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ARTICLE

Paternal deletion of the GNAS imprinted locus (including *Gnasxl*) in two girls presenting with severe pre- and post-natal growth retardation and intractable feeding difficulties

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Deletions of the long arm of chromosome 20 are rare. Here, we report on two girls with a very small interstitial deletion of the long arm of chromosome 20 presenting with severe pre- and post-natal growth retardation, intractable feeding difficulties, abnormal subcutaneous adipose tissue, similar facial dysmorphism, psychomotor retardation and hypotonia. Standard cytogenetic studies were normal, but high-resolution chromosomes analysis showed the presence of a chromosome (20)(q13.2–q13.3) interstitial deletion. Karyotypes of both parents were normal. Molecular studies using FISH and microsatellite polymorphic markers showed that the deletion was of paternal origin and was approximatively 4.5 Mb in size. A review of other reported patients with similar deletions of the long arm of chromosome 20 shows that the observed phenotype might be explained in the light of the GNAS imprinted locus in particular by the absence of the *Gnasxl* paternally imprinted gene and the *TFA2PC* gene in the deleted genetic interval.
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Introduction

Constitutional aberrations of the long arm of chromosome 20 are rare. The most common anomaly being described is ring chromosome 20. Such patients usually present themselves with facial dysmorphism, severe mental retardation, normal pre- and post-natal growth, seizures and a specific EEG pattern.[1] To date, only six patients with an interstitial chromosome 20q deletion have been described in the literature.[1–5] Here, we report on two girls with a very small interstitial deletion of the long arm of chromosome 20 presenting with severe growth retardation, mental retardation and intractable feedings difficulties. The phenotype observed in our patients is discussed in light of the deleted genes in the critical region in particular the GNAS imprinted locus.

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Patients report

Patient 1 was the second child born from non-consanguineous Caucasian healthy parents. The mother was 25 and the father 26 years of age at the time of pregnancy. The father had a history of ulcerative colitis since the age of 6 years. Severe intrauterine growth retardation and oligoamnios were detected during pregnancy but the parents refused prenatal investigations. She was born at 40 weeks of gestation and delivery was uneventful. Birth measurements showed severe growth retardation (length = 38 cm, −6SD; weight = 1320 g, −3.5SD and occipito-frontal circumference (OFC) = 27.6 cm, −5SD). Failure to thrive, respiratory distress, feeding difficulties requiring tube feeding and hypotonia were noted in the neonatal period. She was first seen in the Genetics Department at 6 months of age. She presented with severe growth retardation (measurements were 51 cm for length, −6SD; 3500 g for weight, −4.5SD and 37 cm for OFC, −4SD). Facial dysmorphism included high forehead, small anterior fontanel, sparse hair and eyebrows, enophtalmia, dysplastic iris, large, simple and floppy ears, broad nasal bridge with a bulbous tip of the nose and nasal septum extending below alae nasi, short and prominent philtrum, thin upper lip, retrognathism and a small chin (Figure 1a). The skin was hypopigmented and blood vessels were particularly prominent on the forehead. Multiple bilateral skin dimples were noted on the shoulders, elbows, hips, knees and ankles (Figure 1a) as well as an abnormal distribution of adipose tissue characterised by a very significant reduction of subcutaneous adipose tissue on legs and trunk but normal adipose tissue on buttocks. She also had a deep sacrocoxygeal dimple. Neurological examination revealed central hypotonia with poor head control and peripheral hypertonia. At the last examination at 32 months of age, she was 5700 g for weight (−6SD), 67 cm for length (−6.5SD) and 43 for OFC (−4SD). Persistent and severe feeding difficulties required enteral tube feeding at night. She sat at 2 years of age but she remained hypotonic and did not walk. She was developmentally delayed and said only a few words. No seizures were observed in our patient. No clinical phenotype of Albright hereditary ostedystrophy was observed.

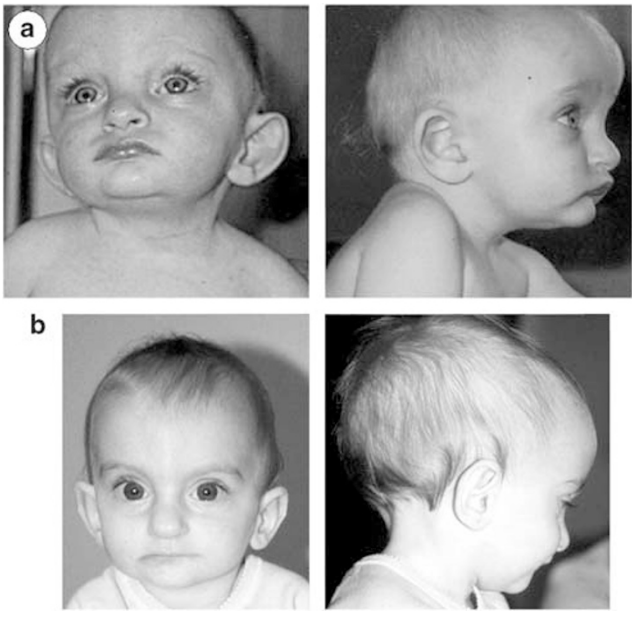

Figure 1 Front and lateral view of patient 1 (**a**) and patient 2 (**b**). Note sparse and blond hair, high forehead with excessive visibility of blood vessels, medial flare of the eyebrows, large and simple ears, beaked nose, short philtrum and small pointed chin.

Patient 2 was the only child born from non-consanguineous Caucasian healthy parents. The mother was 26 and the father 35 years of age at the time of pregnancy. Familial history was unremarkable. Severe intrauterine growth retardation was detected during pregnancy but no prenatal investigation was performed. She was born by caesarean section at 35 weeks gestation for IUGR. Birth measurements showed severe growth retardation (length = 39 cm, −2.5SD; weight = 1570 g, −3SD and OFC = 29 cm, −2.5SD). Failure to thrive, feeding difficulties requiring tube feeding and hypotonia were noted in the neonatal period. At 9 months of age, she was 4610 g for weight (−4SD) 58 cm for length (−5SD), and 41.5 for OFC (−2.5SD). She presented with facial dysmorphism similar to the one observed in the first patient, namely high forehead, small anterior fontanel, sparse hair and eyebrows, enophtalmia, dysplastic iris, large, simple and floppy ears, broad nasal bridge with a bulbous tip of the nose and nasal septum extending below alae nasi, short and prominent philtrum, thin upper lip, retrognathism and a small chin (Figure 1b). The skin was also hypopigmented with excessive visibility of blood vessels but no skin dimple was observed. The subcutaneous distribution of adipose tissue was similar to patient 1. She remained hypotonic with feeding difficulties requiring tube feeding, poor head control and peripheral hypertonia. No clinical phenotype of Albright hereditary ostedystrophy was observed.

Methods

Cytogenetic and molecular studies

Chromosome studies using standard R and G banding and high-resolution banding techniques were performed on peripheral blood lymphocytes of the patients and the parents. The chromosomes were classified according to the international nomenclature.[6]

In an attempt to further delineate the size of the deletion, FISH studies were performed as described previously.[7,8] Hybridisations were performed with (centromere to telomere) PAC RPCI-4 724E16 clone (D20S840), BAC clone RPCI-11 126L5 (D20S120), YAC clone 761C3, kindly provided by Dr Thomas Haaf, PAC clone RPCI-1

309F20 (corresponding to the GNAS imprinted gene), PAC clone RPCI-5 1043L13 (D20S173) and PAC clones RPCI-5 1107C24, RPCI-4 697K14 and 81F12 (Cytocell).[9]

Cytogenetic studies were supplemented with molecular studies using polymorphic microsatellite markers namely (centromere to telomere) D20S119 (20q13.1), D20S178 (20q13.1), D20S196 (20q13.1), D20S857 (20q13.2), D20S840 (20q13.2), D20S120 (20q13.2), D20S100 (20q13.2), D20S102 (20q13.2–q13.3), D20S430 (20q13.2–q13.3), D20S171 (20q13.2–q13.3) and D20S173 (20q13.3). DNA was extracted from the patients and their parents' peripheral blood lymphocytes according to standard techniques.[10] Heterozygozity scores and chromosome location of the microsatellite markers were obtained from either the GENETHON database (http://www.genethon.fr/) or the GENOME DATABASE (http://www.gdb.org/). Alleles were compared between these two patients and their parents.

We also performed expression studies of transcripts at the GNAS locus on cultured fibroblasts from skin biopsy of patient 2. Namely, we studied the bi-allelic expression of the *Gnas* gene, the maternally imprinted *Nesp55* gene and the paternally imprinted *GnasXl* gene (which has been described to be responsible for feeding impairment in mice when deficient).[11,12] We extracted mRNA from cultured fibroblasts using Qiagen RNeasy kit. RT-PCR were performed using 20 μl of cDNA and specific primers for *Gnas*, *Nesp55* and *Gnasxl* genes. Sequences of the primers are available on request.

Results

Laboratory investigations

Metabolic and endocrine studies including plasma and urinary amino-acids and organic acids, plasma T3, T4, TSH, protein glycosylation tests, blood lactate, growth hormone (GH), insulin growth factor 1 (IGF1), cerebral MRI, cardiac and renal ultrasound were unremarkable in both patients. Skeletal X-rays were also normal with no findings reminiscent of Albright hereditary ostedystrophy.

Cytogenetic and molecular studies

High-resolution chromosome analysis of both patients showed the presence of a 46,XX,del(20)(q13.2–q13.3) karyotype in all cells examined (Figure 2). The karyotypes of both parents were normal suggesting that the deletions occurred *de novo*.

FISH studies of the first patient revealed a single signal only of the YAC clone 761C3, and the presence of a signal using PAC clone 81F12 (Cytocell)[9] (Figure 2). This result confirmed the presence of a small interstitial deletion on the long arm of chromosome 20. In an attempt to further delineate the size of the deletion, other probes were used. The PAC clone RPCI-1 309F20 (corresponding to the GNAS imprinted locus), the PAC clone RPCI-5 1043L13 (D20S173), and the PAC clone RPCI-5 1107C24 were deleted, but studies using the PAC clone RPCI-4 724E16 (D20S840), the BAC clone RPCI-11 126L5 (D20S120) and the PAC clone RPCI-4 697K14 revealed one signal on each chromosome 20 (data not shown—results summarised in Figure 3). FISH studies of the second patient revealed a deletion of the BAC clone RPCI-11 126L5 (D20S120), the YAC 761C3, the PAC clone RPCI-1 309F20 (GNAS) and the PAC clone RPCI-5 1043L13 (D20S173). No deletion of PAC clones RPCI-4 724E16, RPCI-5 1107C24 and RPCI-4 724E16 (D20S840) 81F12 was observed (partial results in Figure 2—results summarised in Figure 3).

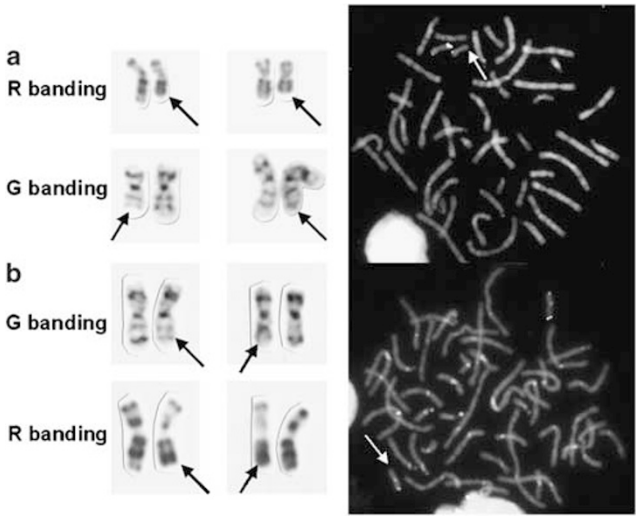

Figure 2 Partial karyotype of patients' chromosome 20 with R and G banding and *in situ* hybridisation studies of patient 1 (**a**) and patient 2 (**b**). The interstitial 20q13.2–q13.3 deletion is shown by black arrows. *In situ* hybridisation studies using PAC clone RPCI-1 309F20 (corresponding to the GNAS imprinted locus—white) and PAC clone 81F12 (20pter—grey) show that one 20q13.3 PAC clone RPCI-1 309F20 (GNAS imprinted locus) corresponding to the white signal is deleted in both patients (white arrows). Chromosomes are counterstained with DAPI.

Molecular studies showed a D20S100 locus deletion of paternal origin in patient 1 and a D20S102 locus deletion of paternal origin in patient 2 (Figure 4). Other microsatellite polymorphic markers were not informative. Using the data from human chromosome 20 working draft sequence, the size of the deletion observed in our patients is estimated to be about 6 Mb in size with a 4.5 Mb common interval including the *TFAP2C* (transcription factor AP-2 gamma) gene and the GNAS imprinted locus (Figure 3).

Analysis of the expression of the *Gnas*, *Nesp55* and *Gnasxl* genes on skin fibroblasts of patient 2 showed the presence of the maternally expressed *Gs*α and *Nesp55* cDNAs, whereas the paternally expressed *XL*α*s* transcript was absent (Figure 5).

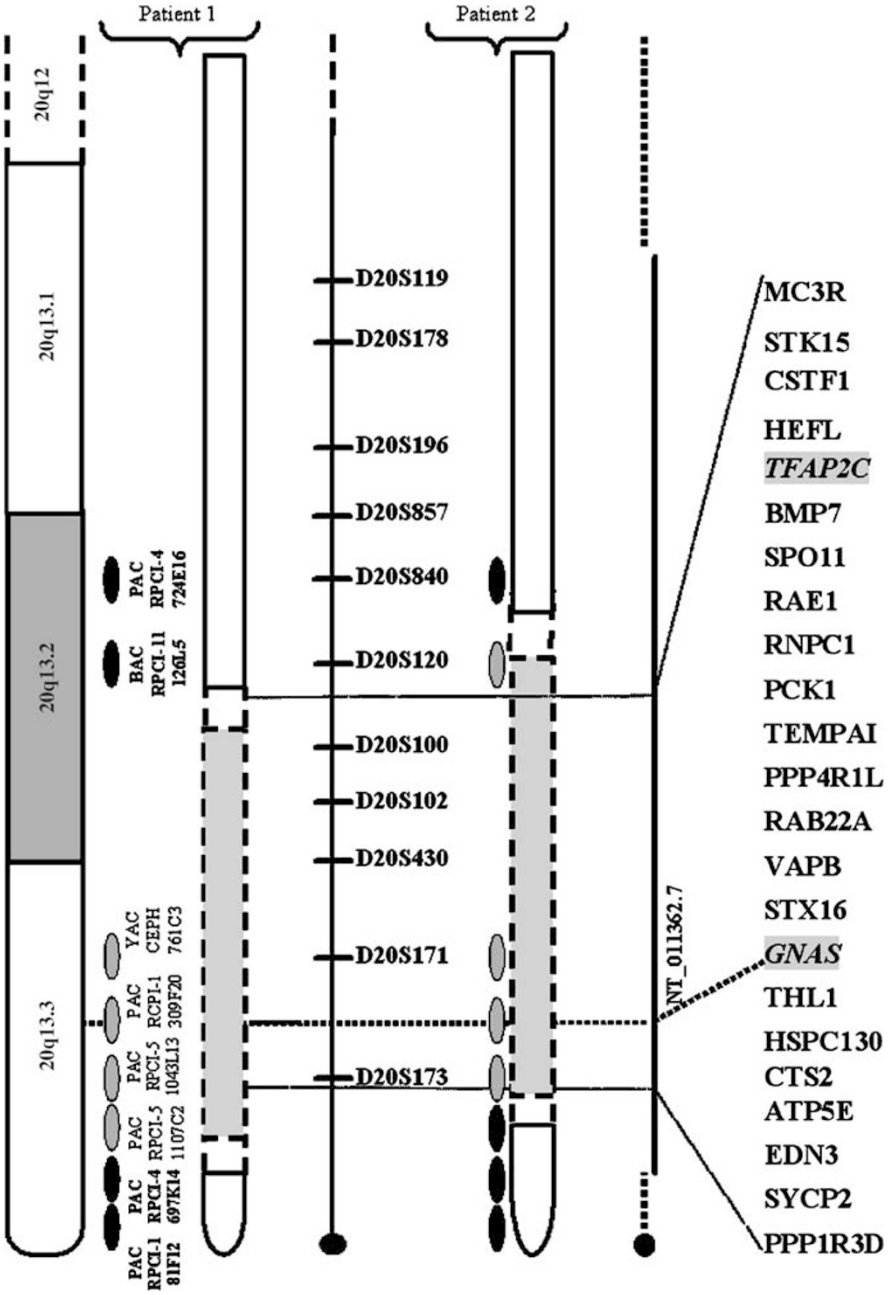

Figure 3 Cytogenetic and molecular analysis of the 20q13.2q13.3 deletion: from left to right: chromosome 20 ideogram, FISH probes used on patient 1, microsatellites markers used for molecular studies in both patients, FISH probes used on patient 2, *Homo sapiens* chromosome 20 working draft sequence corresponding to the deleted region and genes in the region of interest. The common deleted region observed in our patients is located between markers D20S100 and D20S173 (4.5 Mb).

Discussion

Here, we report on two unrelated patients with an interstitial deletion of the long arm of chromosome 20 presenting with severe pre- and post-natal growth retardation, intractable feeding difficulties, facial dysmorphism, mild psychomotor retardation and hypotonia. Constitutional deletions of the long arm of chromosome 20 have been reported rarely. To our knowledge, only six cases have been described in the literature so far.[1–5] Since the breakpoints of the deletion reported by Petersen *et al*[3] were different, we excluded it from our analysis. Clinical and chromosome findings of the five remaining cases as well as the present observation are summarised in Table 1. From this comparison it appears that some clinical

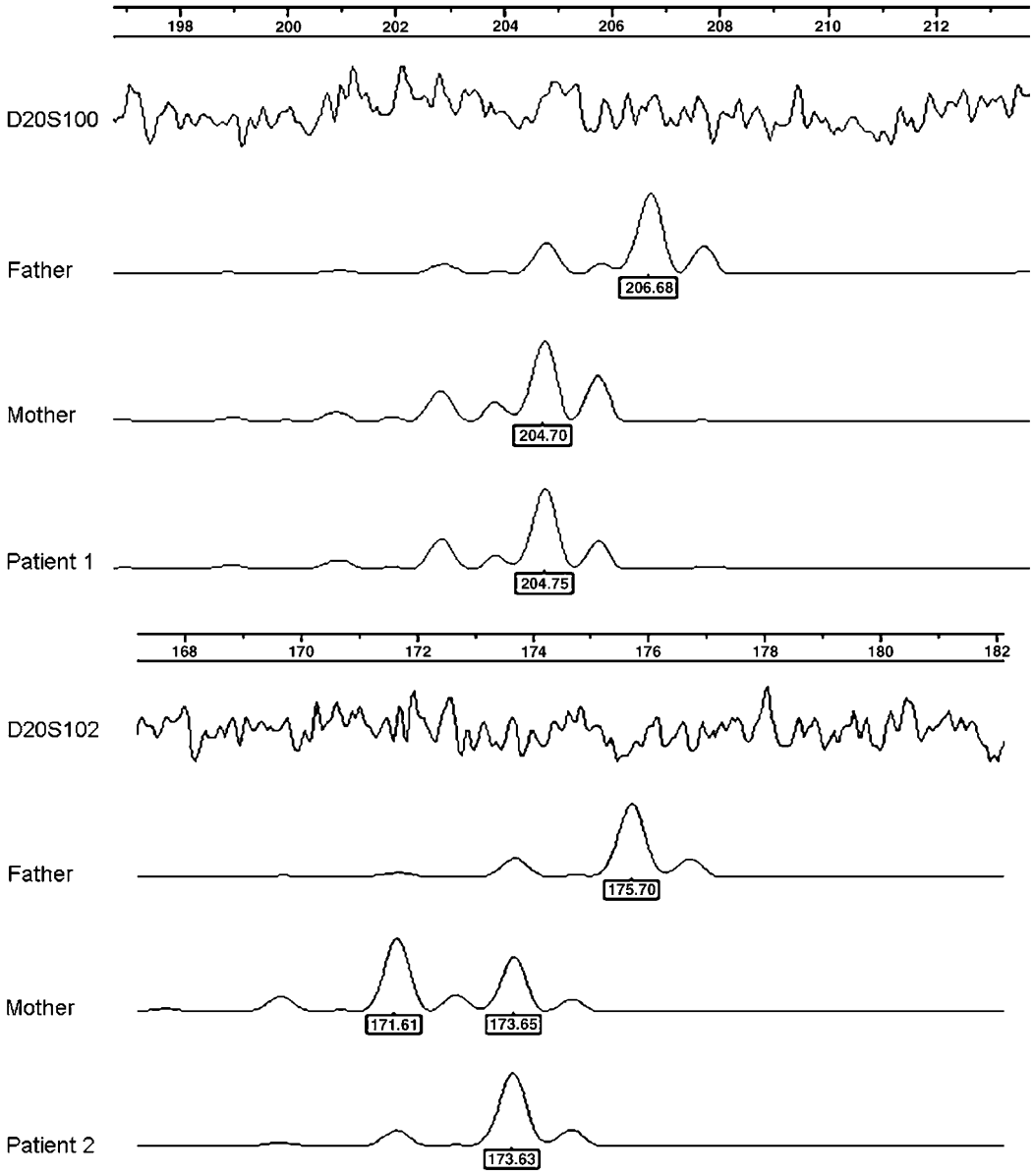

Figure 4 Parental origin of the 20q13.3 deletion. Note the absence of paternal contribution for the D20S100 microsatellite marker for patient 1 and D20S102 microsatellite marker for patient 2.

features, namely mild mental retardation, short stature, facial dysmorphism including high forehead, broad nasal bridge, thin upper lip, small chin, as well as malformed ears and hands are commonly observed in patients with similar deletions of the long arm of chromosome 20. Other features such as microcephaly, cardiac malformation and hypotonia might also be observed. In addition, our patients presented with skin, iris and hair hypopigmentation and abnormal adipose tissue distribution.

Molecular studies performed in this study revealed that the common deletion in our patients was approximately 4.5 Mb in size and mapped between D20S100 and D20S173 polymorphic microsatellite markers. Only a small number of known genes such as the GNAS imprinted locus and *TFAP2C* are included in this deleted genetic interval (Figure 3).

The GNAS imprinted locus is a chromosome region containing four genes.[11–14] Three of them namely, *Gnasx1*, *Nesp* and *Nespas,* are imprinted. *Gnasx1* and *Nespas* are paternally expressed whereas *Nesp* is maternally expressed. Several transcripts have been identified namely *Nesp55*, *Gsα*, *GsαN1*, *ex 1A*, *XLαs* and *XLN1*, but the function of some of these transcripts remains unknown. In mice, maternal duplication of chromosome 2 distal segment

Table 1 Clinical features in patients with deletion of the long arm of chromosome 20

	Fraisse et al[2]	*Porfirio et al*[3]	*Shabtai et al*[4]	*Aldred et al,* [5]		*Present study*	
				Patient 1	*Patient 2*	*Patient 1*	*Patient 2*
Clinical findings	*q13.11-qter*	*Ring q13-qter*	*q13.11–q13.33 (mosaic)*	*q13.13–q13.32*	*q13.31–q13.33*	*q13.2–q13.3*	*q13.2–q13.3*
Mild mental retardation	+	+	+	+	+	+	+
Prenatal growth retardation	–	–	–	+	U	+	+
Postnatal growth retardation	+	+	–	+	+	+	+
Microcephaly	+	–	–	+	–	+	+
Hypotonia	+	–	?	+	U	+	+
Intractable feeding difficulties	–	–	–	+	–	+	+
High forehead	+	+	–	+	U	+	+
Broad nasal bridge	+	–	+	+	U	+	+
Thin upper lip	+	+	–	–	U	+	+
Small chin	+	+	–	–	U	+	+
Malformed ears	+	+	–	–	U	+	+
Hypertelorism	+	–	–	–	U	+	+
Bulbous tip of the nose	+	–	–	+	U	+	+
Long philtrum	+	+	–	–	U	–	–
Malformed Hands/feet	+	+	+	–	U	–	–
Cardiac malformation	–	+	+	–	U	–	–
Seizures	+	–	–	–	–	–	–
Origin of the deletion	?	?	?	Paternal	Maternal	Paternal	Paternal

UUnknown.

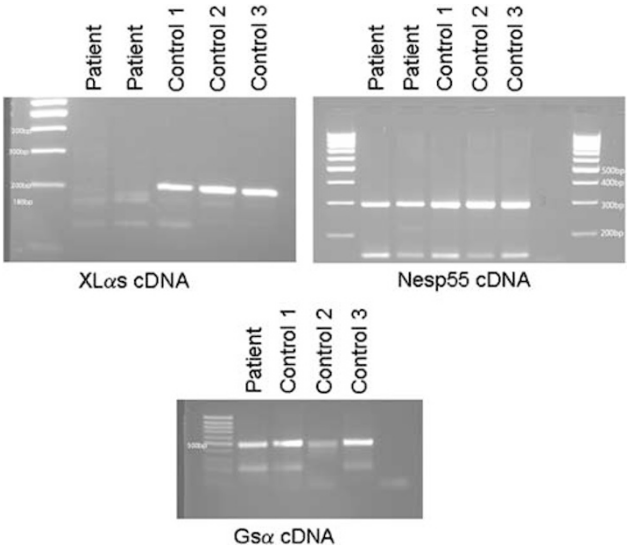

Figure 5 Analysis of the expression of *Gnas*, *Nesp55* and *Gnasxl* genes on skin fibroblasts of patient 2. Note the presence of the maternally expressed *Gsα* and *Nesp55* cDNAs; the paternal expressed *XLαs* transcript is absent.

(MatDp(dist2)), a segment homologous to 20q13 in humans, produces a hypokinetic mouse with prenatal growth retardation and severe feeding difficulties.[15] The MatDp(dist2) mouse dies a few hours after birth whereas mice with a paternally derived deletion of the GNAS imprinted locus survive despite the existence of feeding difficulties and show pre- and post-natal growth retardation.[16] To date only three patients with a chromosome 20 maternal uniparental disomy, involving the GNAS gene complex, have been described in the literature.[17–19] All patients presented with severe pre- and post-natal growth retardation as observed in the MatDp(dist2) mouse. In addition, mutation of the paternally expressed *Gnasx1* gene encoding the XLαs protein has been recently demonstrated to be responsible for pre- and post-natal short stature, abnormal adipose tissue and severe feeding difficulties in mice.[11,12]

Recently, Aldred *et al*[5] reported on two patients with Albright hereditary osteodystrophy and a small interstitial deletion of the long arm of chromosome 20 including the GNAS gene complex. One of the two patients had a paternal deletionand the other, a maternal deletion. Interestingly, the patient with the paternal deletion presented with pre- and post-natal growth retardation and intractable feeding difficulties as observed in our patients. Therefore, we would like to speculate that in the present observation, as well as in the observation of Aldred *et al*,[5] the paternal deletion of the GNAS imprinted locus might account for the severe pre- and post-natal retardation and intractable feeding difficulties observed in our patients.

Other genes might also been involved. For example, *TFAP2C* modulates the transcriptional activity of vitamin A target genes via the retinoic acid receptors.[20] In the mouse, *TFAP2C* is expressed in migrating neural crest cells of the frontonasal, maxillary, mandibulary and second branchial arch mesenchyme, in the developing brain, the limb buds and in cells of the basal layer of the skin.[21] Lack of AP-2γ results in lethality during early gestation due to a blastogenesis defect, whereas heterozygous mice are slightly growth retarded after birth but ultimately reach normal size.[22,23]

Extrapolating from observations made in the mouse, and because we show that the expression of the *Gnasx1* paternal imprinted gene is lacking in patient 2 (Figures 4 and 5), we propose that the severe growth retardation, abnormal subcutaneous adipose tissue and intractable feeding difficulties are due to the loss of the paternal *Gnasx1* imprinted gene.

Other deleted genes in this region such as the *TFAP2C* gene and the paternal *Nespas* as well as the *XLN1* transcripts might also contribute to the clinical features observed in our patients such as mental retardation and facial dysmorphism.

In conclusion, we report on two girls with a chromosome (20)(q13.2–q13.3) subtle interstitial deletion presenting with severe pre- and post-natal growth retardation, intractable feeding difficulties, abnormal adipose tissue, unusual facial dysmorphism, mild psychomotor retardation and hypotonia. We demonstrated that the *Gnasx1* gene is paternally imprinted in fibroblast and that its transcript (XLαs) is lacking in patient 2. These observations suggest that all patients with severe pre- and post-natal growth retardation, abnormal adipose tissue and intractable feeding difficulties should be carefully investigated for deletion of the GNAS complex gene at the 20q13.3 locus and/or abnormal paternal expression of the *Gnasx1* gene. In addition, molecular studies of the *Gnasx1* gene and its transcript should be performed in patients carrying a chromosome 20 maternal uniparental disomy to confirm that the paternal *Gnasx1* imprinted gene is responsible for pre- and post-natal growth retardation and feeding difficulties.

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