CASE REPORT

Two cases of deletion 2q37 associated with segregation of an unbalanced translocation 2;21: choanal atresia leading to misdiagnosis of CHARGE syndrome

Eduardo Fernández-Rebollo^{1,5}, Olga Pérez², Cristina Martínez-Bouzas³, Maria Carmen Cotarelo-Pérez⁴, Intza Garin^{1,5}, Jose Luis Ruibal², Gustavo Pérez-Nanclares¹, Luis Castaño^{1,5} and Guiomar Pérez de Nanclares^{1,5}

¹Endocrinology and Diabetes Research Group, Molecular Genetics Lab, Hospital de Cruces, Cruces-Barakaldo E48903, Bizkaia, Basque Country, Spain, ²Pediatric Endocrinology Unit, Department of Pediatrics, Hospital Clínico Universitario San Carlos, Madrid, Spain, ³Molecular Genetics Lab of Service of Biochemistry, Hospital de Cruces, Barakaldo, Bizkaia, Spain, ⁴Department of Clinical Genetics, Hospital Clínico Universitario San Carlos, Madrid, Spain and ⁵Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ISCIII, Barakaldo, Bizkaia, Spain

(Correspondence should be addressed to G Perez de Nanclares; Email: gnanclares@osakidetza.net)

Abstract

Context: The phenotypic variability of patients with syndromes presenting with dysmorphism makes clinical diagnosis difficult, leading to an exhaustive genetic study to determine the underlying mechanism so that a proper diagnosis could be established.

Objective: To genetically characterize siblings, the older sister diagnosed with Albright hereditary osteodystrophy and the younger one with CHARGE syndrome.

Design: Clinical case report.

Methods: Clinical, biochemical, and radiological studies were performed on the family. In addition, molecular genetic studies including sequencing of *GNAS*, typing of microsatellites on 2q and 21q, and multiplex ligation-dependent probe amplification of subtelomeric regions were performed, as well as confirmatory fluorescent *in situ* hybridization analysis.

Results: The genetic analysis revealed that both sisters presented a 2q37 deletion due to the maternal unbalanced segregation of a 2;21 translocation.

Conclusions: This is the first report of a 2q37 deletion where differential diagnosis of CHARGE syndrome is needed due to the appearance of choanal atresia.

European Journal of Endocrinology 160 711–717

Introduction

Albright hereditary osteodystrophy-like (AHO-like) syndrome (OMIM 600430) has recently been defined as a rare syndrome characterized by features including distinctive dysmorphism, developmental delay with mild to severe mental retardation, stocky build and short stature in the majority of cases, brachymetacarpia (most commonly on 3rd, 4th and 5th fingers), brachyphalangia of terminal phalanges, and brachymetatarsia with a characteristic large first interdigital space. Dysmorphic features include rounded face with frontal bossing, upslanting palpebral fissures, strabismus, low flattened nasal bridge, sparse and fine hair, low-set ears with large fleshy lobes and prominent columnella (1–3).

This combination of brachymetaphalangia and mental retardation also occurs in AHO (4), a dysmorphic syndrome that is also associated with rounded face, short stature, obesity, brachydactyly, ectopic ossifications, and/or mental retardation (4, 5). Individuals affected with AHO may have either pseudohypoparathyroidism

(PHP), with end organ resistance to PTH and certain other cAMP dependent hormones, or pseudopseudohypoparathyroidism (PPHP) with normal hormone responsiveness (6).

The molecular defect responsible has been identified as a reduction in membrane levels of the α subunit of stimulatory protein G ($G_{s\alpha}$) that transduces signals between hormone receptors and adenylyl cyclase, and deactivating mutations have been reported in *GNAS*, the gene encoding $G_{s\alpha}$, located on chromosome 20q13 (7).

The main feature that distinguishes between AHO and AHO-like is the normal activity of the $G_{s\alpha}$ in the latter, so there are no abnormalities in PTH or calcium metabolism and the *GNAS* gene is not involved. Terminal deletions on chromosome 2 with breakpoints at or within band 2q37, ranging from visible abnormalities to cryptic, and subtelomeric deletions have been described in patients with AHO-like syndrome (8).

A recent review of the clinical features associated with chromosome 2q37 deletions revealed that apart from the AHO-like phenotype, major malformations occur in about 30% of cases. Congenital heart

malformations have been noted in up to 20% of these patients, with aortic arch hypoplasia or coarctation occurring in a disproportionately high percentage of children (8). Other major features include gastrointestinal and renal anomalies (3), genitourinary malformations (horseshoe kidney, hypospadias, hypoplastic or dysgenetic gonads, bifid uterus and undescended testes) (9), and CN malformations (3). Other features have occasionally been observed such as aggressivity, seizures, eczema, nipple abnormalities (inverted, supernumerary, widely-placed or low-set), scoliosis and bone anomalies such as hypoplastic ulna, vertebral abnormalities, pectus excavatum, and bilateral dislocated hips with acetabular dysplasia (1, 3, 8).

We report a family in which a 2;21 translocation is segregating and the clinical phenotype of these family members is described.

Materials and methods

Case report

The index case is a 14.9-year-old female, the third child of healthy unrelated parents, born after a normal pregnancy (though little fetal movement was reported by the mother) and delivery at 40 weeks of gestation (weight 4000 g, length: 56 cm). The mother was 28, and the father 32 years of age at the time of the pregnancy. Hypotonia was diagnosed in the first days of life. She suffered from viral meningitis at the age of fifteen days and presented with several urinary tract infections in early infancy. Imaging techniques showed a right renal ptosis and duplication of the collecting system.

She was referred to us at the age of 2 due to obesity. The patient also had developmental delay, initially thought to be due to the meningeal infection contracted as a neonate. Physical examination revealed some dysmorphic features including a rounded face with blepharofimosis, divergent strabismus, cupping of pinnae, short and wide neck, wide thorax and widely-spaced nipples, abundant nevus, kyphoscoliotic deformity, and short stature for her target height (-2.7 SDS) (Fig. 1A and B). Both hands and digits appeared short and stubby, with markedly short third, fourth, and fifth metacarpals and fourth metatarsals (Fig. 1C and D).

Plasma calcium, phosphate, urea, creatinine, magnesium, vitamin D, and intact PTH were all within normal limits, as was thyroid function. $Gs\alpha$ activity levels were not available. The patient had a normal karyotype 46,XX (Turner syndrome excluded) and AHO was considered the most likely diagnosis.

On follow-up her height has been in p25–50 for the Tanner population, with a normal pre-pubertal growth velocity, but without a clear pubertal growth spurt. Bone age is not delayed. Thelarche was apparent by age 12. The patient is now 14 years old and 156 cm tall which is normal for the reference population, but short

for her genetic target height (-3.7 SDS). Pubertal development is at Tanner stage III and menarche has not begun yet. The excess weight (maximum body mass index $+2.1$ SDS) has been controlled through diet and exercise.

The family history was significant for a previous miscarriage, and a sister was diagnosed as having CHARGE syndrome. In addition, there was an unaffected healthy brother.

Her sister is a 19-year old girl with mental retardation and similar phenotype (Fig. 1E–G). She was born after a normal pregnancy at 40 weeks of gestation by instrumental delivery, with a birth weight of 4000 g and microcephaly. In the immediate neonatal period, she was diagnosed with type III esophageal atresia, unilateral choanal atresia, and congenital heart malformation including intraventricular septal defect and aortic coarctation that required surgery. Gastroesophageal reflux required gastrostomy for nutritional support. She also presents right hemihypertrophy. The phenotype includes round faces with diminished palpebral fissure, convergent strabismus, low-set and cup-shaped pinnae, bilateral brachydactyly of the fourth digit on hands and feet and fifth finger clinodactyly on the left hand. Her pubertal development was completed with menarche at age 13. She is 162 cm tall, on p50 for Tanner reference population but short for her genetic height (-2.7 uds).

Molecular studies

GNAS gene analysis Genetic analyses were performed after informed consent of the parents, upon approval of the study by the Institutional Review Board. Genomic DNA was extracted from peripheral blood leukocytes using a commercial kit (NucleoSpin Blood, Macherey-Nagel, Germany). Sequencing of the thirteen coding exons of *GNAS* and methylation analysis of the region was performed as previously described (10).

2q37 and 21q analysis The polymorphic microsatellite markers *D2S125*, *D2S140*, *D2S395* mapping within the 2q37; *D21S337* (21q22.13), *D21S156*, *D21S155*, *D21S1840* (21q22.2); *D21S1974*, *D21S1937*, *D21S2057*, and *D21S1446* (21q22.3) were typed by fluorescent PCR using primers published in the public genome database. Amplified products were run on an ABI3130XL instrument (Applied Biosystems, Foster City, CA, USA) and analyzed by GeneMapper v4.0 software (Applied Biosystems).

Multiplex ligation-dependent probe amplification Subtelomeric regions were also studied by multiplex ligation-dependent probe amplification (MLPA) using the P036B kit (MRC-Holland, Amsterdam, The Netherlands). The protocol was performed following the manufacturer's recommendations. Analysis of the

Figure 1 Facial appearance of the index (A) and sister (E) showing the round face and strabismus. Both sisters have somewhat unusual eyebrow configuration, abnormal nasal alae, and thin upper lips. Profile of the index (B) and sister (F) showing the cupping of pinnae and short and wide neck. Hands of the index (C) and sister (G), and feet of the index (D) showing shortened metacarpals and metatarsals. Written consent was obtained from the patients for publication of these images.

MLPA PCR products was performed on an ABI3130XL genetic analyzer using GeneMapper software (Applied Biosystems). Target imbalances were determined based on ratios of the relative peak areas. Duplications and deletions were considered when outside the ± 0.30 range.

Cytogenetic and fluorescent in situ hybridization studies The cytogenetic study in the index case and family was made using G-banding techniques on cultured peripheral lymphocytes. Fluorescent *in situ* hybridization (FISH) analysis was performed on lymphocyte metaphase chromosomes with chromosome specific subtelomeric probes (mixtures two and four) according to the manufacturer's specifications (ToTel-Vysion TM Multi-color DNA Probe Mixtures, Vysis, Inc., Downers Grove, IL, USA). Images visualized on a fluorescence microscope were captured on a MetaSystems workstation (Altussheim, Germany).

Results

The molecular study of the *GNAS* gene revealed no alteration in the index case confirming a putative diagnosis of AHO-like syndrome. Subsequent typing of the whole family for a range of microsatellites located at the 2q37 band revealed loss of the maternal allele in both sisters. The maternal genotype was ambiguous as both homo- and hemizygosity were possible for the more telomeric markers (Table 1).

These initial results prompted us to investigate the subtelomeric region of the genome in the mother, the index, and the sister. The telomeric MLPA analysis confirmed both the balanced translocation of 21q and 2q in the mother and the amplification of the 21q

Table 1 Results from analysis of 2q polymorphic markers in the reported family.

	Father	Mother	Index	Sister
D2S125	86/88	86/90	88	88
D2S140	149/155	151	149	149
D2S395	145	165	145	145

subtelomeric region and deletion of the maternal 2qter region in the daughters due to unbalanced segregation (Fig. 2).

The proband's karyotype was defined as 46,XX. Given the MLPA result, FISH was performed on cultured peripheral lymphocytes and revealed a lack of hybridization on one chromosome 2q, due to the 2q subtelomere deletion; FISH with the 21q subtelomere probe showed three hybridization spots: two on chromosome 21q and one on 2q, corresponding to the 21q translocated segment. Only one signal was detected on the 21q22 region (LSI AML) of each chromosome so this locus is not involved in translocation (Fig. 3). Microsatellite typing (Table 2) also confirmed that most of the markers show biparental inheritance and only the most telomeric marker was triallelic. Cytogenetic studies of the family members revealed normal karyotypes but FISH studies showed the presence of the reciprocal balanced translocation in the mother (Fig. 4) and the same derivative chromosome on the proband's sister. The derivative chromosome is due to the malsegregation of a maternal balanced translocation leading to distal 2q monosomy and distal 21q trisomy. The combination of these different techniques allowed us to define the karyotype

Figure 2 MLPA results of (A) a healthy woman control compared with (B) the mother, (C) the index case and (D) her sister where black bars represent the normalized quantity of peak-area corresponding to 2q and 21q telomeres; white bars correspond to the rest of telomeres.

Figure 3 FISH on proband metaphase spreads with (A) mixture 2 (TelVysion 2p SpectrumGreen, TelVysion 2q SpectrumOrange, TelVysion Xq/Yq SpectrumOrange, and SpectrumGreen, CEP X SpectrumAqua) and (B) mixture 4 (TelVysion 4p SpectrumGreen, TelVysion 4q SpectrumOrange, TelVysion 21q SpectrumOrange and SpectrumGreen, LSI AML (21q22) SpectrumAqua). No red fluorescent signal can be seen on one 2q telomere (arrowed) but two green signals are present on 2p, whereas an extra red and a green 21qter probe can be seen on chromosome 2q (arrowed) and no aqua fluorescent signal is present on the derivative chromosome.

as 46,XX.ish der(2)t(2;21)(qter;qter)mat(D2S447-, D21S1146+) on both sisters and 46,XX.ish t(2;21)(qter;qter)(D2S447-,D21S1146+;AML+,D21S1146-, D2S447+) on the mother.

Discussion

We report a new family case of a typical AHO-like syndrome associated with a cryptic translocation between the telomeres of chromosomes 2 and 21 detected by molecular analysis. AHO-like syndrome has been mainly associated with isolated, primarily terminal 2q37 deletions. In addition to the pure deletions, monosomy of 2q37 has also been described

Table 2 Results from analysis of 21q polymorphic markers in the reported family.

	Father	Mother	Index	Sister
D21S337	265/265	265/265	265/265	265/265
D21S156	77/97	90/101	77/90	90/97
D21S2055	157/157	131/153	131/157	131/157
D21S1840	199/199	199/199	199/199	199/199
D21S1974	102/104	102/104	102/104	102/104
D21S1937	90/96	90/96	90/96	90/96
D21S2057	192/192	192/192	192/192	192/192
D21S1446	209/219	213/223	213/219/223	213/219/223

due to unbalanced *de novo* (11–14) or familial translocations (15, 16), though the described partner chromosomes have not included chromosome 21. Complete or partial trisomy 21 has been associated with Down syndrome, which is characterized by

Figure 4 FISH on metaphase from the proband's mother with (A) mixture 2 and (B) mixture 4. The red fluorescent signal of a 2q subtelomere probe is present on 21qter (arrows indicate both derivative chromosomes), whereas the 21q subtelomere specific probes (red and green) are detected on 2qter and the LSI AML (21q22) probe (aqua signal) remains on the derivative chromosome 21.

distinctive facial features, such as eyes with inner epicanthic folds, oblique eye fissures, a small mouth, and a flat nasal bridge. It also presents with mental retardation, delayed motor development, congenital heart disease, loose skin, muscular hypotonia, short and broad hands, short neck, and a gap between the first and second toes (17). However, recent studies have described the subtelomeric trisomy 21q as a benign chromosomal variant (18, 19), indeed the critical region for Down syndrome seems to be 21q22.2 between D21S55 and MX1 loci (19), flanked by the D21S337 and D21S1974 microsatellites analyzed. On the other hand, the 2q37 phenotype not only includes the AHO-like syndrome but also mental retardation, seizure disorder, autistic features, a recognizable pattern of dysmorphism, and major malformations such as congenital heart malformation (8), gastrointestinal and renal anomalies (3), genitourinary malformations (9), and CN malformations (3). We have analyzed the 21q region to confirm a putative trisomy at the Down syndrome region and found that neither sister presented any trisomy at this locus, so we can conclude that the phenotype is exclusively due to the 2q37 alteration.

The differential diagnosis of 2q37 carriers is not easy as both inter- and intrafamilial variability has been described (8, 16). In our family, both sisters present similar phenotypes but the eldest child was diagnosed as CHARGE syndrome (OMIM 214800) (which includes coloboma of the eye; heart anomalies; bilateral choanal atresia; retardation of mental and somatic development; genital and ear abnormalities and/or deafness (20); and is genetically linked to *CHD7* (21) and *SEMA3E* (22)), mainly due to the simultaneous presentation of choanal atresia (even if unilateral), heart anomalies, mental retardation, and ear abnormalities. Some of these features have indeed been associated with 2q37 deletion. For example, even if in CHARGE syndrome the most common heart defect is tetralogy of Fallot (23), ventricular septal defects are present in both AHO-like (8) and CHARGE syndromes (23, 24); various pinna anomalies have also been described both in CHARGE (23, 24) and AHO-like syndromes (8); and moreover esophageal atresia has already been reported in a patient with deletion at 2q37.1 (25) and some CHARGE patients (26). This variation in the phenotype of both sisters has made clinical diagnosis difficult and only with the genetic results has the counseling and follow-up been improved.

In summary, the present report adds choanal atresia to the list of major malformations reported in the AHO-like syndrome, underscores the difficulty of diagnosis and possibility of misdiagnosing this disorder, and extends our understanding of the natural history of the condition by reporting two new cases of older individuals. The reporting of several familial cases, including ours, reveal that a translocation, if present, might well be cryptic so that interphase FISH with a subtelomeric probe may provide incomplete

elucidation of the findings and either FISH with a subtelomeric probe set or a CGH should be considered in these cases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was partially funded by grant GV2008/111035 from the Basque Department of Health and BIO06/DI/005. The group is also supported by the Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ISCIII.

Acknowledgements

We thank all members of the affected family for their collaborative participation in this study. The authors would like to thank Oscar Rubio-Cabezas for carefully reviewing the manuscript. G Pérez de Nanclares and G Pérez-Nanclares is a FIS researcher and Higher Technician respectively supported by the Instituto de Salud Carlos III of the Spanish Ministry of Health (grants CP03/0064 and CA05/0158). I Garin has received a grant from the Basque Department of Education (BFIO6.266).

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Received 29 December 2008

Accepted 30 December 2008