Original articles

General overgrowth in the fragile X syndrome: variability in the phenotypic expression of the FMR1 gene mutation

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Abstract
The fragile X syndrome, which often presents in childhood with overgrowth, may in some cases show some diagnostic overlap with classical Sotos syndrome. We describe four fragile X patients with general overgrowth, all of whom are from families with other affected relatives who show the classic Martin-Bell phenotype. Molecular studies of the FMR1 gene in all cases showed the typical full mutation as seen in males affected by the fragile X syndrome. Endocrine studies were unremarkable, except in one case where there were raised levels of insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein-3 (IGFBP-3)

These cases illustrate the clinical variability of the fragile X syndrome and the necessity of performing analysis of the FMR1 gene in mentally retarded patients presenting with general overgrowth.

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The fragile X syndrome is the most common cause of familial mental retardation with an estimated prevalence of 1 in 1250 for males, and 1 in 2000 for females in western countries.[1 2]

Identification of the FMR1 gene in 1991, and the classification of the implication of its full and premutation states, allow definite DNA molecular diagnosis in patients and carriers in nearly all cases.[3] Nearly all fragile X patients show an amplification of the CGG repeat (>200) in the 5' exon of the FMR1 gene (full mutation), as compared to 6 to 54 repeats in controls and 43 to 200 CGG repeats in unaffected carriers of the premutation.[3–7]

Most affected males present with the Martin-Bell phenotype, a combination of mental retardation, a long, large face with prominent, everted ears, and macro-orchidism.[8–11] A phenotype less frequently observed in fragile X patients consists of extreme obesity, short stature, stubby hands and feet, and diffuse hyperpigmentation, which has been designated the Prader-Willi-like subphenotype.[12–14]

In addition, a Sotos-like phenotype was reported in 1986 in two fragile X boys featuring large size at birth, unusual length, large head circumference, and minor facial anomalies.[15]

Here we report clinical, endocrine, and DNA studies in four fragile X patients with overgrowth.

Patients and methods
Four mentally retarded males with phenotypic features resembling Sotos syndrome were identified as fragile X positive either by cytogenetic analysis (cases 1, 3, and 4) or by gene mutation analysis (case 2) and are the subjects of this report.

DNA ANALYSIS
Genomic DNA (8 µg) isolated from blood leucocytes[16] was digested with the restriction enzyme *Hind*III according to the manufacturer's instructions, separated by gel electrophoresis, and subjected to Southern blot analysis according to standard procedures.[17] The intragenic DNA probe pP2 was used for analysis of the FMR1 gene.[18] The probe was labelled by the random oligonucleotide priming method.[19]

ASSAYS OF IGF-I AND IGFBP-3
IGF-I in serum was determined by specific radioimmunoassay (RIA) after acid Sep-Pak C18 (Wares Associates, Milford, MA, USA) chromatography.[20 21]

IGFBP-3 levels were determined by RIA. IGFBP-3 was isolated from human plasma using the purification as modified by Martin and Baxter.[22] IGFBP-3 was iodinated using the chloramine-T method (specific activity 50–100 µCi/µg). New Zealand white rabbits were immunised with 110 µg purified IGFBP-3 in complete Freund's adjuvant by multiple subcutaneous injections along the back and proximal limbs. After 80 days an antiserum was obtained from one rabbit, which precipitated 50% of [^{125}I]IGFBP-3 at a 1:10 000 dilution. The antiserum did not cross react with IGF-I, IGF-II, or IGFBP-1. The assay buffer used in

Clinical manifestations in the fragile X patients with symptoms of overgrowth

	1	2	3	4	Total
Current age (y)	4	3	29	26	
Birth weight (g)	3830	3800	3950	3125	
Adult height (cm)	X	X	184*	193	
Obesity (>2 SD for age and height)	+ +	−	+	+ +	3/4
General features of Sotos syndrome[31] in cases 1–4					
Large body size and early accelerated growth	+	+	+	+	4/4
Acromegaloid features	+	−	+	+	3/4
Advanced bone age	NS	−	X	NS	0/3
Developmental delay	+	+	+	+	4/4
Facial features of Sotos syndrome[31] in cases 1–4					
Frontal bossing	+	+ +	−	−	2/4
High hairline	+	+ +	+	+	4/4
Prominent jaw	−	+	+	+ +	3/4
Downward slanted palpebral fissures	+	+	−	+	3/4
Facial flushing	+	+	−	−	2/4
Dolichocephaly	−	+	+	+ +	3/4
High palate	+	+	+	+ +	4/4
Laboratory findings					
Growth hormone	N	N	X	N	3/3 normal
Insulin-like growth factor-I	↑	N	X	X	1/2 raised
Insulin-like growth factor binding protein-3	↑	N	X	X	1/2 raised
FMR1 gene	P + F	F	P + F	F	4/4 full mutation

+ = present, − = absent, N = normal, P = premutation, F = full mutation, X = not assessable, NS = not significant (advancement of 10 months and 7 months, respectively)
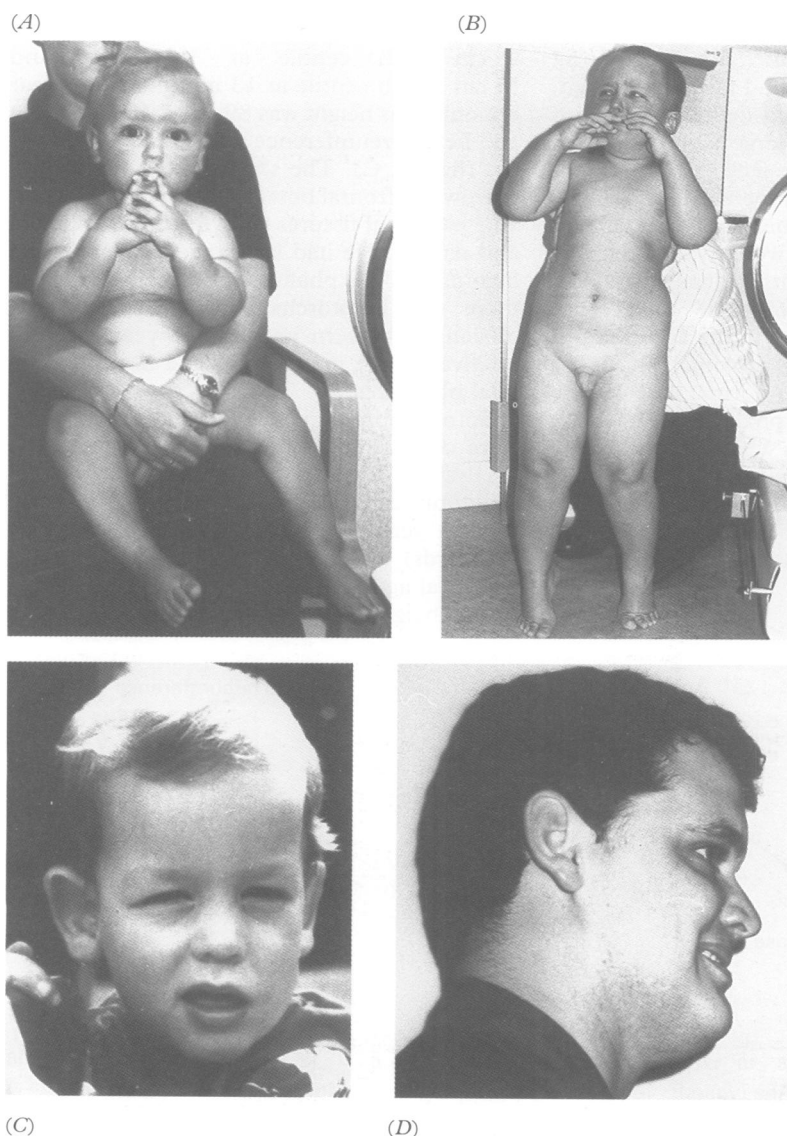* After testosterone therapy

(A) (B) (C) (D)

Figure 1   (A) Case 1 at the age of 1 year 5 months developing overgrowth, (B) case 1 at the age of 3 years 7 months showing progression of the general overgrowth. (C) Case 2 at the age of 3 years. Note bitemporal narrowing, frontal bossing, a high hairline, large, everted ears, and narrow, downward slanted palpebral fissures. (D) Case 3 at adult age. Note long face with high forehead and prominent mandible.

the RIA was composed of 0·05 mol/l sodium phosphate (pH 7·4), 0·01 mol/l EDTA, 0·05% Tween-20, 0·2% BSA, and 0·02% NaN.[22] Standards were prepared from purified IGFBP-3, stored at −70°C. Standard dilutions ranged from 0·06 to 8 ng/tube. Duplicates of serum samples were diluted 1:400 in assay buffer. The incubation mixture consisted of 100 µl assay buffer, 100 µl standard or diluted sample, 50 µl antibody (1:16 000), and 50 µl tracer (10 000 cmp). After incubation for 18 hours at room temperature in polystyrene tubes, 100 µl Sac-Cel solid phase antirabbit coated cellulose suspension (Innogenetics, Nijmegen, The Netherlands) was added. Complex formation was complete after 30 minutes at 20°C. Then 0·6 ml distilled water was added and the samples were subsequently centrifuged at 10 000 g for three minutes. Pellets were washed once with 0·6 ml distilled water and counted in a τ-counter (Packard Instrument Co Inc, Downers Grove, IL). The sensitivity of the assay was 0·5 ng/ml. Intra-assay variation was 6·9% at 2·52 mg/l and 12·9% at 0·83 mg/l. The interassay variation was 10·8% at 2·88 mg/l and 9·9% at 1·67 mg/l. Serum levels showed parallelism with the purified human as well as recombinant glycosylated human IGFBP-3 (kindly provided by Dr C Maack, Celtrix Pharmaceuticals Inc, Santa Clara, CA, USA).

Results
CASE REPORTS
Physical signs, not mentioned in the case histories, are shown in the table.

Case 1
This boy (fig 1A, B) was born after an uneventful pregnancy and delivery with a birth weight of 3830 g (75th centile) and a height of 52 cm (70th centile). His development was slow, crawling at 12 months, sitting at 15 months, and walking with support at 20 months. At the age of 18 months, he was
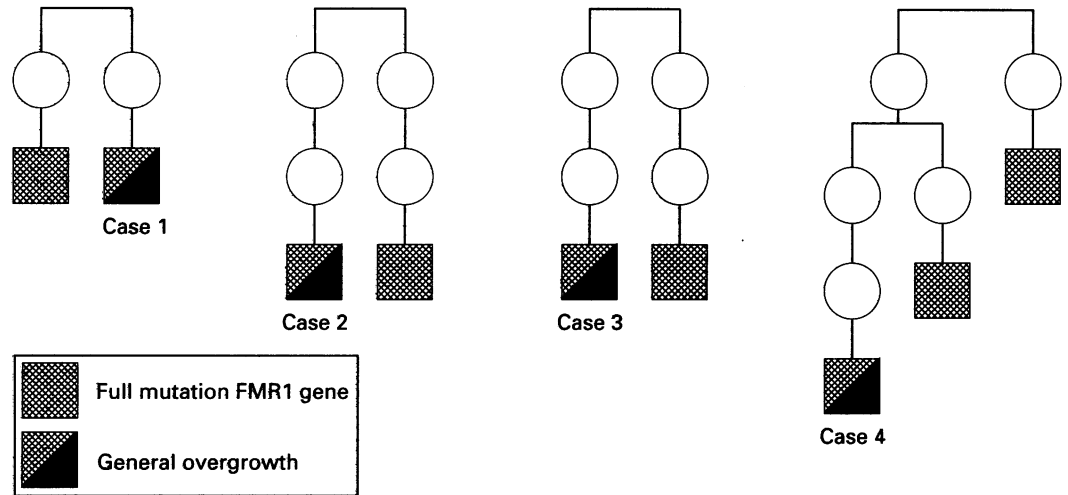
Figure 2   Family pedigrees of cases 1 to 4.

cytogenetically diagnosed as having the fragile X syndrome (40% fragile X expression) after the same diagnosis in his mentally retarded 4 year old maternal cousin (fig 2). At $2\frac{1}{2}$ years his height was 98 cm (90th centile), weight 23 kg ($> +4$ SD for age and for height), and head circumference 52 cm (85th centile) (fig 3A, B). He had a full, round face with a high, prominent forehead, broad dental ridges, and normal sized ears. He had large hands (12·2 cm, >98th centile) and large feet (17 cm, >98th centile) with short, broad toenails. His phallus and testes were of normal size, but he had a shawl scrotum. There were hyperextensible metacarpophalangeal joints, valgus position of the knees, and flat feet. The metacarpophalangeal pattern profiles at the age of 3 and 4 showed relatively long proximal phalanges and first metacarpal, with normal metacarpals 2–5, middle, and distal phalanges. His bone age was 3 years 3 months when he was 2 years 5 months old. At 4 years 2 months his height increased to 116 cm (>98th centile) and his head circumference to 55 cm (>98th centile).

The diagnosis of the fragile X syndrome was confirmed by showing a full mutation of the FMR1 gene.

Case 2

This boy (fig 1C) was born after a normal pregnancy and delivery with a birth weight of 3800 g (75th centile) and height of 52 cm (70th centile). During his first year he developed progressive macrocephaly (head circumference 48 cm = 50th centile at 3 months and 51 cm >98th centile at 13 months). At 1 year 8 months his height was 89·5 cm (90th centile) and head circumference 52·5 cm (>98th centile) (fig 3B, C). The skull showed bitemporal narrowing, frontal bossing, and a high hairline. The palpebral fissures were downward slanted and narrow. He had large, everted ears and a high palate. His phallus was a normal size and there was cryptorchidism. The metacarpophalangeal pattern profile at 3 years showed relatively short metacarpals and normal proximal phalanges.

Sotos syndrome and the fragile X syndrome were considered in the differential diagnosis. Analysis of the FMR1 gene showed the full mutation, confirming the fragile X syndrome.

At 3 years, bone age (Greulich and Pyle standards) was in accordance with the chronological age.
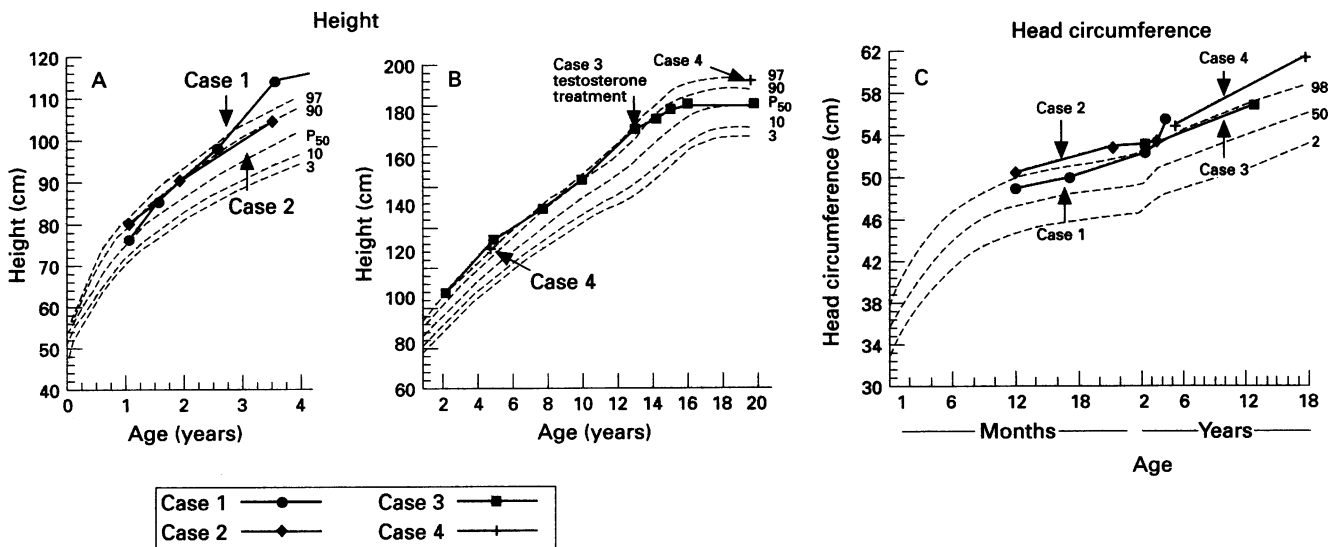
The height and head circumference of both

Figure 3   (A, C) Growth and head circumference charts for cases 1 and 2, (B, C) growth and head circumference charts for cases 3 and 4.
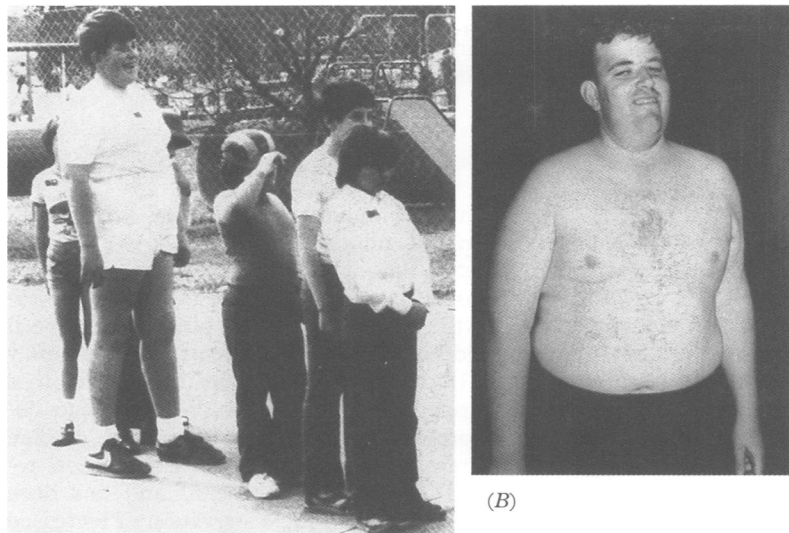
(B)

(A)

Figure 4 (A) Case 4 at the age of 9 years among classmates, (B) case 4 at adult age. Note the general overgrowth, long face with high forehead, broad chin, large, everted ears, and downward slanted palpebral fissures.

parents were normal: mother's head circumference 53 cm and height 169 cm; father's head circumference 56·5 cm and height 170 cm.

Case 3
This male (fig 1D) was born at term after a normal pregnancy; birth weight was 3950 g (80th centile) and height 56 cm (>98th centile). Neonatal weight gain was above average. He sat alone at 10 months and walked alone at 14 months. At 2 years 10 months he had mastered about 20 single words, his height was between the 90th and 97th centile, and head circumference 52·4 cm (98th centile). He had a broad, large nose and valgus deformity of the feet. A diagnosis of cerebral gigantism was considered at that time. At 13 years of age, the diagnosis of the fragile X syndrome was made by cytogenetic testing (28% fragile X expression).

At 12 years of age, testosterone enanthate treatment was started in an attempt to limit his

final height. At that age, his height was 172·2 cm (98th centile) with a weight of 60 kg (90th centile) (fig 3B, C). His final height after three years of testosterone treatment was 183·5 cm (50th centile). His weight was 97 kg (>98th centile).

The height and head circumference of both his parents were normal: mother's head circumference 55 cm and height 172 cm; father's head circumference 57·5 cm and height 176 cm.

Case 4
This male (fig 4A, B) was born at term with a birth weight of 3125 g (25th centile). The neonatal period was uneventful. He was found to be obese at the age of 3 months with a weight of 7000 g (98th centile). His developmental milestones were moderately delayed and he was thought to have "mild non-specific mental retardation" at the age of 18 months.

At the age of 4 years 11 months his height was 121 cm (>98th centile), weight 35·8 kg (>98th centile), and head circumference 54·2 cm (>98th centile) (fig 3B, C). He was an obese and retarded child without major abnormalities, except for a large penis with slightly enlarged testes for his age. His bone age was 5½ years (7 months advanced according to the Greulich and Pyle standards). Skull x rays were normal for age and random growth hormone levels were not raised (<2 ng/ml). The diagnosis of cerebral gigantism was considered.

At the age of 18, the diagnosis of fragile X syndrome was made by cytogenetic testing (18% fragile X expression). Two male relatives (the son of the maternal grandmother's sister and the son of the sister of the maternal great grandmother) (fig 2) were also affected by the fragile X syndrome (including FMR1 gene full mutations) but showed the Martin-Bell phenotype without any symptoms of overgrowth.

At 24 years of age, height was 193 cm (>98th centile) and head circumference 61 cm (+4 SD). The height and head circumference of both his parents were normal, except for the father's head circumference: father's height 180 cm and head circumference 60 cm (>98th centile); mother's height 168 cm and head circumference 57 cm.
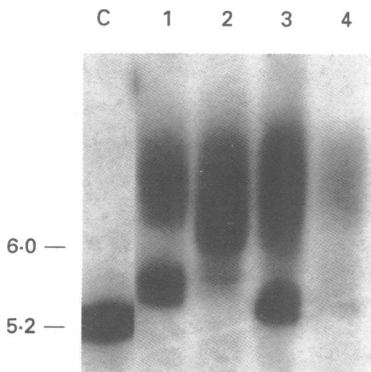

Figure 5 Analysis with probe pP2 of HindIII digested DNA of one unaffected male (lane 1) and cases 1 to 4 (lanes 2 to 5). In addition to a full mutation, cases 1 and 3 have a premutation.

MOLECULAR AND ENDOCRINE FINDINGS
Molecular studies showed a full mutation of the FMR1 gene in all cases, with an additional premutation in cases 1 and 3 (fig 5).

In case 1, both the insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein 3 (IGFBP-3) were raised. At the age of 2 years 9 months the IGF-1 was 138 ng/ml (>+2 SD) and IGFBP-3 was 2·14 mg/l (>+1 SD), at the age of 3 years 7 months the IGF-I was 85 ng/ml (>+1 SD) and IGFBP-3 was 2·52 mg/l (>+2 SD). His thyroid function, GH, LH, FSH, and plasma testosterone levels were normal.

In case 2, the IGF-I and IGFBP-3 were both normal at the age of 3 years 4 months, 82 ng/ml and 1·16 mg/l respectively.

Additional endocrine studies could not be performed in cases 3 and 4.

The clinical, molecular, and endocrine findings of cases 1 to 4 are summarised in the table.

Discussion

Overgrowth as a feature of the fragile X syndrome has been reported in childhood, but mostly relating to head size.[23-26] Head circumference, in comparison with the normal population[24-27] and with non-fragile X retarded males,[28] was found to be increased in children and adults. One study reported an increased head circumference in fragile X males as infants and children but not as adults.[23] Another study showed an increase in height (>95th centile) in nine out of 29 fragile X boys in childhood, with normal height in adult fragile X males.[25] Several groups have described normal height in young fragile X patients, with adults being below mean normal height.[24 27-29]

The four present cases are special in that the enlarged head circumference, together with extreme overall body overgrowth, was similar to that described earlier in two fragile X boys.[15] The latter observation led to the proposition that a "Sotos-like" phenotype of the fragile X syndrome might exist. This study extends these observations with respect to the overgrowth. In all four cases reported here, a full mutation in the FMR1 gene was found with an additional premutation in cases 1 and 3, providing a clear diagnosis of the fragile X syndrome. The two adult cases (3 and 4) had previously been diagnosed as Sotos syndrome at a time when the fragile X syndrome was unknown. Originally, Sotos syndrome was characterised by large body size and early accelerated growth in combination with acromegaloid features, advanced bone age, developmental delay, and a non-progressive neurological disorder.[30] Developmental delay was observed in all four present cases (mental retardation is the major feature of the fragile X syndrome) and other features of Sotos syndrome are also apparent in the present cases (table 1), including large body size (all cases) and acromegaloid features (cases 1, 3, and 4). The advanced bone age in cases 1 (10 months advanced) and 4 (7 months advanced) could be considered within the normal variation of the population.

Sotos syndrome is further characterised by additional features[31] which are helpful for clinical differential diagnosis (table).

The facial appearance in case 2 shows some similarities to Sotos syndrome; however he lacks acromegaloid features and advanced bone age. In cases 1, 3, and 4 the facial features, which are difficult to observe owing to extreme obesity, seem atypical of Sotos syndrome. Obesity is rarely observed in patients with Sotos syndrome[31] but is occasionally seen in fragile X patients.[12-14] Other diagnostic differences are normal or high normal birth weights (all cases) and normal birth lengths (cases 1 and 2, case 4 unknown), which are clearly less extensive than in Sotos syndrome patients. Only case 3 had a birth length of 56 cm (>2 SD) as has

been observed in most patients with Sotos syndrome.[31]

The metacarpophalangeal pattern profile (MCPP) in case 1 corresponds with the profiles observed in patients with Sotos syndrome,[32-34] while the MCPP in case 2 resembles the profiles seen in fragile X patients.[34]

The phenomenon of general overgrowth prompted endocrine studies in the two younger patients. Raised IGF-I and IGFBP-3 was found in case 1, perhaps related to the extreme overgrowth. Raised IGF-I by bioassay has been found before in Sotos patients.[35 36] The family of IGFs and their binding proteins are involved in growth processes, including cartilage development, tissue regeneration and ovarian function.[37 38] Early menopause has been reported in fragile X carriers[39] and has been linked recently to the observation of increased dizygotic twinning among offspring of these carriers.[40] The latter phenomenon might also be related to local IGF-I function.

The general overgrowth in the present cases might be considered as a distinct manifestation of the fragile X syndrome. Although "Sotos-like" might seem a descriptive term for this variant of the fragile X syndrome in the present cases, this term may be both confusing and lacking precision in the absence of the full characteristics of Sotos syndrome in all cases.

The fragile X syndrome is phenotypically heterogeneous with some patients showing a tendency to general overgrowth (presented here) and another minority of fragile X patients presenting with phenotypes suggesting the Prader-Willi syndrome.[12-14] This has important implications for clinical and differential diagnosis.

In conclusion, the present four cases illustrate the clinical variability of the fragile X syndrome and the necessity of performing analysis of the FMR1 gene in mentally retarded patients presenting with general overgrowth, and in cases suspected of having Sotos syndrome.

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