CLINICAL AND BEHAVIORAL FEATURES OF PATIENTS WITH BORJESON-FORSSMAN-LEHMANN SYNDROME WITH MUTATIONS IN *PHF6*

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Objective To describe clinical and behavioral features of 10 men from 2 families with Borjeson-Forssman-Lehmann syndrome (BFLS) and missense mutations in the *PHF6* zinc-finger transcription factor gene.

Study design BFLS behavioral features were compared with other age-matched men with other syndromes and similar intellectual functioning through the use of standardized questionnaires: the Child Behavior Checklist, the Vineland Adaptive Behavior Scales, and the Reiss Personality Profile. Participants included 10 with BFLS, 10 with Prader-Willi syndrome, and 23 with Klinefelter syndrome variants (13 with 48,XXYY, 4 with 48,XXXY, and 6 with 49,XXXXY).

Results Contrary to initial reports, our men with BFLS had no microcephaly, seizures, or short stature. They manifested deep-set eyes with large ears, coarse facial features, small external genitalia, gynecomastia, and obesity. Family A had mild to moderate mental retardation, whereas family B was more severely affected. On Vineland Adaptive Behavior Scales, men with BFLS had higher daily living and social skills than communicative skills. Men with BFLS also had lower internalizing and externalizing symptoms and appeared more social and helpful than men with Prader-Willi syndrome or Klinefelter syndrome variant.

Conclusions Men with *BFLS* from 2 families with mutations in the PHF6 gene manifested distinctive clinical features and a low risk for maladaptive behaviors. *(J Pediatr 2004;145:819-25)*

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Genetic disorders influence various aspects of behavior, ranging from cognition and language to adaptive and maladaptive behaviors. It is important to understand the distinctive behavioral features associated with different genetic mental retardation syndromes and to compare behavioral phenotypes between syndromes with similar physical characteristics. Borjeson-Forssman-Lehmann syndrome (BFLS) is a rare X-linked recessive mental retardation syndrome consisting of obesity, hypogonadism, and dysmorphic features. It was first described in 1962, in 3 men from one family.[1] The uncle and two nephews had coarse facial features, large ears, deep-set eyes, short stature, and obesity. They also had hypogonadism, gynecomastia, and epilepsy (in 2 of the 3 cases). They were severely retarded, and pedigree analysis suggested X-linked recessive inheritance with variable milder expression in female heterozygotes.

Subsequently, 50 cases were reported, and the gene for BFLS was localized to Xq26-q27.[2-4] Recently this gene localization was reduced to a 9 Mb region containing more than 62 genes.[5] Among those genes, a novel zinc-finger transcription factor gene, *PHF6*, was identified, and among 7 familial and 2 sporadic cases of BFLS, 8 different missense and truncation mutations were identified in this gene.[5,6] We report clinical and behavioral features in 10 men with BFLS and a mutation in *PHF6*.

METHODS

Molecular Analysis of *PHF6*

A base substitution, c.134G→A, giving rise to a missense mutation, p.C45Y, has previously been reported [Lower et al, 2002, family 4] in Family A. The c.134G→A

BFLS	Borjeson-Forssman-Lehmann syndrome	KS	Klinefelter syndrome
CBCL	Child Behavior Checklist	PWS	Prader-Willi syndrome

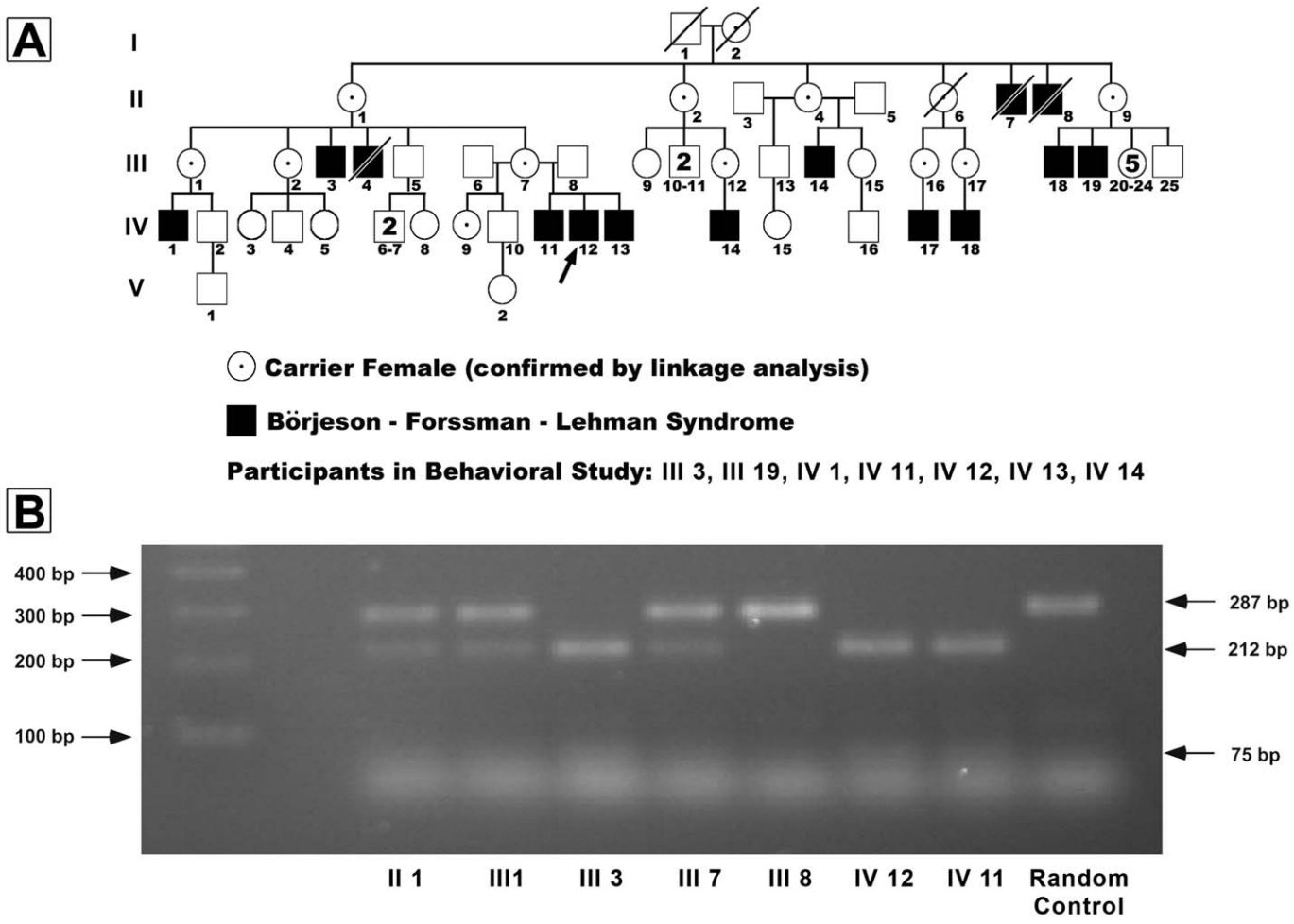

Figure 1. A, Family A pedigree. **B,** TatI analysis of the c.134G→C alteration in PHF6 in family A. Presence of the c.134G→C alteration is indicated by 2 bands at 212 bp and 75bp (numbers below lanes reflect pedigree numbers).

substitution creates a TaqI site in the mutant genomic DNA, which was used to confirm the presence of the c.134G→A change in the affected male subjects and obligate carriers in family A (Figure 1, *B*). Genomic DNA was used to PCR exon 2 of the PHF6 gene using primer BFLS exon 2 F (5′ AAA ATT AAC ATT GTC GCC CTTC 3′) and BFLS exon 2 R (5′ GAA CAT TCA TGT GTT ATT AAG AG 3′). PCR was carried out in a 30-μL volume containing 52.5 ng of genomic DNA, 1× buffer, 50 μmol/L dNTPs, 1 μmol/L of each primer, 1.5 units of Sigma Taq Polymerase (Boehringer Mannheim, Indianapolis, Ind), and 0.33 μg TaqStart Antibody (Clonetech, Palo Alto, Calif). The initial denaturing of 95°C for 3 minutes was followed by 30 cycles of denaturing at 95°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 7 minutes. After PCR amplification, 10 μL of the reaction was digested in 20 μL final volume, using 50 units of TaqI (New England Biolabs, Beverly, Mass) and spermidine at a final concentration of 1 μmol/L. The digestion products were separated on a 2% agarose gel containing ethidium bromide.

Family B was analyzed and reported by Lower et al (2002) as family 7, with a base substitution c.769A→G giving rise to a missense mutation p.A257G,[5] and clinical details were reported by Turner et al as family 7.[6]

Comparison Subjects

Parents or caretakers completed the questionnaires regarding the clinical and behavioral aspects of men with BFLS.[6] Comparison groups of men in the same age range were selected from a larger data set of men with Prader-Willi syndrome (PWS) and Klinefelter syndrome variants (48,XXYY; 48,XXXY; and 49,XXXXY). The men with 48,XXXY and 49,XXXXY were combined into one group. These comparison groups included 10 men with PWS (mean age, 25.58 years; SD, 13.37), 10 men with 48,XXYY (mean age, 18.75 years; SD, 8.54), and 12 men with 48,XXXY/49,XXXXY (mean age, 21.92 years; SD, 15.09).

Procedure and Measures

Parents completed the Child Behavior Checklist,[7] a widely used measure of maladaptive behavior in children

Table I. BFLS participants

Pedigree	Age	Height (cm)	Weight (kg)	BMI (kg/m^2)	Degree of MR
Family A					
III:3	45	172 (25%ile)	90 (90%ile)	30.4	Mild
III: 14	42	182 (75%ile)	159 (>97%ile)	48	Moderate
III: 19	32	177 (50%ile)	90 (90%ile)	28.7	Mild
IV: 1	26	172 (25%ile)	106 (>97%ile)	35.8	Mild
IV: 11	17	185 (90%ile)	148 (>97%ile)	43.3	Mild
IV: 12	16	182 (90%ile)	125 (>97%ile)	37.8	Mild
IV: 13	14	187 (>97%ile)	185 (>97%ile)	52.9	Mild
Family B					
V: 1	48	168 (10-25%ile)	100 (97%ile)	35.4	Severe
V: 4	41	170 (25%ile)	62.6 (25-50%ile)	21.7	Severe
V: 5	39	154 (<3%ile)	46.3 (<3%ile)	19.5	Severe

and adolescents. For this measurement, a 3-point scale is used to assess 112 items (0 = not true; 1 = sometimes true; 2 = often true) to assess internalizing problems (withdrawn, somatic complaints, anxious/depressed) and externalizing problems (delinquent behavior, aggressive behavior). Remaining clinical domains include social problems, thought problems, attention problems, and other problems. In this study, we used the Child Behavior Checklist raw scores; total Child Behavior Checklist *t* scores were also examined to assess clinical severity. The reliability and validity of this measure have been well established.

The Vineland Adaptive Behavior Scales[8] allow for the evaluation of three distinct domains of adaptive behavior consisting of specific subdomains: communication, daily living skills, and socialization.

The Reiss Profiles of Fundamental Goals and Motivation Sensitivities for Persons with Mental Retardation[9] uses a 5-point likert scale (strongly disagree to strongly agree) to assess 100 questions about personality. The Reiss profiles consist of a 15-factor domain, which is consistent across people with and without mental retardation. The Reiss profiles differ from many other available instruments for persons with mental retardation because they do not measure maladaptive behavior or psychopathology but instead analyze motivational strengths and styles.

RESULTS

The study subjects included 3 affected brothers and 4 affected male cousins and uncles from one 5-generation family. This family (family A) contained 7 affected men with a p.C45Y missense alteration (Figure 1, *A* and *B*), whereas family B contained 3 affected men with a p.A257G missense mutation.[5,6] Participants were 10 men with BFLS ages 14 to 48 years (mean age, 32.59 years; SD, 12.89) from two unrelated families (Tables I and Table II).

Results from the Vineland Adaptive Behavior Scales are shown in Table III. On the Vineland Scales, men with BFLS demonstrated strengths in daily living and socialization skills, with some difficulty in communication. Their daily living skills score of 72.90 (SD = 29.63) was an area of strength when compared with socialization and daily living skills. Men with PWS clearly had weaknesses in all three domains, with more impairment in daily living skills.

Results from the Child Behavior Checklist (CBCL) are shown in Table III. Men with BFLS had significantly fewer maladaptive internalizing and externalizing behaviors compared with the PWS and Klinefelter syndrome (KS) variants groups. The BFLS group demonstrated the lowest total CBCL score. Only the PWS and 48,XXYY men had total CBCL T-scores that exceeded the clinical cutoff of 64.

Further analysis of the subdomains revealed men with BFLS to be at a lower risk of internalizing and externalizing maladaptive behaviors. PWS had the highest internalizing symptoms, such as anxiety and withdrawal, followed by 48,XXYY and 48,XXXY/49,XXXXY, with men with BFLS having the lowest score. Men with BFLS also had fewer externalizing behaviors, aggression and delinquency. PWS showed the most aggression and delinquency compared with KS variants and men with BFLS. 48,XXYY had the highest score in the social subdomain.

On the Reiss Personality Profile (data available from authors), men with BFLS were comparable to PWS in their inactivity domain, probably as a result of their obesity. However, BFLS had the lowest score in the domains of anxiety, attention-seeking behavior, frustration, and vengeance. Furthermore, subjects with BFLS showed elevated scores in helping others, social contact, and sexuality. The group with PWS was high in their motivations for food and order.

DISCUSSION

Borjeson-Forssman-Lehmann syndrome is a rare X-linked recessive form of mental retardation first described in 1962 in three related men with coarse facial features, deep-set eyes, large ears, short stature, and obesity. Hypogonadism, gynecomastia, and epilepsy were also noted. These men were severely impaired, with IQ ranging from 20 to 30.[1] Two BFLS case reports published subsequently also described other men

Table II. BFLS findings

Parameters	Previous cases		New cases: Family A	New cases: Family B	Summary
Craniofacial					
Microcephaly	11/15	73%	0/5	0/3	0/8
Deep-set eyes	16/16	100%	5/5	3/3	8/8
Nystagmus	6/7	85%	0/5	0/3	0/8
Large ears	17/17	100%	5/5	3/3	8/8
Coarse face	14/16	87%	5/5	3/3	8/8
Endocrine					
Gynecomastia	14/14	100%	5/5	3/3	8/8
Small external genitalia	16/17	94%	4/5	3/3	7/8
Hypogonadism	13/13	100%	3/5	3/3	6/8
Obesity	7/12	58%	7/7	1/3	8/10
Short stature	10/12	83%	0/7	1/3	1/10
Neurologic					
Seizure	6/11	54%	0/7	0/3	0/10
Hypotonia	13/13	100%	0/5	1/3	1/8

Table III. Means and standard deviations for the Vineland Adaptive Behavior Scales

	BFLS		PWS		48,XXYY		48,XXXY/49,XXXXY	
	M	SD	M	SD	M	SD	M	SD
Daily living skills	72.9	29.63	34	13.25	72.3	21.68	50.6	28.71
Communication	49	29.26	47	17.16	62.7	23.73	37.2	8.96
Socialization	61.5	26.8	40.5	19.87	62	23.6	54.8	25.81

Mean domains raw scores and SDs on the Child Behavior Checklist

	BFLS		PWS		48,XXYY		48,XXXY/49,XXXXY	
	M	SD	M	SD	M	SD	M	SD
Internalizing	11.8	6.94	18.1	9.92	15.4	7.83	12.33	9.87
Externalizing	12.1	8.07	22.7	9.32	17.9	4.25	14.25	10.19
Total CBCL	45	21.13	79.11	26.93	65.6	17.87	56.16	28.46

Means, standard deviations, and Fs for CBCL subdomains

	BFLS		PWS		48,XXYY		48,XXXY/49,XXXXY		
	M	SD	M	SD	M	SD	M	SD	F
Withdrawn	4.2	3.39	6.8	3.99	5.5	3.2	3.75	2.56	1.85*
Anxiety	5.2	5.49	8.6	5.27	6.2	4.18	5.41	5.8	.898*
Somatic	2.4	1.42	2.7	2	3.7	3.19	3.16	3.35	0.456
Social	6.5	2.46	7.8	2.97	8.7	2.26	6.16	2.79	2.084†
Thought	1.5	1.71	4.66	1.65	2.4	2.01	2.25	2.8	3.77†
Attention	5.7	2.62	9.3	4.02	9.5	2.46	7.33	3.42	3.15†
Delinquent	3.1	2.02	7.4	3.09	5.2	2.09	4.16	4.66	3.22†
Aggressive	9	7	15.3	7.07	12.7	4.11	10.08	6.96	1.96*

*$P < .5$
†$P < .05$

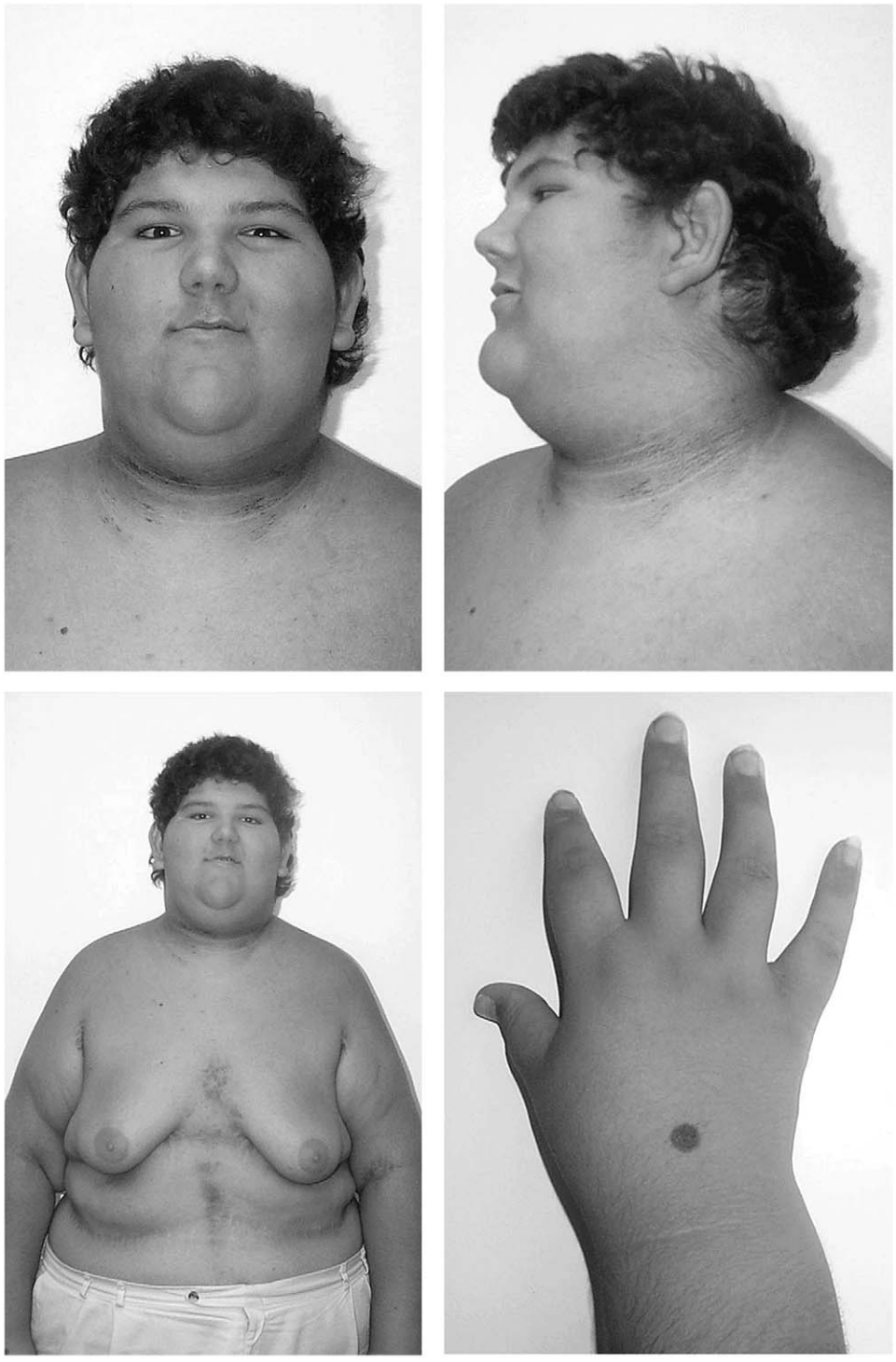

Figure 2. Clinical features of family A IV:13 showing large ears, deep-set eyes, tapered fingers, gynecomastia, acanthosis nigricans, and obesity.

with severe mental retardation, round face with large normally formed ears, obesity, hypogonadism, and short stature.[10,11] Familial cases of BFLS were later identified and these men had similar facial characteristics with prominent supraorbital ridges, microcephaly, deep-set eyes, ptosis, and large ears as well as obesity, severe mental retardation, hypotonia, and hypogonadism.[4,12,13] Heterozygous women with BFLS were reported with a wide range of phenotypic features and varying IQ levels. Recently, BFLS mutations were identified in the PHF6 zinc-*finger* transcription factor gene located at Xq26-q27.[5,6]

Severe mental retardation was present in many of the previously reported cases; however, family A in this study functions in the mild range (Table I). These affected men share similar facial features with previously reported cases; however, they do not have microcephaly, short stature, or

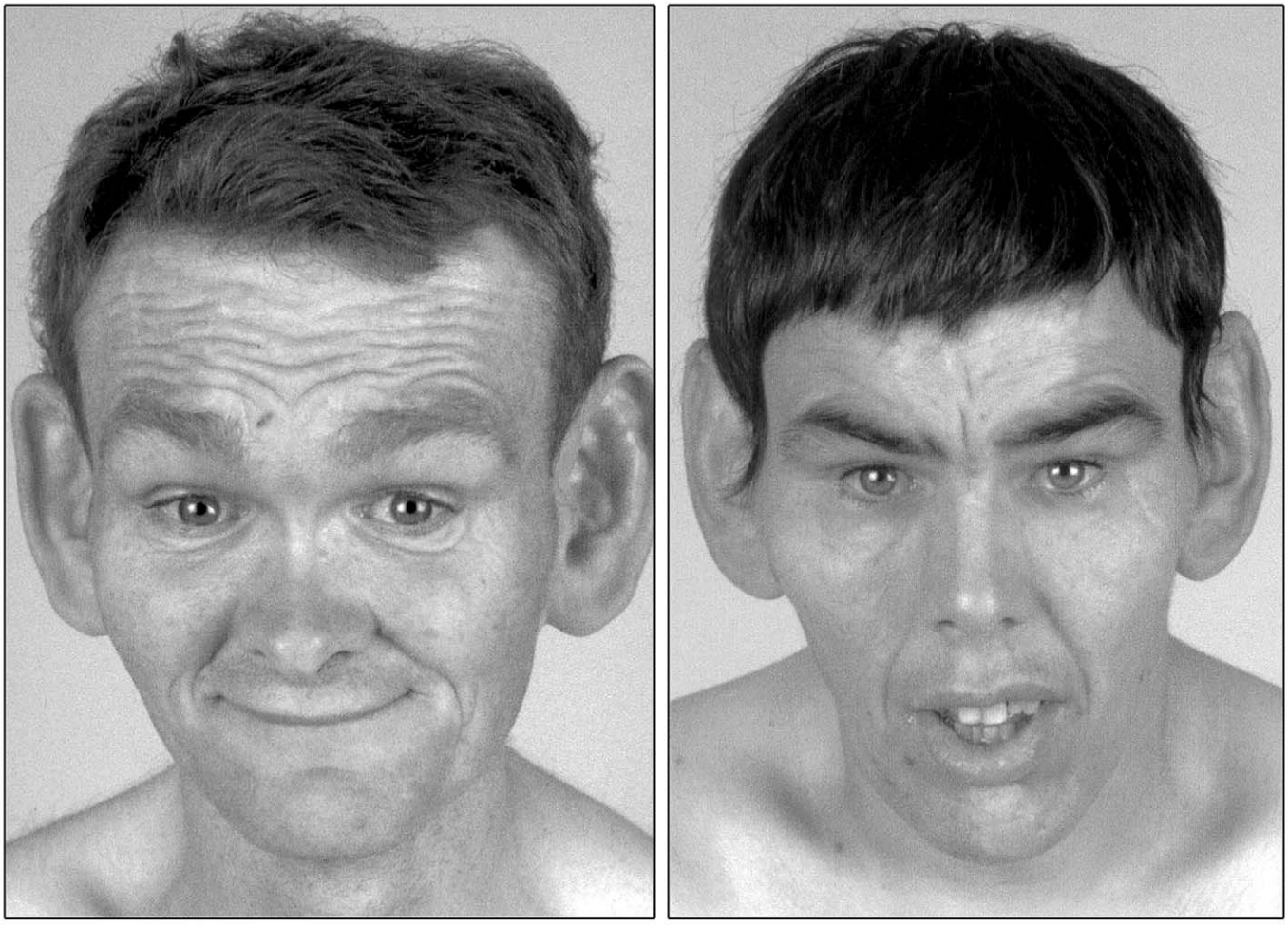

Figure 3. Photographs of individuals family B V:4 and V:5, demonstrating deep-set eyes and large ears, but neither individual is obese.

epilepsy (Table II and Figure 2). Family B has severe mental retardation, short stature, and distinct facial characteristics consistent with BFLS (Figure 3). Individuals from both families have mutations in PHF6 and were used to identify the mutated gene involved in this syndrome.[5]

Although the clinical features of BFLS are well described in literature, the behavioral phenotype has not been studied. This study is designed to determine whether BFLS is associated with a distinctive pattern of behavior functioning. To achieve this, we compared 10 men with BLFS with other men with mental retardation syndromes that share similar physical characteristics such as hypogonadism and obesity. Individuals with PWS and KS variants (48,XXYY; 48,XXXY; 49,XXXXY) are compared with BFLS to assess their similarities and differences in behavior.

Men with BFLS have lower global ratings of maladaptive behavior than PWS and KS variants. PWS shows a distinctive behavioral phenotype of preoccupations and repetitive, compulsive-like behaviors that are not related to food. In several studies, it has been shown that individuals with PWS manifest significant maladaptive behaviors.[14,15] Men with BFLS are at a low risk for externalizing and internalizing maladaptive behavior.

Obesity in BFLS and PWS causes avoidance of physical activity, as revealed on the Reiss Personality Profiles. Unlike men with PWS, individuals with BFLS are not highly motivated by food and are more motivated by sexuality. Men with PWS are impatient and have low frustration tolerance, characteristics that are well established in this syndrome.[15,16] Men with BFLS in our study were motivated to help others and socialize, as opposed to men with 48,XXYY.

Men with BFLS are higher-functioning than men with PWS and 48,XXXY/49,XXXXY and this results in their relative strengths of daily living and socialization skills on the Vineland Adaptive Behavior Scales 48,XXYY IQ level usually ranges from 60 to 80, whereas 48,XXXY and 49,XXXXY are lower-functioning.[17] Men with 48,XXYY demonstrated fewer problems in communicative skills as compared with BFLS, PWS, and 48,XXXY/49,XXXXY. Communicative skills appeared to be problematic for men with BFLS and PWS; however, men with BFLS have significantly higher skills in their daily living and socialization domains.

Although the sample size remains a limitation of this study, findings for men with BFLS are relevant with regard to the delineation and interventional implications of distinct behavioral phenotypes. Men with BFLS demonstrate a deficit

in the communicative domain, and this weakness may be masked by their relative strengths in socialization and daily living skills. Hence, it is imperative for men with BLFS to receive early speech and language therapy to improve their communicative skills. Despite this weakness, they have many positive attributes in their personality style, as seen in their desire to help and interact with others. They also have low risk of delinquency or aggressive behaviors. For these reasons, they are usually capable of living independently. Relative to the general population of persons without intellectual disabilities, we find men with BFLS present with the capability of leading productive lives within the society in which they live if given sufficient early interventional speech therapy, access to educational assistance to address specific cognitive, behavioral, and adaptive concerns. In addition, obesity can be a serious problem for boys with BFLS as well as for other boys with hypogonadism, and it merits early anticipatory guidance and consideration of testosterone therapy.

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