as well as
mild talipes and some limitation of elbow and
knee extension, presumed secondary to oligo-
hydramnios. Bilateral inguinal hernias were
repaired. Hearing impairment was detected on
routine screening and tympanograms indicated
middle ear fluid for which grommets were
inserted. Subsequent hearing tests have shown
additional mild sensorineural hearing loss.
Bone biochemistry profiles and thyroid func-
tion were normal at 8 months. Further inves-
tigation at 21 months showed normal serum cal-
cium but slightly raised phosphate (1.6 mmol/
l), urea (11.2 mmol/l), and creatinine (73
µmol/l), and normal TRH, arginine stimula-
tion, and synacthen tests. Renal ultrasound
showed bilateral hydronephrosis and mega-
ureters secondary to reflux in micturating
cystography. Renal function has remained sta-
bly reduced on antibiotic prophylaxis. Head
circumference, initially progressing along the
3rd centile, increased to the 50th centile when
assessed at 2 years 7 months. CT scan of the
head was normal. Currently, at 4 years 9
months, his height and weight are on the 3rd
centile and OFC is on the 50th-75th centile.
He has pronounced midfacial hypoplasia (fig
2A), a flat nasal bridge, small anteverted nose
with a short columella, and striking shortening
of all the tubular bones of the hands and feet
(fig 2B). Developmental assessment has con-
firmed mild global developmental delay. His
father's height is above the 97th centile and
mother's between the 10th and 25th centiles.

MEASUREMENT OF GSL BIOACTIVITY
Five millilitres of venous blood in citrate
anticoagulant was frozen on dry ice and stored
at -70°C. Measurement of erythrocyte GSL
bioactivity was by cysc reconstitution assay as
described by Bourne et al (1983) and mem-
branes were kindly provided by Dr C Van Dop.
Purification of [32P]cAMP was as described by
Salomon et al (1974) using Dowex Alumina
chromatography and [3H]cAMP to monitor
Table 1. Results of erythrocyte Gsa bioactivity measurement expressed as a percentage of that of a concurrent sample from a healthy unrelated control.

<table>
<thead>
<tr>
<th>Assay 1 (%)</th>
<th>Assay 2 (%)</th>
<th>Assay 3 (%)</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>93</td>
<td>103</td>
<td>107</td>
</tr>
<tr>
<td>Case 2</td>
<td>126</td>
<td>101</td>
<td>122</td>
</tr>
</tbody>
</table>

recovery. Sample eluates were counted in 8 ml of scintillation fluid (Universal ES) in a dual channel scintillation counter. Values are the mean of duplicates corrected for cAMP recovery and expressed as a percentage of the [³²P]cAMP production in a concurrent sample from an unrelated healthy control.

DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) ANALYSIS OF EXONS 2-13 OF THE GSL GENE

Genomic DNA was extracted from peripheral lymphocytes using standard methods and screened by the mutational analysis procedure of DGGE as described elsewhere (Oude Luttikhuis et al., manuscript in preparation). Positive controls for exons 3-10 and 12 were included.

Results

GSA BIOACTIVITY

Both patients had normal erythrocyte Gsa bioactivity as measured on three separate assays (table 1). Six unrelated subjects diagnosed with AHO (either PHP based on physical and endocrine findings or PPHP based on an AHO appearance without hormone resistance but either a first degree relative with PHP or the presence of cutaneous ossification) had Gsa bioactivity ranging from 53-73% (mean 62%) of the same control sample over these assay runs.

GSA GENE MUTATION SCREENING

No sequence variations were detected on screening of exons 2-13 inclusive by DGGE (data not shown).

Discussion

We have shown normal Gsa bioactivity in two unrelated patients with acrodysostosis. Furthermore, we did not detect any sequence variation in a screen of 12 of the 13 exons and the adjacent splice sites of the Gsa gene assessed by DGGE, a procedure reported to approach 100% efficiency. The remaining exon 1 was not analysed because the very high GC nucleotide content makes it unamenable to screening by DGGE. In 17 unrelated patients with AHO in whom we have detected Gsa mutations, including those with exon 1 mutations detected by direct sequencing, all had reduced Gsa bioactivity (Oude Luttikhuis et al., manuscript in preparation). These results indicate that acrodysostosis in these patients is not caused by deactivating mutations in the Gsa gene, in contrast to AHO.

The clinical features of AHO and acrodysostosis are summarised in table 2. Although translated literally "acro"-dysostosis and "peripheral" dysostosis are synonymous, the latter is generally used in a descriptive sense. As such, peripheral dysostosis is a prominent feature of both acrodysostosis and AHO and may be a feature of other conditions including acromesomelic dysplasia, acromicric dysplasia, brachdactyly E, trichorhinophalangeal syndrome, and the Turner syndrome. In AHO, the resulting pattern of brachymetaphalangism may be as severe and generalised as in acrodysostosis, but is usually milder and more marked in the ulnar metacarpals and distal phalanges. When present, other features may be helpful in distinguishing the two. Endocrine disturbance and cutaneous ossifications are not features of acrodysostosis. The short stature of acrodysostosis is often of prenatal onset and shortening of the extremities may be noted at birth, in contrast to AHO where birth weights and lengths are often normal and bony shortening of the hands and feet is frequently not manifest until 3 or 4 years of age (unpublished data). Epiphyseal stippling, reported in the first few months of life in seven children with acrodysostosis, has not been reported in AHO, although this may reflect less obvious clinical indications for radiographs in infancy. Shortening of the nose and flattening of the nasal bridge is common in AHO but it is not usually striking, unlike acrodysostosis where there is marked nasal hypoplasia, anteverision of the nares, a deficient columella, proagmatism, and flattening of the zygomas with prominence of the upper alveolar process causing a rather concave midface and open mouth appearance.

Occasional patients have been reported where the diagnoses of acrodysostosis and AHO were felt to coincide or to be indistinguishable. Ablow et al. reported two unrelated people of whom the first had clinical features, including soft tissue calcifications and resistance to PTH, which leaves little doubt about a diagnosis of PHP. However, the nasal bridge was felt to be flattened to a greater extent than is usual in AHO. In contrast, the second patient had typical facial features of acrodysostosis without endocrine abnormalities or cutaneous ossification. In the report of Davies and Hughes, the absence of generalised severe brachymetaphalangism or marked nasal hypoplasia with deficient columella are more consistent with the PPHP form of AHO. In a further family, two sibs with typical features of acrodysostosis have been described.

Table 2. Summary of clinical features of acrodysostosis and AHO.

<table>
<thead>
<tr>
<th>Peripheral dysostosis</th>
<th>Severe, generalised</th>
<th>Variable, may be generalised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facial features</td>
<td>Severe nasal and maxillary hypoplasia</td>
<td>Round face, mild midfacial hypoplasia</td>
</tr>
<tr>
<td>Short stature</td>
<td>Frequent, prenatal onset</td>
<td>Frequent, postnatal onset</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>Mild/moderate</td>
<td>Mild/moderate</td>
</tr>
<tr>
<td>Hormone resistance</td>
<td>Absent</td>
<td>PHP type I only</td>
</tr>
<tr>
<td>Soft tissue ossification</td>
<td>Absent</td>
<td>~ 50%</td>
</tr>
<tr>
<td>Epiphyseal stippling</td>
<td>Neonatal period</td>
<td>Not reported</td>
</tr>
<tr>
<td>Bone age</td>
<td>Frequently advanced</td>
<td>Frequently advanced</td>
</tr>
<tr>
<td>Vertebral interpedicular distance</td>
<td>Loss of caudal widening</td>
<td>Loss of caudal widening</td>
</tr>
<tr>
<td>Forearm brachymelia</td>
<td>Common</td>
<td>Not usually marked</td>
</tr>
<tr>
<td>Relative 1st ray hyperplasia</td>
<td>Common</td>
<td>Not usually marked</td>
</tr>
<tr>
<td>Delayed dentition</td>
<td>Common</td>
<td>Common</td>
</tr>
<tr>
<td>Otitis media/glue ear</td>
<td>Common</td>
<td>Common</td>
</tr>
<tr>
<td>Inheritance</td>
<td>Probably AD</td>
<td>AD with parental origin effect</td>
</tr>
</tbody>
</table>
whose mother had normal stature and intelligence, mild nasal hypoplasia, and variable bony shortening in the hands and feet, closely resembling PPHP phenotypically. She may represent a somatic mosaic for acrodysostosis. These families illustrate the difficulties that may occur differentiating these diagnoses clinically.

The two patients we report closely resemble the original phenotypic descriptions of acrodysostosis. While at a molecular level there may be heterogeneity underlying the acrodysostosis phenotype, our findings of normal Gsα bioactivity in conjunction with the negative Gsα mutation screen support the contention that at least in a proportion of patients this condition is aetio logically distinct from AHO.

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