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Lack of evidence for monosomy 1p36 in patients with Prader-Willi-like phenotype

V.R. Rodríguez, L.F. Mazzucato and J.M. Pina-Neto

Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

Correspondence to: J.M. Pina-Neto, Departamento de Genética, FMRP-USP, Av. Bandeirantes, 3900, 14049-900 Ribeirão Preto, SP, Brasil
Fax: +55-16-3633-0069. E-mail: jmdpneto@fmrp.usp.br

Monosomy 1p36 is the most common subtelomeric microdeletion syndrome with an incidence rate estimated to be 1 in 5000 births. A hypothesis of a similarity between patients with 1p36 deletion and those with Prader-Willi syndrome and the existence of two different phenotypes for 1p36 microdeletion has been suggested. The main objective of the present study was to determine the existence of 1p36 microdeletion in a sample of patients with mental retardation, obesity and hyperphagia who tested negative by the methylation test for Prader-Willi syndrome. Sixteen patients (7 females, 9 males), 16-26 years old, were evaluated with high-resolution cytogenetic analysis at 550-850 band levels and with 11 polymorphic microsatellite markers located in the 1p36 region. All patients had normal cytogenetic and molecular results. The results obtained by high-resolution cytogenetic methodology were confirmed by the molecular analyses. We did not detect a 1p36 microdeletion in 16 subjects with the Prader-Willi-like phenotype, which reinforces that no correlation seems to exist between Prader-Willi-like phenotype and the 1p36 microdeletion syndrome.

Key words: Monosomy 1p36; 1p36 microdeletion syndrome; Prader-Willi syndrome; Prader-Willi-like phenotype

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We tested for the 1p36 microdeletion in 16 patients (16-26 years old) with the Prader-Willi-like phenotype who tested negative for the Prader-Willi syndrome using the methylation test by Southern blot with the Kb17 probe (1).

Clinical characterization of the patients (Table 1) was performed using a protocol with the characteristics of Prader-Willi phenotype and 1p36 microdeletion syndrome based on the literature (2-11). The protocol was approved by the institutional Ethical Review Board and written informed consent was obtained from the parents of all patients. Twelve patients were examined by the authors, and the other 4 were studied by the University Hospital of Rio Grande do Sul (Porto Alegre, RS, Brazil).

Two methods were used to identify the 1p36 microdeletion. First, the high-resolution 1p36 cytogenetic region studies. For this, we obtained metaphases from phytohemagglutinin-stimulated peripheral blood lymphocytes using the methotrexate and thymidine synchronization method (12) with GTG banding for cytogenetic analysis performed at 550-850 band levels. We analyzed 40 metaphases for each patient. The levels reached to exclude mosaics were $\leq$6% for a confidence interval of 99%, and $\leq$8 and $\leq$11% for 95 and 90% confidence intervals, respectively (13). Secondly, polymorphic markers of the 1p36 region were analyzed using 11 dinucleotide or tetranucleotide polymorphisms (D1S243, D1S468, D1S2660, D1S2795, D1S2870, D1S2145, D1S214, D1S2663, D1S450, D1S244, D1S2667). For this assessment, we collected blood samples from patients and their parents and extracted genomic DNA by a standard method, and later performed amplification in a volume of 15 µL with 50 ng genomic DNA, 6 pmol of each primer, and 0.5 unit of thermostable DNA polymerase in a buffer containing 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, and 100 µmol/L of each dNTP, and 0.2 µL 32-P dNTP was added per reaction. Thirty-one amplification cycles were used with 35 s at 95°C, 35 s at 55-62°C for primer annealing, and 35 s at 72°C for extension. Products

were separated on 6% polyacrylamide/urea gels and analyzed based on mapping data obtained from <http://www.Ensembl.org/Homo_sapiens> for chromosome 1. In order to establish which polymorphic loci were deleted, alleles were compared between the patient and their parents.

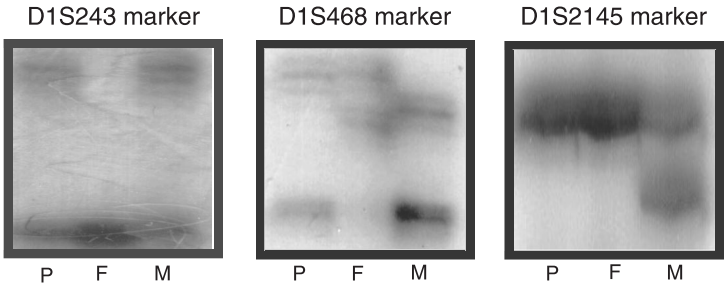

Figure 1. Polymorphic marker analysis for 1p36 deletion. Analyses of three chromosome 1p36 markers are shown (D1S243, D1S468 and D1S2145) for patient 8 (P = patient, F = father, M = mother). The allelic distribution was considered to be normal.

Table 1. Clinical characteristics of Prader-Willi phenotype and specific characteristics of 1p36 microdeletion syndrome observed in 16 patients using a protocol based on the literature (2-11).

Clinical characteristics of Prader-Willi phenotype	Number of patients (%)
Developmental delay	13/16 (81%)
Mental retardation	16/16 (100%)
Hypotonia	15/16 (94%)
Obesity	16/16 (100%)
Before 6 years of age	9/16 (56%)
After 6 years of age	7/16 (44%)
Hyperphagia	16/16 (100%)
Before 6 years of age	9/16 (56%)
After 6 years of age	7/16 (44%)
Infant feeding problems	15/16 (94%)
Hypogonadism	2/16 (13%)
Hypogenitalism	4/16 (25%)
Small hands	4/16 (25%)
Small feet	5/16 (31%)
Down-turned mouth	3/16 (19%)
Middle face hypoplasia	6/16 (38%)
Sleeping disturbance	1/16 (6%)
Strabismus	4/16 (25%)
Abusive behavior	12/16 (75%)
Specific clinical characteristics of 1p36 monosomy present in our patients	**Number of patients (%)**
Large anterior fontanel	4/16 (25%)
Middle face hypoplasia	6/16 (38%)
Short fifth finger	3/16 (19%)
Fifth finger clinodactyly	4/16 (25%)
Strabismus	4/16 (25%)
Hypothyroidism	1/16 (6%)

After the high-resolution cytogenetic analysis, no subtelomeric deletion in the 1p36 region was observed in 40 metaphases for each patient. All 11 dinucleotide and tetranucleotide repeat polymorphic markers for each family were negative for 1p36 microdeletions. In cases of homozygosity in one marker, the heterozygosity of other markers was used to indicate lack of deletion (Figure 1).

Our patient sample (Table 1) was consistent with the Prader-Willi-like phenotype because 100% had mental retardation associated with obesity and hyperphagia, and a high prevalence of other important signs of Prader-Willi syndrome, i.e., developmental delay (81%), hypotonia (94%), infant feeding problems (94%), abusive behavior (75%), and other associated dysmorphic features.

The number and position of the polymorphic markers in the 1p36 region used are located in all six intervals of the microdeletion distribution, as suggested by Wu et al. (14) and observed by Heilstedt et al. (11). Furthermore, we used two polymorphic markers (D1S243 and D1S468) mapped inside the obesity/hyperphagia chromosomal segment 1p36.33-36.32 suggested by D'Angelo et al. (15). All results obtained in the polymorphic marker analyses in our patient sample were normal. On the other hand, all 40 metaphases analyzed by the high-resolution technique were also normal, revealing a correspondence between cytogenetic and molecular analysis and reinforcing the observation of Heilstedt et al. (16) that 98% of this microdeletion could be detected by high-resolution chromosome analysis.

Slavotinek et al. (8) reviewed 39 cases reported to have pure 1p36 deletion and only 2 cases (5.1%) had the Prader-Willi-like phenotype. Shapira et al. (7) described 13 cases of the pure 1p36 deletion and 2 cases (15%) had obesity. D'Angelo et al. (15) have described 1 case of pure 1p36 deletion within a group of 41 cases with Prader-Willi-like phenotype. The phenotype defined as monosomy 1p36 by Heilstedt et al. (11) excluded hyperphagia/obesity and the relationship between this microdeletion syndrome and the Prader-Willi-like phenotype. Our results do not confirm the suggestion of D'Angelo et al. (15) about a specific hyperphagia/obesity chromosomal region in 1p36 but reinforce the opinion of Heilstedt et al. (11).

Shaffer and Heilstedt (17) affirm that the phenotype of 1p36 monosomy is very characteristic with high similarities among the patients. If we consider that obesity is a very common clinical sign associated with mental retardation, and that it is now a public health problem, we question if these patients, who were described as being obese, are really representative of this microdeletion syndrome.

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