

Clinical and molecular studies in fragile X patients with a Prader-Willi-like phenotype

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Abstract

A special subphenotype of the fragile X syndrome is reported which is characterised by extreme obesity with a full, round face, small, broad hands/feet, and regional skin hyperpigmentation. It resembles the Prader-Willi syndrome (PWS) and might therefore be named 'Prader-Willi-like'. Unlike the PWS, these PW-like fragile X patients lack the neonatal hypotonia with feeding problems during infancy followed by hyperphagia from toddlerhood. We describe five new fragile X patients and present a clinical update of three previously described patients with the PW-like phenotype. In one family, segregation of either the classical Martin-Bell or the PW-like phenotype was observed and in another family there was repeated transmission of the PW-like phenotype. Previously, one of the patients had been misdiagnosed as having classical PWS, based on clinical findings.

Molecular studies of the FMR-1 gene showed the typical full mutations as seen in fragile X syndrome males. Molecular analysis of the 15q11-13 region, which is deleted in the majority of classical PWS patients, did not show any detectable abnormalities.

In a group of 26 patients with suspected Prader-Willi syndrome but without detectable molecular abnormalities of chromosome 15, one fragile X patient was found.

These clinical and molecular findings illustrate the necessity to perform DNA analysis of the FMR-1 gene in mentally retarded patients presenting with a PW phenotype but without the PWS specific cytogenetic/molecular abnormalities of chromosome 15.

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The fragile X syndrome is the most frequent form of familial mental retardation. The syndrome occurs in approximately 1/1250 males and 1/2000 females.^{1,2} The clinical diagnosis can be confirmed by cytogenetic analysis in folic acid deficient medium showing a fragile site at Xq27.3.^{3,4} The majority of male fragile X patients show the typical Martin-Bell phenotype consisting of mental retardation, long face with large everted ears, and megalo-testes.⁵⁻⁸ Less frequently described phenotypes include short stature, extreme obesity, short, broad hands and feet, and hypogonadism with

hyperpigmentation of the periorbital, axillary, and genital region. Two prepubertal fragile X boys and two adult males were described with this phenotype, which resembled the Prader-Willi syndrome.⁹

Recently, the FMR-1 gene implicated in the fragile X syndrome was isolated and characterised.¹⁰ This gene has a trinucleotide CGG rich repeat in a 5' exon, which consists of six to 54 copies of CGG.^{10,11} Premutation alleles are characterised by an increase in the number of triplets to between 52 and 200.¹⁰⁻¹⁴ Male and female premutation carriers are mentally normal and do not show cytogenetic expression of the fragile site Xq27.3.

In male patients the repeat exceeds 200 triplets (designated the full mutation) and the fragile site becomes apparent on cytogenetic testing as well as the clinical features of the fragile X syndrome.

We report five new cases of male fragile X patients with a 'Prader-Willi-like' phenotype and an update of three previously described patients.^{9,15} The recognition of this Prader-Willi-like phenotype among fragile X males prompted us to analyse the FMR-1 gene in 26 patients suspected clinically to have classical Prader-Willi syndrome (PWS) but without any detectable cytogenetic or molecular abnormalities of the 15q11-13 region.

Materials and methods

Seven mentally retarded males with a Prader-Willi phenotype, in whom fragile X syndrome was identified by cytogenetic expression of the fragile site at Xq27.3, were included in the clinical and molecular study. From another 26 patients cytogenetic and DNA analyses were requested because of clinical suspicion of the classical Prader-Willi syndrome. These patients, who had not shown any detectable molecular abnormalities of the 15q11-13 region, were studied for mutations in the FMR-1 gene.

DNA ANALYSIS

The intragenic DNA probe pP2 was used for analysis of the FMR-1 gene.¹⁶ Genomic DNA was isolated from blood leucocytes.¹⁷ DNA (8 µg) was digested to completion with the restriction enzyme *EcoRI* according to the manufacturer's instructions, separated by gel electrophoresis, and subjected to Southern blot analysis according to standard procedures.¹⁸ The probe was labelled by the random oligonucleotide priming method.¹⁹ After pre-

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hybridisation and hybridisation, the filters were washed in $0.1 \times \text{SSC}$ at 65°C before autoradiography.¹⁸ DNA analysis of the chromosomal region which is involved in classical PWS was performed, using standard methods, with markers at GABRB3, D15S9-13, and D15S15-18.²⁰⁻²²

CYTOGENETIC TESTING

Leucocytes were cultivated under conditions designed to show the fragile site at Xq27.3.^{23,24} At least 50 metaphase spreads were examined from each patient.

Results

CASE REPORTS

Case 1

This 15 year old boy (figs 1 and 2) was born at term after a normal gestation and delivery with a birth weight of 3330 g and length of 51 cm. The umbilical cord was wound three times around the neck. His development was slow; at the age of 6 years he was estimated (PEP-test) to be functioning at the level of 2.5 to 3 years. Up to the age of 10, he was a slender boy (fig 1A), but subsequently he gained weight within a few months without any change in diet.

At the age of 12, the fragile X syndrome was diagnosed (fragile site Xq27.3 in 38% of the cells investigated). At this age he had a small phallus with small retractable testes. At 13 years 7 months, his weight was 94.5 kg (+6 SD for height), height 173 cm (80th centile), head circumference 57 cm (90th centile), and he had a short arm span (161 cm).²⁵ He had a full, round face, truncal obesity, short, broad hands and feet with tapering fingers, and narrow, deep nails (fig 1B,C,D). He had normally sized testes for his age (10 ml bilaterally) and sparse pubic hair.

His mother and half sister (who is mildly retarded and slightly obese) both showed expression of the fragile site Xq27.3 in 14% of their blood lymphocytes.

Case 2

The patient (figs 2 and 3) was born after a normal gestation and delivery with a birth weight of 3425 g. At the age of 2 years, he was found to be mentally retarded and he was diagnosed as having the fragile X syndrome (17% fragile X expression) after a similar diagnosis in his mentally retarded older brother. At the age of $6\frac{1}{2}$ years, he gained weight without change in his diet and became obese within about 5 months. At $9\frac{1}{2}$ years his weight was 44 kg (+8 SD for height), height 129 cm (-2 SD), and head circumference 52.5 cm (20th centile). He had a full, round face, ears with a prominent helical root and large lobes, dental crowding, truncal obesity, short, broad hands and feet, stubby fingers with small nails, and short, hyperconvex toe nails (fig 3B,C,D). He had hyperextensible metacarpophalangeal joints and flat feet. His phallus was small with small descended testes and hyperpigmentation of the scrotal skin.

His three year older brother is mentally retarded and shows the classical Martin-Bell phenotype with a long face, large everted ears, and macro-orchidism.

Case 3

This boy (figs 2 and 4A) was delivered by caesarean section because of a breech presentation with a birth weight of 4000 g. He was weak and floppy during the first few months of life. His development was retarded and he walked at 15 months. At the age of 6 years, he attained a full scale IQ of 50 (WISC-R). At that age, he suddenly gained weight without increased food intake. Two years later his

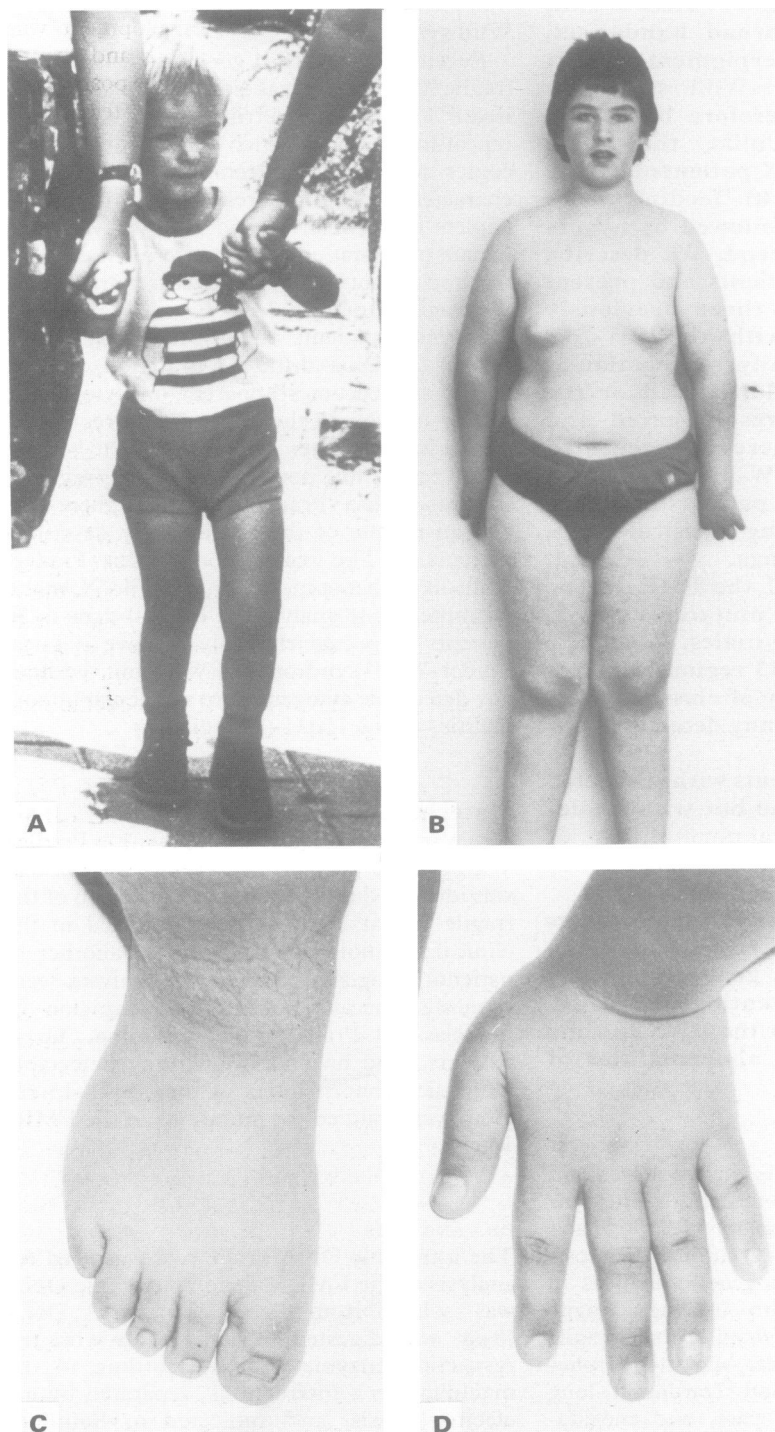


Figure 1 Case 1 (A) at the age of 4 years showing slender build; (B, C, D) at the age of 12 years showing full body, foot, and hand. Note full round face, truncal obesity, short, broad foot, and hand with tapering fingers and narrow, deep set nails.

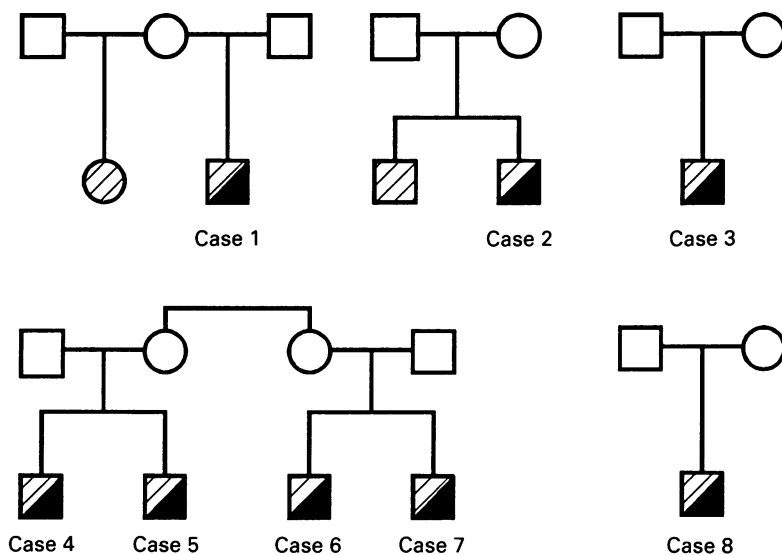


Figure 2 Family pedigrees of cases 1 to 8. Half hatched symbols = patients with fragile X expression and the full mutation in the FMR-1 gene (for cases 6 and 7, fragile X expression only). Half filled symbols = patients with Prader-Willi like phenotype.

weight was 60 kg (+7 SD for age). He was suspected of having the Prader-Willi syndrome. Nine years later, at the age of 15, the diagnosis of the fragile X syndrome was made by cytogenetic testing (fragile site Xq27.3 in 30% of the cells investigated). By then, his weight was 99 kg (+6 SD for height), height 177 cm (40th centile), and head circumference 57.5 cm (80th centile). He had large, prominent ears (7.2 cm, >95th centile), large testes (28/16 ml), hyperextensible joints, and a plantar crease.

Cases 4, 5, 6, and 7

These are an update on two earlier reported patients (cases 4 and 5) and addition of two other affected family members (cases 6 and 7) (figs 2 and 4B,C).

The two brothers described by Fryns *et al*⁹ in 1987 are now aged 14½ and 12½ years respectively. Their mental function is moderately to severely retarded (IQs 30 and 32, WISC-R). Their phenotype is unaltered from the previous report. The older brother has weight 79 kg (+7 SD for height), height 155 cm (-2 SD), and head circumference 54.5 cm; the younger brother has weight 70 kg (+6 SD for height), height 153 cm (30th centile), and head circumference 53 cm. Their faces are round and full and they have truncal obesity and short, broad hands and feet with stubby fingers and toes. The skin hyperpigmentation in the periorbital, axillary, and genital region became more manifest with age and pubertal development was markedly delayed. In the younger brother the genital status remained identical with a small phallus, hypoplastic scrotum, and two small maldescended testes palpable in the inguinal channel. In the older boy the first signs of pubertal development became apparent with the appearance of pubic hair and both testes (8/12 ml) were descended in a small scrotum.

Further evaluation of this family showed that two maternal cousins are equally moderately to severely mentally retarded (cases 6 and 7). The diagnosis of the fragile X syndrome has been confirmed in both with fragile X expression in 18% and 12% of the cells. They present with an identical phenotype of truncal obesity, skin hyperpigmentation in the periorbital, axillary, and genital regions, and short, broad hands and feet. They are now 19 and 17 years old, respectively, with weights of 98 and 92 kg, heights of 172 and 168 cm, and head circumferences of 56.5 and 54 cm.

Fig 4B and C shows patients 6 and 7 at the age of 15 and 13 years, respectively. In the older patient, the face became somewhat longer with age. Pubertal development was equally markedly delayed in both and started only after the age of 14 years. Their postpubertal status is normal with normal testes and normal scrotum. The hyperpigmentation of the genital region increased after puberty.

All four patients became obese at the age of 5 to 7 years.

Case 8

This 5 year old boy was born at term after a normal pregnancy and delivery with a birth weight of 3100 g. His development was retarded; he walked at 18 months and he spoke only a few words at 5 years. He was obese with cryptorchidism and a small penis. Because he was suspected of having the Prader-Willi syndrome, his DNA sample was referred to our laboratory for analysis of the 15q11-13 region; however, no specific abnormalities on chromosome 15 were found. He subsequently was studied for the FMR-1 gene mutation, as he belonged to the group of patients with PWS-like clinical features without the specific molecular findings in chromosome 15.

In all patients, thyroid function, LH, FSH, and plasma testosterone levels were normal for their age. The clinical features and molecular findings in cases 1 to 8 are summarised in the table.

MOLECULAR FINDINGS

In case 1, analysis of the FMR-1 gene showed a full mutation in addition to a premutation allele (insert size 0.4 kb) (fig 5, lane 2). Patient 2 showed a full mutation in addition to a deletion in the FMR-1 gene in a part of the cells (fig 5, lane 3). The size of the deleted part is 250 bp and is located around the CGG repeat.¹⁵ Proximal to the CGG repeat 53 base pairs are deleted and distal to the repeat 178 base pairs are deleted. The allele with the deletion was unmethylated. The patient's brother, with the Martin-Bell phenotype, had a full mutation only and the mother had a premutation (data not shown).

Molecular studies in cases 3, 4, and 5 showed a full mutation of the FMR-1 gene (fig 5, lanes 4, 5, and 6). DNA from cases 6 and 7 was not available for study.

In cases 1, 2, and 3 a deletion in chromo-

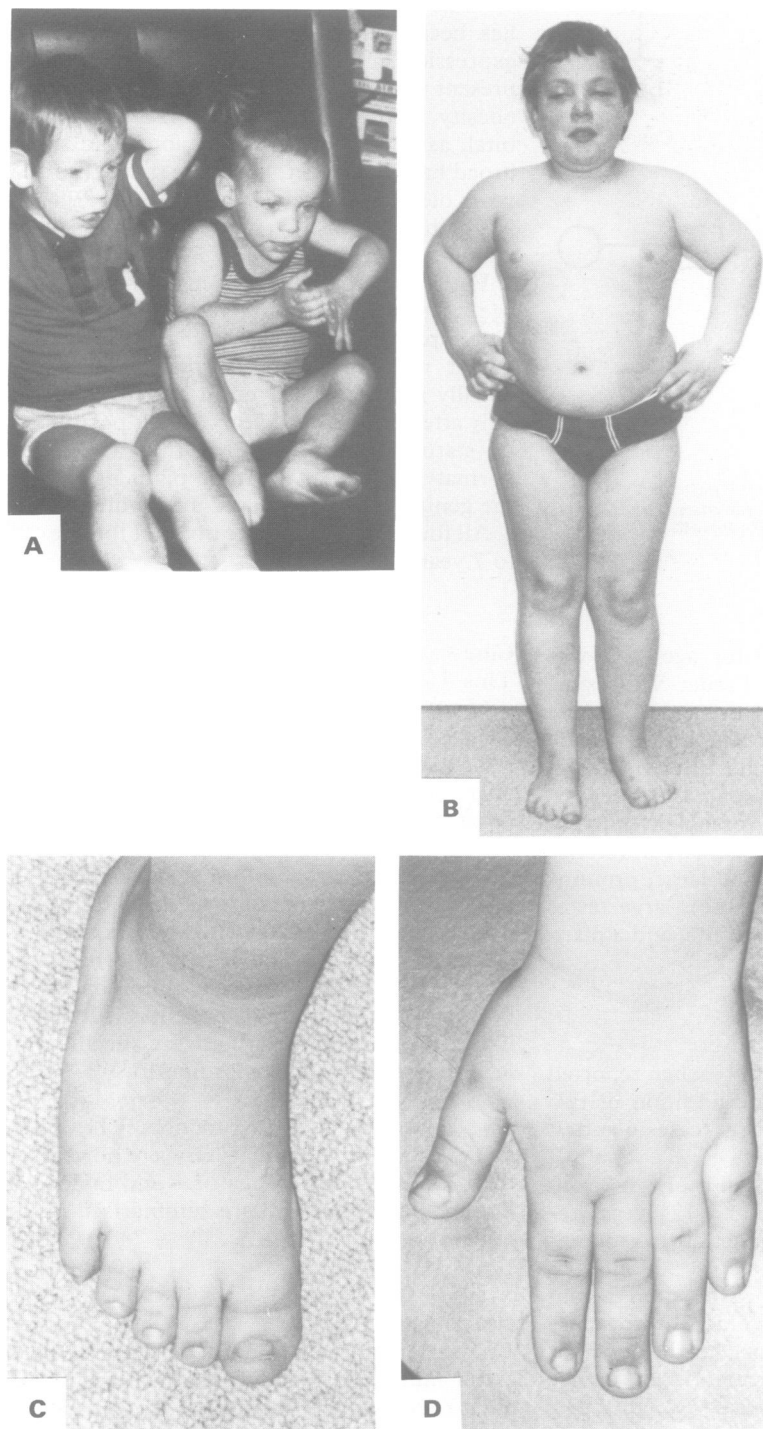


Figure 3 Case 2 (A) on right at the age of 4 years with his brother aged 7 on left. Note the slender build of case 2 and the Martin-Bell phenotype in his brother (long face with large everted ears). (B, C, D) Case 2 at the age of 9 years showing full body, foot, and hand. Note full round face, truncal obesity, short, broad foot with short, hyperconvex toe nails and hand with stubby fingers.

some 15q11-13 was not found and maternal disomy of chromosome 15 could be excluded in cases 2 and 3 (data not shown).

From the group of 26 patients with clinical features suggestive of the Prader-Willi syndrome but without the specific cytogenetic/molecular abnormalities of chromosome 15,

analysis of the FMR-1 gene showed a full mutation in one patient, case 8.

Discussion

The eight fragile X patients described here show features resembling the Prader-Willi syndrome (PWS), such as truncal obesity, hypogenitalism, and small hands and feet. Consequently, these fragile X patients might be erroneously diagnosed as having Prader-Willi syndrome, as occurred in cases 3 and 8. It is proposed to call this genotype 'Prader-Willi (PW)-like' to highlight the resemblance between both phenotypes. However, some major differences are observed between the classical Prader-Willi syndrome and the PW-like subphenotype in these fragile X patients. Unlike PWS patients, PW-like fra(X) patients have a normal birth weight and show no hypotonia with feeding problems during infancy (except for case 3). Furthermore, seven patients (cases 1 to 7) developed a sudden gain of weight at the age of 5 to 10 years without any change in diet. This is not observed in PWS patients who become obese because of a change in eating pattern which often occurs at a younger age.²⁶ Another diagnostic difference is the typical fragile X behaviour, including poor eye contact, hyperactivity, short attention span, and perseverative speech,²⁷ which is expressed by the fragile X patients with the PW-like subphenotype.

It seems that an additional metabolic defect might cause the sudden weight change in these patients. Although routine hormonal investigations in the patients were normal, the typical obesity, short hands/feet, and hyperpigmentation suggest an unrevealed metabolic disturbance and further studies have been initiated.

In most patients with the Prader-Willi syndrome, a paternal contribution to 15q11-13 is absent, caused by a deletion in the paternal chromosome or by maternal disomy. Because of the similarity in phenotype, we examined the contribution of maternal and paternal alleles at 15q11-13 in three patients (cases 1 to 3) and no abnormalities were found. Molecular studies of the FMR-1 gene in the PW-like patients showed full mutations. In one patient (case 1) a full mutation with an additional premutation was observed. As this mosaic pattern is seen in around 15% of fragile X patients,²⁸ it is not specific for the PW-like phenotype. One patient (case 2) had a full mutation and a deletion of 250 bp in the FMR-1 gene in a part of the cells.¹⁵ Considering the mutation pattern found in other PW-like patients, it is not likely that this deletion is involved in the development of the PW-like phenotype.

It is intriguing to observe the occurrence of the classical Martin-Bell phenotype and the PW-like phenotype in two brothers with the fragile X syndrome (case 2, fig 2) and the repeated transmission of the PW-like phenotype in another family (cases 4 to 7, fig 2). At present, there is no molecular explanation for these phenomena. It is conceivable that the

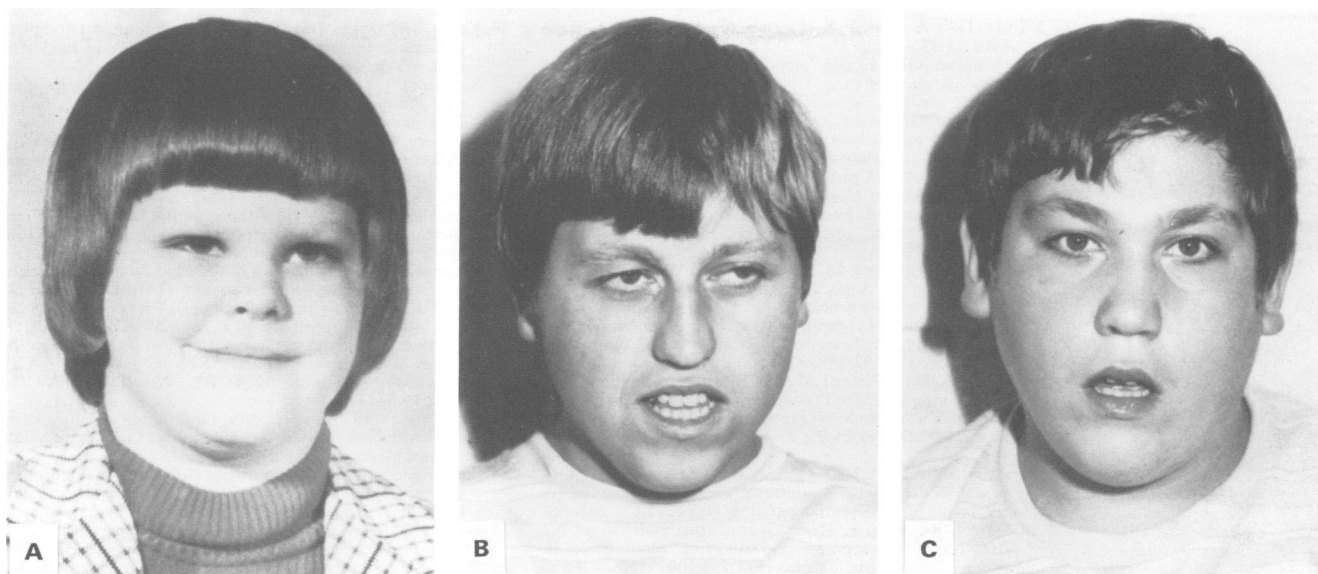


Figure 4 (A) Case 3, (B) case 6, (C) case 7. Note full found face in all three patients.

Clinical manifestations in the PW-like fragile X patients

	De Vries <i>et al</i> ¹⁵			Fryns <i>et al</i> ⁹				
	1	2	3	4	5	6	7	8
Centre of diagnosis	R	R	N	L	L	L	L	P
Present age (y)	15	11	24	14	12	19	17	5
Round, full face	+	+	+	+	+	+	+	+
Obesity (> 2 SD)	+	+	+	+	+	+	+	+
Short stature	-	+	-	+	-	-	-	-
Short, broad hands/feet	+	+	-/+	+	+	+	+	+
Hyperpigmentation								
Periorbital	-	+	-	+	+	+	+	-
Axillary	-	-	-	+	+	+	+	+
Genital	+	+	-	+	+	+	+	-
Age of onset of weight change (y)	10	6	7	5-7	5-7	5-7	5-7	ND
Delayed puberty	+	NA	+	+	+	ND	ND	NA
Hormonal investigations (TSH, LH, FSH, test)	N	N	N	N	N	N	N	N
Fra(X) expression	38%	17%	30%	12%	8%	18%	12%	ND
DNA analysis FMR-1 gene	P+F	D+F	F	F	F	ND	ND	F

+ = present, - = absent, N = normal, ND = no data available, NA = not yet assessable, P = premutation, F = full mutation, D = deletion. Centre: R = Rotterdam, N = Nashville, L = Leuven. P = Palermo.

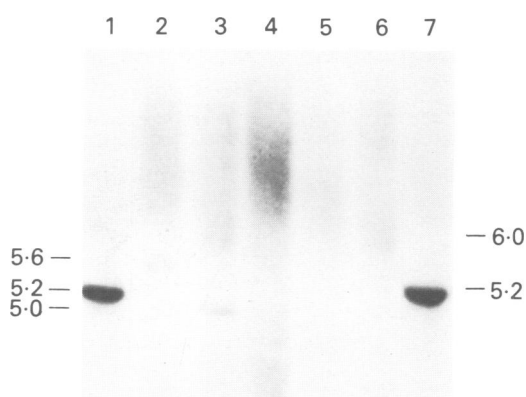


Figure 5 Analysis with probe pP2 of EcoRI digested DNA of two unaffected males (lanes 1 and 7) and five affected males (lanes 2 to 6, patients 1 to 5 in text). In addition to a full mutation, case 1 (lane 2) has a premutation and case 2 (lane 3) a deletion of 250 bp. Cases 3 to 5 (lanes 4 to 6) have a full mutation only.

mutation at the FMR-1 locus might reduce expression of (nearby) genes. The activity of a gene at the iduronate sulphatase (IDS) locus just distal to the FMR-1 locus is reduced in fragile X patients but a correlation between methylation at the IDS locus and the fra(X) status could not be found.²⁹

In the group of 26 patients, referred because of a suspected Prader-Willi phenotype but without detectable molecular abnormalities of chromosome 15, another fragile X patient with a full mutation in the FMR-1 gene was found (case 8). Of course, such a finding has major implications for diagnosis and genetic counselling.

These eight (including three previously described) cases of the 'Prader-Willi like' phenotype confirm this phenotype as a distinct manifestation of the fragile X syndrome, as described by Fryns *et al*⁹ in 1987. It seems advisable to perform DNA analysis of the FMR-1 gene in all patients presenting with a Prader-Willi phenotype without the specific molecular and cytogenetic findings on chromosome 15q.

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