Familial cryptic translocation between chromosomes 2qter and 8qter: further delineation of the Albright hereditary osteodystrophy-like phenotype

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Abstract
Recently five patients with an Albright hereditary osteodystrophy (AHO)-like phenotype were reported to have a subtelomeric deletion of the long arm of chromosome 2. These patients showed a striking resemblance to a number of patients from a large pedigree known to us for a long time. After molecular confirmation of a subtelomeric deletion in one patient, FISH analysis was used and a cryptic translocation between the long arms of chromosomes 2 and 8, t(2;8)(q37.3;q24.3), was detected. Remarkably, five proven and 10 probable cases with a 2qter deletion were found in the family, but none with an 8qter deletion. This was not explained by increased fetal loss.

The major clinical characteristics of terminal 2q deletion are a short, stocky build, round face, sparse hair, deeply set eyes, bulbous nose, thin vermilion border, brachymetaphalangism, seizures, and developmental delay. A specific behavioural phenotype consisting of periods of hyperkinesia and aggression can develop with age. The overall phenotype is sufficiently characteristic to allow clinical recognition.

The cytogenetic and molecular studies did not narrow down the common deleted region. Both testing of additional 2q markers and characterisation of other AHO-like patients with 2q37 microdeletions may help to define the candidate gene region.

Keywords: cryptic translocation; t(2;8); 2q deletion; AHO-like phenotype

Clinical geneticists are regularly confronted with patients in whom clinical features or the pedigree suggest a chromosome abnormality, but in whom cytogenetic analysis fails to show an unbalanced karyotype, even on high resolution banding. In such cases, a submicroscopic chromosome abnormality can be present. Fluorescence in situ hybridisation (FISH) studies may be very helpful in this respect.

In 1968, a member of such a family was referred to our centre, followed by numerous others. These family members all showed mental retardation in combination with skeletal abnormalities. At that time the diagnosis of Albright hereditary osteodystrophy (AHO) was made (see Wilson et al for review). After more specific investigations, the patients were considered to have pseudopseudohypoparathyrodism (PPHP). In 1980 it became known that Gsα activity was reduced in patients with PHP and PPHP. Measurement of Gsα in one patient in this family, however, failed to show any abnormalities, raising doubts regarding the diagnosis. When Wilson et al recently described five patients with an AHO-like phenotype, including a normal Gsα activity, and with subtelomeric deletions of the long arm of chromosome 2, the resemblance to the phenotype and history of our family was striking.

Here, we report on the results of molecular testing of one of our patients in whom molecular studies showed a terminal 2q deletion and on the clinical findings and cytogenetic studies using fluorescence in situ hybridisation (FISH) in the extended family. A detailed clinical description of the patients will hopefully contribute to the spectrum of the AHO-like phenotype.

Patients
The pedigree of the family under study is presented in fig 1.

No family member is known to have had recurrent miscarriages. Six out of 13 children of II.1 died shortly after birth (III.7-12). On four of them there is no information; one died at 6 weeks and one at 8 days, both of unknown causes.

INDEX CASE (V.10)
Patient V.10 was not the first patient seen in this family, but as her sister asked for genetic counselling, and at that time the definitive diagnosis was made, we regard her as the index patient in this family.

V.10 is a 31 year old female, born after an uneventful pregnancy and delivery. In the neonatal period she suffered from recurrent periods of vomiting, for which no definite cause could be found. In infancy she had recurrent bladder infections and upper airway infections. Furthermore, she repeatedly had convulsions without fever. Developmental delay became apparent in early childhood. At the age of 3 years 3 months bilateral inguinal herniae were surgically corrected. She had extreme hyperkinesia and aggressive behaviour, necessitating institutionalisation from the age of 3 years. At present she is severely mentally retarded and lives in an institution for the mentally handicapped.
The results of physical examination at the age of 9 years are summarised in table 1. Many symptoms, such as mental retardation, stocky build, epilepsy, facial dysmorphism (fig 2A, B), and brachymetaphalangism (figs 3 and 4A), fit the Albright hereditary osteodystrophy-like phenotype.

Internal screen, EEG, 24 hour urinary screen for metabolic disorders, blood investigation (including calcium, phosphate, PTH, urea, creatinine, vitamin D), response to exogenous PTH, and cytogenetic analysis (850 bands) were all normal.

X-ray showed an advanced bone age (2½ years at the age of 2 years, 10½ years at the age of 9½ years). As with other patients in this family, she did not have any (sub)cutaneous calcifications.

OTHER PATIENTS (V.3, V.19, V.21)

Patients V.3, V.19, and V.21 were alive at the time of this study. Patients IV.6, IV.17, V.7, and V.20 have died, but were carefully examined in the past. All showed symptoms compatible with the AHO-like phenotype. A summary of the major clinical findings is provided in table 1. Clinical photographs of the patients are shown in figs 2C-H and 3A (patient V.19) and B (patient V.21); characteristic radiographs are shown in fig 4B (patient V.19) and C (patient IV.6).

POSSIBLE PATIENTS (III.6, III.19, IV.3, IV.4, IV.19, IV.20, IV.27)

According to anamnestic data, these family members may have been patients, but firm data are lacking. All were reported to have had developmental delay or mental retardation, combined with poor speech in III.19. Apart from IV.3 and IV.4, all were known to have suffered from convulsions.

Skeletal abnormalities are reported less frequently: III.19 was said to have a "disabled left foot and right arm" and IV.27 had short stature (about 1.5 m as an adult).

BEHAVIOURAL PHENOTYPE

Most patients are friendly (V.3, V.19, V.20, V.21), though with advancing age occasional outbursts of aggression (V.19, V.20) and obsessive behaviour (V.20) have been reported. Hyperkinetic behaviour is reported frequently (IV.3, V.7, V.20), sometimes only in childhood (V.19). Patient V.21 self-mutilates when she is frustrated (hand biting).

Patient IV.27 showed destructive behaviour, which necessitated institutionalisation from the age of 7, but patient V.19 lives with her parents and is able to join her family in outdoor activities, such as cycling, swimming, and skating. Patient IV.6 was known to have mental retardation in combination with a psychiatric disorder, probably psychosis.

GENERAL HEALTH

In the neonatal period patient V.19 suffered from unexplained cyanotic episodes, and she had feeding difficulties and a weak cry. During infancy some patients suffered from recurrent upper airway (V.3 and V.20) and ear infections (V.3). Menarche occurred around the age of 14 years (V.19, V.20, V.21).

CAUSES OF DEATH

Most of the (possible) patients died from infectious diseases. Patients III.6, IV.4, and...
IV.17 died from pneumonia at the ages of 15 years, 16 months, and 40 years respectively. III.19 died from an influenza infection aged 21 years. IV.5 died at the age of 30 years from peritonitis, and IV.20 died from diphtheria at the age of 3 years. Patient V.20 had recurrent pneumonia, but died from choking at the age of 38 years. Patients IV.19 and IV.27 died from status epilepticus, aged 16 and 19, respectively. V.7 died at the age of 19 years from a mediastinitis resulting from a perforated gastric ulcer.

Additional Investigations
A CT scan of the brain of V.3 at the age of 19 years showed minimal peripheral atrophy, but was otherwise normal. Plasma calcium and phosphate levels have been found to be normal in IV.17, V.3, and V.7. Investigations in V.3 showed normal levels of urea, creatinine, vitamin D, and intact PTH. At the age of 4 years the bone age in patient V.3 was three years advanced.

Material and methods
Genetic marker studies
Genomic DNA from the index patient (V.10) and her parents was prepared from peripheral blood lymphocytes by standard methods. Thirteen polymorphic 2q markers were typed as described previously.

Cytogenetic studies
Prometaphase chromosomes from peripheral lymphocytes were obtained using a double

Table 1: Summary of clinical features

<table>
<thead>
<tr>
<th>Wilson et al</th>
<th>This study</th>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>V.10</td>
</tr>
<tr>
<td>Age at examination (y)</td>
<td>12</td>
</tr>
<tr>
<td>Gender</td>
<td>F</td>
</tr>
<tr>
<td>Developmental delay (1)</td>
<td>+++</td>
</tr>
<tr>
<td>Height (&lt;3rd centile)</td>
<td>50</td>
</tr>
<tr>
<td>Weight (centile)</td>
<td>25</td>
</tr>
<tr>
<td>Stocky build</td>
<td>+</td>
</tr>
<tr>
<td>Behaviour (2)</td>
<td>A, M</td>
</tr>
<tr>
<td>Seizures</td>
<td>+</td>
</tr>
<tr>
<td>OFC (centile)</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Promont upper lip</td>
<td>+</td>
</tr>
<tr>
<td>Prominent upper lip</td>
<td>+</td>
</tr>
<tr>
<td>Prominent forehead</td>
<td>+</td>
</tr>
<tr>
<td>Prominent columella</td>
<td>+</td>
</tr>
<tr>
<td>Ears (8)</td>
<td>P</td>
</tr>
<tr>
<td>Fleshly earlobes</td>
<td>+</td>
</tr>
<tr>
<td>Eczema</td>
<td>+</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>+</td>
</tr>
<tr>
<td>Brachymetacarpalism</td>
<td>+</td>
</tr>
<tr>
<td>Contractures</td>
<td>+</td>
</tr>
<tr>
<td>Scoliosis</td>
<td>+</td>
</tr>
<tr>
<td>Feet (9)</td>
<td>S, B</td>
</tr>
<tr>
<td>Prominently placed thumbs</td>
<td>+</td>
</tr>
<tr>
<td>Hypoplastic ulna</td>
<td>+</td>
</tr>
<tr>
<td>Menses (10)</td>
<td>A</td>
</tr>
<tr>
<td>Other</td>
<td>I</td>
</tr>
</tbody>
</table>

+ definitely positive, − definitely negative, (+) probably positive, (−) probably negative
(1) +, mild; ++, moderate; ++++, severe
(2) A, aggressive; M, self-mutilation; H, hyperkinetic
(3) S, sparse; F, fine; W, white; A, alopecia areata; AT, alopecia totalis
(4) R, round; S, asymmetrical
(5) P, prominent; S, sparse; A, absent; med, medial; lat, lateral
(6) B, bulbous; S, short
(7) Ma, macrognathia; Mi, micrognathia
(8) L, large; P, prominent
(9) S, small; B, broad
(10) A, secondary amenorrhoea; i, irregular; r, regular
(11) U, umbilical; I, inguinal; H, hiatus hernia
(12) Crouzon syndrome
(13) Slow growing hair, ptosis, upward slanting palpebral fissures, bilateral cataract
(14) Keratocutaneous, beaked nose, growth hormone deficiency
(15) Flat midface, myopia, bilateral hip dislocation, dislocated radial heads, bowing of radius and ulna, joint laxity, mild epiphyseal dysplasia
(16) Hypothyrominaemia in neonatal period, feeding difficulties
(17) Short philtrum, thick neck
(18) Mild pectus excavatum, bilateral hallux valgus, lumbar lordosis, shortening of the right leg
(19) Rotating nystagmus, low nasal bridge, ileus, diplagia, flat feet
(20) Neonatal feeding difficulties, upward slanting palpebral fissures, protruding maxilla, small mouth, small, thick tongue, hyponoamyia
(21) Psychiatric disorder, exanthema affecting the skin of his arms, legs, and trunk, bilateral cubital valgus, genu valgum, pes planus and valgus, dystrophic nails of the halluces, ileus
(22) Pale fundus, hearing loss, prominent ribs secondary to scoliosis, recurrent urinary tract infections
(23) Poor speech, upward slanting palpebral fissures, bilateral cubital valgus
synchronisation technique. Fluorescence in situ hybridisation (FISH) was performed as described previously. Slides were examined under a Zeiss Axioplan epifluorescence microscope. For digital imaging microscopy the Cytovision Probe system (Applied Imaging) was used.

Cosmids cCI2-3 (2q36.3-37.1) and cCI2-60 (2q37.1-37.3), and cos2003 were used for in situ hybridisation. Cos2003 is a subclone from half-YAC yRM2003 (D2S446, D2Z4) and recognises subtelomeric DNA in the long arm of chromosome 2 (2q37) and sequences on chromosome 8p23.

**Results**

**ANALYSIS OF MICROSATellite MARKERS**

A total of 13 polymorphic markers mapping within 2q37 were analysed, indicating in V.10 a paternal deletion of markers D2S395, D2S90, D2S140, and D2S125 (provisional centromeric to telomeric order according to Wilson et al). No informative marker centromeric to D2S395 or telomeric to D2S125 has yet been found. Given this result and the pedigree structure, the possibility of a cryptic translocation involving 2q37 was raised.

**FISH STUDIES**

In earlier studies, chromosome analysis (both routine and prometaphase) had never shown any chromosomal abnormality in patients IV.6, IV.17, V.3, V.7, V.10 or their parents (fig 5A). Blood lymphocytes from patients V.3, V.10, V.19, and V.21 and their parents IV.1, IV.18,
IV.26, and IV.28 were studied with FISH probes. Fibroblasts from V.7 had been stored for over 20 years and were used for FISH analysis.

In all patients hybridisation of metaphases with the 2q probe (cos2003) showed a signal on one chromosome 2 and, because this probe cross hybridised with chromosome 8p, showed signals on the short arms of both chromosomes 8 (fig 5B). This hybridisation pattern with cos2003 confirmed the molecularly detected deletion of chromosome 2q. Hybridisation with cosmids cCI2-3 and cCI2-60 showed normal hybridisation patterns, indicating a position proximal to the breakpoint on chromosome 2q.

To establish the other chromosome in a presumed translocation, we hybridised cos2003...
Familial t(2;8) and AHO-like phenotype

with metaphases of an obligate carrier (fig 5C).
The translocated fragment was located on 8q,
indicating the presence of a cryptic transloca-
tion t(2;8)q37.3;q24.3.

In this family, all available patients with
Albright hereditary osteodystrophy-like syn-
drome and mental retardation (V.3, V.7, V.10,
V.19, and V.21) showed a terminal deletion of
chromosome 2q. No family member showed a
terminal deletion of chromosome 8q24.3.

Discussion
A familial cryptic translocation between the
long arms of chromosomes 2 and 8,
t(2;8)(q37.3;q24.3), is reported, giving rise to
a proven subtelomeric deletion of chromosome
2q in five family members and a probable dele-
tion in another 10 members. An AHO-like
phenotype is linked to this unbalanced karyo-
type. The other unbalanced karyotype (trisomy
of the subtelomeric region of chromosome 2q
and a deletion of the subtelomeric region of
chromosome 8q) was not detected. There may
be several explanations for its absence. It is
possible that one or more of the patients who
had died carried this genotype. However, as
most members were reported to have had mental retardation and convulsions and some
had short stature and brachydactyly, the 2qter
deletion is more likely in them. Not all
members were examined personally, however,
hence some of them may still have had an 8qter
deletion/2qter duplication. Alternatively, the
8qter deletion genotype may have a very mild
phenotypic effect, if any, and may go unrecog-
nised. This possibility cannot be excluded, as
too few family members with a normal pheno-
type may have been karyotyped. A third explana-
tion may be either early fetal loss, which is not
known to have occurred repeatedly in the fam-
ily, but may have gone unrecognised if
occurring in the first weeks of pregnancy, or
prenatal and perinatal death, known to have
occurred nine times in three branches of the
family.

The clinical features of AHO are short,
stocky build, round face, cutaneous
ossifications, hypocalcaemia, and hyperphosphatae-
ia. In the AHO-like phenotype, cutaneous
ossification has not been described and
calcium and phosphate levels are normal. To
date there are several case reports describing patients with deletions of chromosome 2q
(recently reviewed by Power et al4). These
reports and the description of our family led to
the delineation of a syndrome consisting of
mental retardation, short stature, brachymeta-
phalangism, epilepsy, and facial dysmorphism
(table 1). Life span appears to be reduced; in
several of the present cases death was caused by
lower airway infections. Though the patients
are generally friendly, their behaviour can be
complicated by hyperkinesia, aggression, self-
mutilation, or frank psychiatric problems.

It is unknown how the AHO-like phenotype
in this family is affected by the duplication of
the terminal region of chromosome 8q. To the
best of our knowledge, duplications of band
8q24.3 only have never been described. There-
fore we are unable to compare a 8qter duplica-
tion phenotype, if any, with the summarised
phenotype in this family.

The chromosome region for one or more
genes for the AHO-like phenotype is defined by
microsatellite marker analysis in a series of five
patients by Wilson et al. The centromeric
boundary of the common deleted region is
marked by D2S338 and the minimal region of
deletion overlap involves D2S125, the most
telomeric 2q37 marker used. Microsatellite
marker analysis in our family has not helped to
define the region, since all markers centromeric
to D2S395 were uninformative and the exten-
sion of the deletion at the molecular level is
therefore unknown. Both testing of additional
2q markers and identification of other AHO-
like patients with 2q37 microdeletions may
help to narrow down the candidate gene
region.

After years of investigation in this family, a
cryptic chromosome translocation was found
to be the cause of the familial mental
retardation syndrome. In patients where an
unbalanced karyotype is highly likely, but can-
not be detected by routine cytogenetic investi-
gations, we recommend careful comparison with
similar cases that have been published or that
are in a cytogenetic database. Corresponding
cases or specific symptoms entered in a
database query may provide a clue regarding
chromosome regions that might be involved.

Based on the localisation of these regions,
probes for FISH analysis can be selected and
tested for imbalances. It can be expected that
the development of new techniques (for exam-
ple, 24 colour FISH) will simplify this strategy
in the near future, allowing a diagnosis in more
of our “unknowns”.

We wish to thank Dr Harold C. Riethman for the YRM2003 subclone, the Japanese Cancer Research Resources Bank for the cCl2-cosmids, and the family for their willingness to participate
in this study.

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