RARE CASE OF WOLF- HIRSCHHORN INVOLVING THE GENES PIGG AND PAOX

LAÍS DUARTE **CABRAL**[1], MONIQUE JARETTA **ARDISON**[1], ROBERTA DE ANDRADE **MACHADO**[1], VINÍCIUS DEL GAUDIO **ALBERGARIA**[1], JAQUELINE MELO **SOARES**[2], MARIANA ANGELOZZI **OLIVEIRA**[3], CATARINA AMORIN BACCARINI **PIRES**[4], CIRO DRESCH **MARTINHAGO**[5], ANALINA FURTADO **VALADÃO**[6*]

1. Medicine student from the Metropolitan Institute of Higher Education / Imes-Univaço, Ipatinga, Minas Gerais, Brazil; 2. Veterinary Medicine. PhD in Sciences. Professor in the Medicine Course at the Metropolitan Institute of Higher Education/IMES - Univaço, Ipatinga, Minas Gerais, Brazil. 3. Biologist. Experience in Genetics, with emphasis on Human and Medical Genetics. 4. Pediatrician. Specialist in neonatology and neonatal intensive care by SBP. Professor in the Medicine Course at the Metropolitan Institute of Higher Education/IMES - Univaço, Ipatinga, Minas Gerais, Brazil. 5.Medical geneticist. Specialist in clinical genetics by SBGC. PhD in Reproductive Genetics, UNESP, Botucatu, member of the genetics team at Albert Einstein Hospital. 6. Pharmacist. PhD in Biochemistry and Immunology. Professor in the Medicine Course at the Metropolitan Institute of Higher Education/IMES - Univaço, Ipatinga, Minas Gerais, Brazil.

* Rua João Patrício Araújo, 157, Veneza I, Ipatinga, Minas Gerais, Brasil. CEP: 35164-251. analina@famevaco.br

Recebido em 21/11/2019. **Aceito** para publicação em 17/12/2019

ABSTRACT

Wolf-Hirschhorn Syndrome is a genetic disorder characterized by craniofacial malformations, convulsions, growth delay and cognitive disability. The etiology is characterized by microdeletions of the most terminal portion of the short arm of chromosome 4. We describe the case of a Male child, 4 years old, low weight, short stature, delayed psychomotor development, congenital dislocation in both knees, absence of tibia, bilateral short femur, malformations in ears and other alterations seen in imaging tests. G-band karyotype examination showed the result: 46, XY, der (4) t (4; 10) (p16.1; q25.2)Pat, including monosomy of the 4p16.1-pter region and trisomy of the region 10q25.2qter. In order to further investigate the altered chromosome region, the Multiple Link Probes Amplification (MLPA) technique was chosen, two genes disorders were identified: deletion of the PIGG gene on the short arm of chromosome 4 and duplication of the PAOX gene on the long arm of chromosome 10. Conclusion: Clinical findings are compatible with the characteristics described for the syndrome combined with a rare genetic phenotype. Monosomy of the 4p16.1-pter region and trisomy of the region 10q25.2qter combined with a double unbalanced genetic segment in a WHS child could be related to severe phenotypical alterations and limited surviving rate.

KEYWORDS: Wolf-Hirschhorn; genotyping; microdeletion; subtelomeric.

1. INTRODUCTION

Wolf-Hirschhorn Syndrome (WHS) was first described in 1961 by the German scientist Ulrich Wolf and the American researcher Kurt Hirschhorn; both studied and published, separately, a case of genetic deletion of the terminal region of the short arm of chromosome 4[1]. In 1965, both characterized the syndrome as causing microcephaly and anomalous morphology of the skull. It's characteristic shape in the classical presentation of the disease received the name of "Greek warrior's helmet".

Approximately 50-60% of the affected individuals have a *de novo* deletion covering the 4p16.3 region, known as the critical region (WHSCR), and around 40-45% have unbalanced translocations. The size of the chromosomal deletion may be variable and these deletions can, in some cases, be inherited from a relative with a balanced rearrangement [2].

According to Shannon, Maltby, Rigby and Quarrell[3], the syndrome affects 1 in every 50,000 live births, with the female population being the most affected in a 2:1 rate. Mortality, according to Battaglia, Fillipi and Carey[4], is estimated at 34% in the first two years of life.

Conventional banding techniques detect between 50-60% of the deletions, while fluorescence in situ hybridization (FISH) detects more than 95% of them, when performed with probes for the critical region[5].

New techniques such as array-based comparative genomic hybridization (aCGH) and Multiple Link Probing Amplification (MLPA) are even more accurate.

Considering all the information above, the purpose of this study is to report a rare genetic alteration in a male child with Wolf-Hirschhorn Syndrome, as well as to describe the phenotypic characteristics of the patient.

2. CASE REPORT

This study was approved by the Research Ethics Committee on Human Subjects (CEP-Unileste/MG), under the number 1,875,173, CAAE - 60445516.3.0000.5095.

In the present article, we report the case of a male child, born in 2013 in a small town of the Brazilian state of Minas Gerais.

There were intercurrences during gestation, such as bleeding and hypertension in the first months. It was his mother's second gestation; in the first there was a spontaneous abortion. The fetus had moved little during intrauterine life. Pregnancy was accompanied by prenatal exams. The mother denied the use of cigarettes, drugs and alcohol before and during

pregnancy.

The child was born premature, at 33 weeks, a Caesarian section was performed due to oligohydramnios and restricted intrauterine growth. Weighing 1.475 kg and 33.7 cm in length, cephalic perimeter of 30.7 cm, Apgar score of5 (1') and 6 (5') and presenting respiratory distress. He received oxygen for 2 days and remained hospitalized for 27 days. The newborn blood screening tests were negative and the hearing tests were abnormal.

The child had lower limb hypotrophy, congenital dislocation in both knees, absence of right and left tibia, bilateral short femur, absence of medial rays of both feet, globally diminished tonus, left cryptorchidism, upward palpebral slit, presence of epicanto, short neck, axial hypotonia, slow movements of the upper limb and until 10 months of age he did not present cervical control.

The echocardiogram detected patent foramen ovale and small shunt from left to right. The transfontanelar ultrasound presented choroid plexus cysts bilaterally and supratentorial ventriculomegaly. Ultrasound of the abdomen showed dilatation of the left renal pelvis.

Computed tomography showed interdigitationsamong cerebral gyri near the falxcerebri, associated with parallelism of the lateral ventricles, whose differential diagnosis includes dysgenesis/agenesis of the corpus callosum.

The morphological examination of the patient showed a broad forehead, prominent metopic suture, epicanthal folds, impression of hypertelorism, upslanted palpebral slits, flattened nasal base, small nose, undeveloped nasal fins, long and well markednasolabial filter, ears rotated posteriorly and previously malformed, fingertips with sharp points, rudimentary feet, two rudimentary fingers on the right foot with syndactyly, two rudimentary fingers on the left foot with interdigital cleft and curved lower limbs.

Genetic Analysis

The parent's genetics were normal for the mother with a 46, XX karyotype and 46, XY, t (4; 10) (p16.1; q25.2) for the father, characterizing apparently, a balanced translocation involving the short arm of chromosome 4 and the long arm of chromosome 10.

The child's karyotype was 46, XY, der (4) t (4; 10) (p16.1; q25.2) pat. This result shows deletion at the end of the short arm of chromosome 4 and trisomy in the end of the long arm of chromosome 10 apparently due to parental balanced translocation between the short arm of chromosome 4 and the long arm of chromosome 10. For more detailed investigation of the observed changes, it was used the Multiplex Ligation-dependent Probe Amplification (MLPA) (MRC-Holland) technique, using probe P036.

In the analyzed sample, according to figure 1, it is shown the partial deletion of the short arm of chromosome 4 and partial duplication of the long arm region of chromosome 10. Based on the panel of probes used, the deletion in the short arm of chromosome 4 compromised the PIGG gene, located on the short arm of chromosome 4, (4p16.3). The partial duplication of the long arm of chromosome 10 (10q26.3) compromised the PAOX gene.

In the analyzed sample, according to figure 1, it is evidenced the partial deletion of the short arm of chromosome 4 and partial duplication of the long arm region of chromosome 10. Based on the panel of probes used, the deletion in the short arm of chromosome 4 compromised the PIGG gene, located on the short arm of chromosome 4(4p16.3). The partial duplication of the long arm of chromosome 10 (10q26.3) compromised the PAOX gene.

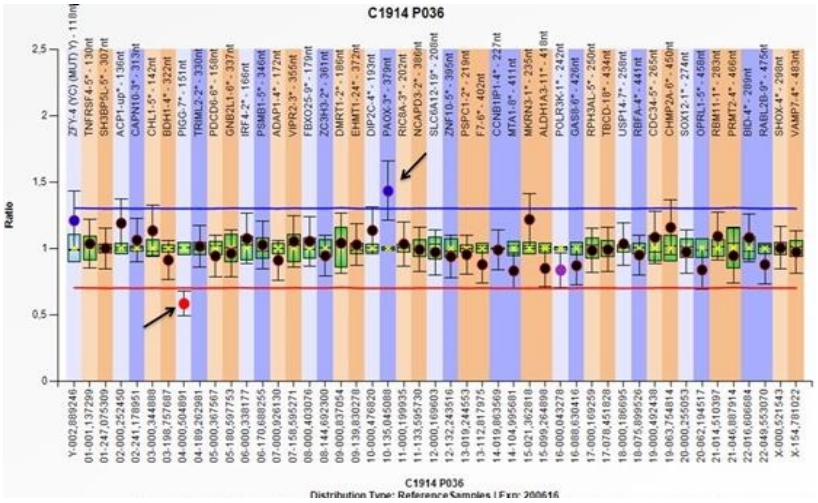

Figure 1. Result of MLPA P036 showing evidence of terminal deletion on the short arm of chromosome 4 and terminal duplication on the long arm of chromosome 10 (indicated by the arrows). Ordinate represent the peak areas of each amplification product. Abscissae represent the location of coordinates of genes. The upper part shows the size of the probes and their abbreviations of the investigated genes. Each point represents the corresponding probe signal. The greens boxes between the two lines represent the normal signal of the probes, the peak ratio of the range from 0.7 to 1.3. The Red dot, below the red line represents the elimination of the gene dosage (deletion). The Blue dot above the blue line means increased gene dosage (doubling). Retained image of the MLPA exam that was performed on the patient in the case.

3. DISCUSSION

In the last five decades, since the original description, knowledge about clinical phenotype, molecular cytogenetics and natural history of Wolf-Hirschhorn Syndrome (WHS) has expanded, especially in recent years[5]. Wolf Hirschhorn Syndrome (WHS) is a condition caused by the deletion of the short arm of the chromosome 4 (region 4p16.3), specifically in the gene locus LETM1, WHSC1 and WHSC2.

In order to elucidate the genotype-phenotype relationship identified in the patient described in the article, the MLPA (MRC-Holland) technique was performed. It identified loss of genetic material at

the end of the short arm of the chromosome 4 involving the PIGG gene and gain of genetic material at the end of the long arm of chromosome 10 involving the duplication of the PAOX gene.

To assess which factors led to the phenotypic and clinical characteristics of the patient, it should be taken into account that two genes were mainly affected: PIGG and PAOX. The PAOX gene has never been previously associated with WHS. This finding reaffirms the comments of Ho et al.[6]: "deletions associated with WHS are highly variable in size and genetic content potentially causing or contributing to the variability of the clinical presentation of the syndrome ".

The PIGG gene is located in position 4p16.3, and its absence is related to mental retardation. The PIGG gene is responsible for encoding a protein involved in the transfer of an ethanolamine phosphate (EtNP) molecule to a second mannose (Man2) molecule of most types of glycosylphosphatidylinositol (GPI) anchors. The encoded PIGG protein has the ability of changing GPIs conformation after binding to membrane proteins[7].

The proteins encoded by the PIGG gene are distributed throughout eukaryotic cells contents. They have as main functions: enzymatic catabolism, cell-cell interaction, the complement system regulation and production of antibodies[8].

In 2014, Zollino et al.[9] described that PIGG gene could be proposed as a candidate for the presence of seizures in syndromic patients. In 2016, Bi, Cheung, Breman and Bacino[10], showed that in addition to the seizure, this gene may also be involved with intellectual disability and hypotonia. In 2016, Ho et al.[6] found a critical region of 197 kbp at the end of chromosome 4, which could be responsible for the convulsive condition. In this region, the PIGG gene was found. This was then described as an "excellent candidate for the gene responsible for susceptibility to seizures".

The PAOX gene is located on chromosome 10, more specifically in the region 10q26 .3. It is a protein coding gene, responsible for the coding of polyamine oxidase[11].

Polyamines are polycations that interact with negatively charged molecules, including DNA, RNA and lipids. This binding ability on different molecules means that polyamines are involved in several important processes, such as DNA stability, cell growth and proliferation, and their death. The metabolism of polyamines is very dynamic and many studies are still in the process of investigating the role of the three main polyamines: putrescein, spermidine and spermine[11].

Considering the extremely low physical development of the patient (malformations of the lower limbs, absences of bone structures and significant delay in the neuropsychomotor development), before explaining that the PAOX gene regulates anabolic and catabolic processes, it is possible to suggest that there is some expression of the PAOX gene in this patient. Disarrangement between genes PAOX and its protein coding ability in conjunction with the other aspects of the syndrome may have compromised the development of weight and height of the child.

No description of the WHS- related PAOX gene wasfound in the literature, which makes this work unprecedented in this respect and it demonstrates the complexity of the phenotypic and genotypic interactions that occur in the reproductive process.

In the case under discussion the size of the observed deletion was not determined. Zollino et al.[12] proposed the classification of clinical signs in three categories related to the amount of genetic material lost. The first category is called "mild" (deletions smaller than 3.5 Mb), "moderate" (deletions between 5 to 18 Mb), and "severe or atypical" (22-25 Mb loss of genetic material).

From this perspective, clinical signs of the patient in question were compared in conjunction with the classification of the clinical signs suggested by Zollino et al. (2008)[12] shown on Table 1.

Comparing the studied patient to the "mild, moderate and severe" WHS categories, we can see that the patient presents clinical signs that are compatible with the "severe" category because these patients have atypical facial features and are not characteristic of classic WHS.

Two characteristics that should be highlighted in this patient are the seizures and the very low neuropsychomotor development. Ho et al. (2016)[6] described that 90% of patients have seizures, and these can greatly influence the individual's quality of life, hence the importance of recognizing the most influential genetic changes for seizures.

Other similar cases of chromosome translocation between chromosomes 4 and 10 using other diagnostic techniques have been reported but, the literature shows that cases of translocations, especially unbalanced, between chromosomes 4 and 10 are rare.

Goodship et al. (1992)[13] reported a case of a 2-year-old female with neuropsychomotor development, seizures, hypertelorism, short lip filter, prominent glabella, and carp mouth. The clinical characteristics were suggestive of the Wolf-Hirschhorn Syndrome, so to elucidate the case a molecular investigation was carried out. With the FISH technique, there was a balanced translocation between the short arm of chromosome 4 and the long arm of the paternal chromosome 10, but in the child there was an unbalanced translocation, in which deletion of the short arm of chromosome 4 and doubling of the long arm of chromosome 10 were observed.

Zollino et al. (2008)[12], described an analysis performed with 80 patients with WHS. Among the several translocations responsible for the etiology of the disease, it is mentioned that in two patients, there was an unbalanced translocation between chromosomes 4 and 10, as well as in our study, but in different regions. The translocations described in the

study were t (4; 10) (p16.3; p15) and (4; 10) (q26.11; p15.2).

Table 1. Clinical signs of the patient compared to the classification proposed by Zollino et al. (2008).

Features	Patient	Tables adapted from Zollino et al. (2008)					
		mild (<3.5 Mb)		Moderate (5 to 18 Mb)		Severe (> 22-25 Mb)	
		N *	%	N	%	N	%
Typical facial dysmorphisms	-	50/50	100	106/106	100	-	-
Typical facial dysmorphisms, not consistent with WHS	+	-	-	-	-	10/10	100
Mild to moderate mental retardation	-	31/41	76	9/37	24	0/5	0
Severe mental retardation	+	10/41	24	28/37	76	5/5	100
Seizures	+	47/49	96	61/76	80	9/10	90
Prenatal growth delay	+	25/26	96	26/31	84	8/8	100
Postnatal growth delay	+	43/50	86	79/87	91	7/9	78
Microcephaly	+	44/47	94	72/76	95	9/9	100
Hypotonia	+	17/19	89	39/43	91	7/7	100
Congenital Heart Defects	+	1/47	2	54/103	52	7/10	70
Cleft Lips or palate	-	4/49	8	25/102	25	4/9	44
Ocular coloboma	+	0/29	0	30/101	30	8/10	80
Hypospadias	NI *	6/10	60	12/29	41	3/8	38
Renal Abnormalities	+	1/42	2	31/83	37	6/16	38
Skeletal Abnormalities	+	8/29	28	23/58	37	6/18	33
Psychotic behavior	NI	-	-	-	-	2/4	50
Clinical signs associated with deletion							

4. CONCLUSION

Genetic diagnosis is fundamental for the resolution of phenotypically complex cases in Wolf-Hirschhorn Syndrome. In this case, the patient had several characteristics associated to WHS, but only the karyotype did not explain the severity of the case. After completion of the MLPA technique, it was clear that the patient suffered from WHS and it was also determined that the genes PIGG and PAOX were affected, attributing to them the serious dysfunctions observed in the patient.

This is the first report of a patient that combined 4p16.1-pter deletion with an associated duplication of the 10q25.2qter genetic region, together with the probably abnormal expressions of the PIGG and PAOX genes. The coexistence of two chromosomal changes generating a chimeric phenotype contributes to the complexity and severity of the patient's clinical condition.

FINACIAL SUPPORT

We thank the laboratory Chromossome – Genomic Medicine for the accomplishment of the examination of MLPA.

To the Association of Parents and Friends of Ipatinga (APAE), the support in the contact with the parents and with the patient.

To the laboratory Dirceu Ferreira, the support in the collection oh the biological material.

To the patient's parents, trust and attention.

REFERÊNCIAS

[1] Bergemann A; Cole F; Hirschhorn K. The etiology of Wolf-Hirschhorn syndrome. Trends Genet. 2005; 21(3): 188-195.

[2] Wieczorek D, Krause M, Majewski F, Albrecht B, Meinecke P, Riess O, *et al.* Unexpected high frequency of non- unbalanced translocation with Wolf-Hirschhorn syndrome (WHS). J Med Genet. 2000; 37(10):798-804.

[3] Shannon NL, Maltby EL, Rigby AS, Quarrell OW. An epidemiology study of Wolf-Hirschhorn: life expectancy and cause of mortality. J Med Genet. 2001; 38(10):674-679.

[4] Battaglia A; Fillippi T; Carey JC. Update on the clinical features and natural history of Wolf-Hirschhorn (4p-) syndrome: experience with 87 patients and recommendations for routine health supervision. Am J Med Genet C Semin Med Genet. 2008; 148C(4):246-51.

[5] Battaglia A; Carey JC; South ST. Wolf-Hirschhorn syndrome: a review and update. Am J Med Genet C Semin Med Genet. 2015; 169(3):216-223.

[6] Ho KS, South ST, Lortz A, Hensel CH, Sdano MR, Vanzo RJ, *et al*. Chromosomal microarray testing identified at 4p terminal region associated with seizures in Wolf-Hirschhorn syndrome. J Med Genet. 2016; 53(4):256-263.

[7] Makrythanasis P, Kato M, Zaki MS, Saitsu H, Nakamura K, Santoni FA, *et al*. Pathogenic variants in PIGG cause intellectual disability with seizures and hypotonia. Am J Hum Genet. 2016; 98(4):615-26.

[8] Paulickm MG; Bertozzic CR. The Glycosylphosphatidylinositol Anchor: A Complex Membrane-Anchoring Structure for Proteins. Biochemistry. 2008; 47(27):6991-7000.

[9] Zollino M, Orteschi D, Ruiter M, Pfundt R, Steindl K, Cafiero C, *et al*. Unusual 4p16.3 deletions suggest an additional chromosome region for the Wolf-Hirschhorn syndrome-associated seizures disorder. Official Journal of the International League Against Epilepsy. 2014; 55(6):849-857.

[10] Bi W, Cheung SW, Breman AM, Bacino CA. 4p16.3 Microdeletions and Microduplications Detected by Chromosomal Microarray Analysis: New Insights Into Mechanisms and Critical Regions. Am J Med Genet A. 2016; 170(10):2540-2550.

[11] Vujcic S, Liang P, Diegelman P, Kramer DL, Porter CW. Genomic identification and biochemical characterization of the mammalian polyamine oxidase involved in polyamine back- conversion. Biochem J. 2003; 15;370(Pt 1):19-28.

[12] Zollino M, Murdolo M, Marangi G, Pecile V, Galasso C, Mazzanti L, *et al*. On the Nosology and Pathogenesis of Wolf-Hirschhorn Syndrome: Genotype-Phenotype Correlation Analysis of 80 Patients and Literature Review. Am J Med Genet C Semin Med Genet. 2008; 148C(4):257-269.

[13] Goodship J, Curtis A, Coss I, Brown J, Emslie J, Wolstenholme J, *et al*. A submicroscopic translocation, t (4; 10), responsible for recurrent Wolf-Hirschhorn syndrome identified by allele loss and fluorescent in situ hybridization. J Med Genet. 1992; 29(7):451-454.