A Peruvian Child with 18p-/18q+ Syndrome and Persistent Microscopic Hematuria

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Abstract

Keywords

- chromosome 18 pericentric inversion
- recombinant chromosome 18
- hematuria
- microarray analysis
- genotype–phenotype correlation

Chromosome 18 pericentric inversion carriers could have offspring with recombinant chromosomes, leading to patients with clinical variable manifestations. Patients with 18p-/18q+ rearrangements share some clinical characteristics, while other characteristics differ. Factors for such divergence include the length of the inverted segment, among others. Here, we describe a Peruvian child with dysmorphic features, intellectual disability persistent microscopic hematuria, aortic pseudocoarctation, and descending aorta arteritis, among others. Karyotype analysis of family members determined the mother as the carrier of a pericentric inversion: 18[inv(18)(p11.2q21.3)]. This child carries a recombinant chromosome 18, with chromosomal microarray analysis detecting two genomic imbalances in patient's chromosome 18: one duplicated region and one deleted segment in the large and the short arms, respectively. Persistent microscopic hematuria has not been reported among 18p-/18q+ phenotypes. Our patient elucidates that other factors play significant and yet unknown roles for not fulfilling the proposed genotype–phenotype correlation associated with hemizygoty in this type of recombinant chromosome 18 or presenting these features as the patient ages.

Introduction

Chromosomal inversions correspond to 10% of chromosomal structural alterations¹ and represent changes where two breaks separate the chromosome material. The region within these breakpoints rotates 180 degrees and reinserts into the same chromosome. Thus, two types of these alterations can emerge, pericentric and paracentric, where the first one contains the centromere and the latter does not. Genetic risks of chromosomal inversion carriers comprise offspring's

unbalanced karyotype, inheritance of chromosomal alteration, infertility, uniparental disomy, and altered phenotypes.¹ Inversions maintaining the chromosomal material integrity (i.e., same copy number variants [CNVs]) keep chromosomes balanced. When a balanced inverted chromosome and its normal homolog crossover during meiosis, some genetic material near the inversion breakpoints could be either lost or gained. This could lead to (1) trisomy and monosomy of short-arm and long-arm terminal regions, respectively, and, (2) monosomy and trisomy (i.e., the other

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Fig. 1 Diagram of inversion loop model depicting the crossing-over during meiosis between an abnormal chromosome with pericentric inversion and its normal homolog. The event within the loop crossover produces two recombinant chromosomes: one with duplicated segment of a long arm segment and the other with duplicated segment of a short arm region.

way around) of these chromosomal segments, respectively (► **Fig. 1**). These chromosomal rearrangements could translate a diversity of clinical manifestations.

Researchers have reported the clinical repercussions of recombinants from pericentric inversion carriers. Hasi-Zogaj et al reported the genotype-phenotype correlation in patients with 18p- syndrome, proposing a list of dosage sensitive genes.² A recent publication of Lustosa-Mendes et al has pinpointed the diversity of clinical findings and chromosomal regions involved in the 18p-/18q+ rearrangements.³ We report the case of a Peruvian boy who carries a recombinant chromosome 18 arising from the crossing-over during meiosis between normal chromosome 18 and the homolog with pericentric inversion. The boy presents altered phenotype with dysmorphic features and intellectual disability, among others. We aimed to compare our findings with the recent reviews of Lustosa-Mendes et al and Hasi-Zogaj et al.

Clinical Report

A 2-year-old male child was referred to our institution after suffering for months with fever of unknown origin. He presented hematuria and tested positive for urine cultures (*Morganella morganis*), for which he received treatment. Latter

urine cultures remained negative, but the fever persisted. He was then diagnosed with bilateral chronic otitis media, needing surgical intervention at another institution. To date, we have found the following clinical features: appendicular hypertonia and hypotonia at early and late infancy, respectively, ataxia, dental malocclusion, strabismus, myopia, keratosis pilaris, persisting microscopic hematuria without infection or other positive examination, bicuspid aortic valve, aortic coarctation (identified by echocardiography) since infancy, intellectual disability, and impulsive and aggressive behavior. We examined our patient aged 6 years and 7 months following Hall et al recommendations,⁴ noticing short height (height: 99.6 cm, <3rd percentile), telecanthus and hypertelorism (inner and outer canthal distances of 3.5 cm [>2 standard deviation] and 9.5 cm [>97th percentile], respectively), microcephaly (49 cm, <3rd percentile), short hands (length 10.5 cm, <3rd percentile) and feet (14.5 cm, <3rd percentile), and no genital abnormalities. Brain magnetic resonance imaging (MRI) detailed white matter lesions not compatible with holoprosencephaly or its microforms. Cardiac computed tomography (CT) angiography demonstrated signs of aortic pseudocoarctation and supra-aortic brachiocephalic branches dilation with descending aorta walls thickening, suggesting arteritis. Serum immunoglobulin levels were within normal range. ►**Fig. 2**, ►**Fig. 3**, and

► **Supplemental Table S1** (available in the online version) show other clinical features.

Materials and Methods

Following the Instituto Nacional de Salud del Niño (INSN) regulations, an informed consent was signed by the patient's mother, and peripheral blood was obtained for cytogenetic studies including karyotyping and chromosomal microarray analysis (CMA).

Cytogenetic Analysis

Conventional cell culture, harvesting, and GTG banding followed standardized procedures. Following the results obtained on the patient, this analysis was performed on the patient's siblings and his parents.

Chromosomal Microarray Analysis

Genomic DNA (deoxyribonucleic acid) was isolated from whole blood using QIAamp DNA Blood Mini kit (Qiagen, Germany). Total DNA (250 ng) was amplified, labeled, and hybridized using GeneChip CytoScan 750K Array protocols (Affymetrix, Santa Clara, California, United States) according to the manufacturer's instructions. The array specifications include 550,000 nonpolymorphic markers and 200,436 single-nucleotide polymorphism markers. CEL files obtained after scanning the arrays were analyzed using the Chromosome Analysis Suite (ChAS) software (Affymetrix) and genome build GRCh37 (hg19). Gains and losses that affected a minimum of 25 markers and loss-of-heterozygosity regions that expand over 5 Mbp were initially considered.

In summary, the CNVs found in the proband were compared with genomic variants in public databases, including Database of Chromosomal Imbalance and Phenotype in Humans using

Ensemble Resources (DECIPHER, <https://decipher.sanger.ac.uk/>), and the UCSC Genome Browser (<https://genome.ucsc.edu/>). CNVs were classified as pathogenic, likely pathogenic, and of unknown clinical significance.

Results

After conventional karyotyping, two kind of chromosomal rearrangements were found in the patient: one deletion of part of the short arm of chromosome 18, and a duplication of a long-arm segment of chromosome 18 (► **Fig. 4**). The mother carries a pericentric inversion in chromosome 18, and the patient's living siblings inherited the same structural alteration from her: 18 [inv(18)(p11.2q21.3)], all of them without any significant clinical manifestation. We were unable to perform cytogenetic analysis in other family members of the maternal lineage (► **Fig. 5**). Thus, the patient's karyotype was written as 46,XY,rec(18)dup(18q)inv(18)(p11.2q21.3)mat.

Chromosomal microarray analysis (CMA) detected two genomic imbalances in patient's chromosome 18: a 15.8-Mbp duplication at 18q22.1q23(62,142,135–78,013,728)x3 and a 11.1-Mbp deletion at 18p11.32p11.21(136,227–11,246,728)x1 (► **Fig. 6**).

Discussion

Pericentric rearrangements occur in 0.01 to 0.02% of general population.^{5,6} Our patient's mother carries a pericentric inversion on chromosome 18 without any clinical manifestation. During crossing-over in meiosis, balanced inverted chromosome and normal homolog aligned segments form a loop, hence recombining regions of chromosome 18 in our patient (► **Fig. 1**). This process has occurred on our patient's

Fig. 2 Phenotypic features of the Peruvian child aged 6 years and 7 months with the 18p-/18q+ rearrangement.

Fig. 3 Diagnostic images showing patient's anomalies. (A) Brain magnetic resonance imaging (MRI) on T1 (sagittal view) and T2 (axial and coronal views) sequences show white matter lesions noted as small spots on white matter. (B) Persistent patent ductus arteriosus and descending aorta walls thickening. (C) Aortic arch bulging and kinking suggesting aortic pseudocoarctation and dilated supra-aortic brachiocephalic branches.

Fig. 4 Chromosomal findings in the mother and the proband using conventional karyotype (left) and schematic figures (right). Red arrows highlight abnormal chromosomes. The mother carries a pericentric inverted segment, whereas the proband has inherited a recombinant chromosome 18.

Fig. 5 Pedigree showing karyotypes of family members. Karyotype analysis could not be performed on miscarriages. yo: years old; mo: months old, inv: chromosomal inversion, rec: recombinant chromosome. Black arrow signals the proband.

chromosome 18: trisomy of a terminal portion on long arm and a monosomy on p-terminal segment.

This recombination depends on length of the inverted segment.⁷ ► **Fig. 7** shows carriers with different lengths of inverted regions on this chromosome, elsewhere reported. Inv (18) carriers seem to produce more offspring with inversions than recombinants, with higher frequencies from maternal

origin for inversions inheritance and paternal transmission of recombinants.³ In our patient's family, more siblings have inherited the inversion in chromosome 18 and only one child (proband) has a recombinant, but we lack information about genetic testing of a deceased sibling at 2 months and a miscarriage at 8 weeks reported by the propositus's mother. According to other patients with 18p-/18q+ rearrangements,

Fig. 6 Results of copy number variants in the present case using GeneChip CytoScan 750K Array protocols (Affymetrix) showing a duplication at 18q22.1q23(62,142,135–78,013,728)x3 and a deletion at 18p11.32p11.21(136,227–11,246,728)x1. At the top line, red and blue boxes denote signal loss and gain of chromosomal material, respectively.

Fig. 7 Pericentric inverted segments of chromosome 18 leading to offspring with recombinants. Our patient rearrangement is depicted in the box with loss (red) and gain (blue) of regions. Other reported carriers with schematic inverted segments are shown in the right, whose offspring demonstrates recombinants with altered phenotypes. Black: inverted segment, green: cases with molecular analysis for chromosomal region confirmation (fluorescent in situ hybridization [FISH] or arrays analysis).

phenotypic features varies from mild to severe,^{3,8–20} including oligoasthenospermia as a unique manifestation in one patient reported by Quiroga et al.²¹

Lustosa-Mendes et al reviewed patients with 18p-/18q+ rearrangements, pinpointing similar characteristics such as elongated face, failure to thrive, developmental delay, frontal bossing, high frontal hairline, arched eyebrows, narrow palpebral fissures, large or low/malformed ears and micrognathia. We noticed (see ► **Supplemental Table S1**, available in the online version) other common characteristics such as telechantus, hypertelorism, strabismus, long philtrum, downturned mouth corners, round face, short neck, heart defects, and clinodactyly. Patients similar to ours exhibited phenotypes more related to 18p- syndrome² than to trisomy 18 syndrome. For instance, several physical findings in our patient were related to 18p- syndrome, such as brain white matter lesions and heart defects. These features have been reported in up to 66 and 56% respectively in these patients.² On the other hand, we think that persistent patent ductus arteriosus could be the only cardiac finding related to trisomy 18 syndrome.²² To our knowledge, our patient represents the first report with diagnosis of aortic pseudo-

coarctation with descending aorta walls thickening, suggesting Takayasu's arteritis.

Phenotype variability of Edwards syndrome could be correlated to length and location of the duplicated segment on long arm of chromosome 18. Boghosian-Sell et al suggested two critical regions along long arm of chromosome 18: (1) 18q12.1–18q21.2 and (2) 18q22.33–18qter.²³ Most cases reviewed here reported long-arm duplications distal to the first suggested critical region, but involving 18q22.33–18qter. This fact could suggest that proximal 18q duplications contribute more than distal, explaining our observation of phenotypes in 18p-/18q+ rearrangements. On the other hand, our patient has an 18p deletion involving several genes, without manifesting all features (► **Table 1**), suggesting that other factors could contribute to the clinical manifestation.^{2,24}

Comparing breakpoints of our patient's chromosomal findings with those closely similar in the literature, we found great variability across clinical features. For instance, our patient has a similar chromosomal rearrangement to the one reported by Lustosa-Mendes et al,³ but with clinical differences, as shown in ► **Supplemental Table S1** (available in the online version). Accurate phenotype description should be

Table 1 Genes with clinical relevance and phenotypic features in our patient

Dosage sensitive genes	Phenotype associated	Genes compromised in our patient	Phenotype in our patient
<i>CENT1</i> ^a	Infertility	+	Not yet reported
<i>TGIF1</i> ^a	Holoprosencephaly or its microforms, conductive hearing loss (?)	+	White matter nonspecific lesions Mild conductive and neurosensorial hearing loss
<i>LAMA1</i> ^a	Keratosis pilaris, ulerythema ophryogenes, Poretti–Boltshauser’s syndrome	+	Keratosis pilaris
<i>GNAL</i> ^a	Dystonia	–	Not yet reported
<i>AFG3L2</i> ^a	Spinocerebellar ataxia (type 28)	+	Only ataxia to date
<i>PTPN2</i> ^a	Autoimmune disease	+	Not yet reported
<i>SMCHD1</i> ^a	Facioscapulohumeral muscular dystrophy	+	Not yet reported
<i>TWSG1</i> ^a	Facial anomalies, holoprosencephaly, neural tube defects	+	White matter nonspecific lesions Facial anomalies
<i>DLGAP1</i> ^a	Autism spectrum	+	–
<i>LRRC30</i> ^a	Autism spectrum		–
<i>ANKRD12</i> ^a	Autism spectrum	+	–
<i>IMPA2</i> ^a	Autism spectrum	–	–
<i>NDUFV2</i> ^b	Hypertrophic cardiomyopathy and encephalopathy	+	Our patient has persistent patent ductus arteriosus, aortic arch bulging and kinking suggesting aortic pseudocoarctation, and dilated supra-aortic brachiocephalic branches and descending aorta walls thickening
<i>ADCYAP1</i> ^b	Myopia, optic disc anomaly	+	Myopia
<i>LPIN2</i> ^b	Myopia, optic disc anomaly	+	Myopia

^aDosage sensitive genes proposed by Hasi-Zogaj et al.²
^bPhenotype identified in mouse.

provided to compare patients with similar age or at least similar follow-up lapse since the reported cases equally compare toddlers, infants, adults, and even fetuses. We think this factor could contribute to reduced comparability. We took into account phenotypic manifestations reported in patients with 18p- syndrome² in our patient’s clinical follow-up, and we think that further publications of patients with 18p-/18q+ rearrangements should complete clinical examination focusing on these clinical findings scrutiny.

Haploinsufficiency, imprinting, and environment effects could lead to diverse phenotypes in patients with chromosomal abnormalities. Five to ten percent of genes have been proposed to show gene dosage sensitivity, with subunit imbalance, metabolic rate limiting, and developmental regulation as mechanisms of haploinsufficiency in subjects with hemizygosity of these genes.²⁴ Few genes have been reported as imprinting-dependent in the long arm of chromosome 18, but none of these is duplicated in our patient (information available at <http://www.geneimprint.com/site/genes-by-species>). A recent publication²⁵ suggests that environmental-induced epimutations could cause genome instability and transmit genomic alterations to the progeny, which could explain part of the phenotypic diversity among 18p-/18q+

syndrome. Factors such as gene–gene interactions, recessive mutations, and possible regulatory effects between genes modifying gene structures could explain the phenotypes in chromosomal disorders.²⁴

To our knowledge, this is the first case reported with persistent microscopic hematuria without an identifiable cause. Glomerular basement membrane (GBM) injury is one of the causes of glomerular hematuria. The earliest precursor of this structure contains laminin α1, the product of *LAMA1*, which is present in early GBM development, but it is absent on the mature GBM. Nevertheless, it is possible that a heterozygous deletion of *LAMA1* would have some negative effect on the development of GBM in our patient. In addition Ning et al found through a knockout mouse model that *LAMA1* may be involved in maintaining mesangial cell populations and mesangial matrix deposition through transforming growth factor-β/Smad signaling.²⁶

This article remarks on clinical variability between patients with 18p-/18q+ rearrangements. Clinical features of our patient have been reported during childhood development. Novel clinical findings in our case adds information to literature: persistent microscopic hematuria and aortic pseudocoarctation with descending aorta arteritis. Further

experiments and clinical correlation studies would be necessary to confirm whether *LAMA1* hemizygozity could explain any grade of hematuria in humans. We highlight the importance of accurately correlating genotypes, that is, with microarray chromosomal or sequencing analysis, with phenotypes, following exhaustive clinical and radiological examination across lifespan and taking into account age-dependent comparability in further reports. This improved diagnostic road could serve for initiating personalized treatment in patients with chromosomal abnormalities.²⁴

Note

Written informed consent was obtained from the patient's mother for publication of this case report and accompanying images.

Conflict of Interest

None.

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References

- Muss B, Schwanitz G. Characterization of Inversions as a Type of Structural Chromosome Aberration. *Int J Hum Genet* 2007;7(02): 141–161
- Hasi-Zogaj M, Sebold C, Heard P, et al. A review of 18p deletions. *Am J Med Genet C Semin Med Genet* 2015;169(03):251–264
- Lustosa-Mendes E, Dos Santos AP, Viguetti-Campos NL, Vieira TP, Gil-da-Silva-Lopes VL. A boy with partial dup(18q)/del(18p) due to a maternal pericentric inversion: genotype-phenotype correlation and risk of recombinant chromosomes based on systematic review of the literature. *Am J Med Genet A* 2017;173(01):143–150
- Hall JG, Allanson JE, Gripp KW, Slavotinek AM. *Handbook of Physical Measurements*. 2nd ed. New York, NY: Oxford University Press; 2007
- Evans HJ. Chromosome anomalies among livebirths. *J Med Genet* 1977;14(05):309–312
- Kaiser P. Pericentric inversions. Problems and significance for clinical genetics. *Hum Genet* 1984;68(01):1–47
- Anton E, Blanco J, Egozcue J, Vidal F. Sperm studies in heterozygote inversion carriers: a review. *Cytogenet Genome Res* 2005; 111(3–4):297–304
- Vianna-Morgante AM, Nozaki MJ, Ortega CC, Coates V, Yamamura Y. Partial monosomy and partial trisomy 18 in two offspring of carrier of pericentric inversion of chromosome 18. *J Med Genet* 1976;13(05):366–370
- Turleau C, de Grouchy J. Trisomy 18qter and trisomy mapping of chromosome 18. *Clin Genet* 1977;12(06):361–371
- Kukolich MK, Althaus BW, Sears JW, Mankinen CB, Lewandowski RC. Abnormalities resulting from a familial pericentric inversion of chromosome 18. *Clin Genet* 1978;14(02):98–104
- Andrews T, Gardiner AC, Boon AR. Recombinant chromosome 18 in two offspring of a chromosome 18 inversion heterozygote. *Ann Genet* 1982;25(03):185–188
- Asano T, Ikeuchi T, Shinohara T, Enokido H, Hashimoto K. Partial 18q trisomy and 18p monosomy resulting from a maternal pericentric inversion, inv(18)(p11.2q21.3). *Jinrui Idengaku Zasshi* 1991;36(03):257–265
- Ayukawa H, Tsukahara M, Fukuda M, Kondoh O. Recombinant chromosome 18 resulting from a maternal pericentric inversion. *Am J Med Genet* 1994;50(04):323–325
- Israëls T, Hoovers J, Turpijn HM, Wijburg FA, Hennekam RC. Partial deletion of 18p and partial duplication of 18q caused by a paternal pericentric inversion. *Clin Genet* 1996;50(06):520–524
- Mejía-Baltodano G, Bobadilla L, Gonzalez RM, Barros-Núñez P. High recurrence of recombinants in a family with pericentric inversion of chromosome 18. *Ann Genet* 1997;40(03):164–168
- Leonard NJ, Tomkins DJ, Demianczuk N. Prenatal diagnosis of holoprosencephaly (HPE) in a fetus with a recombinant (18)dup(18q)inv(18)(p11.31q11.2)mat. *Prenat Diagn* 2000; 20(12):947–949
- Vermeulen SJ, Speleman F, Vanransbeeck L, et al. Familial pericentric inversion of chromosome 18: behavioral abnormalities in patients heterozygous for either the dup(18p)/del(18q) or dup(18q)/del(18p) recombinant chromosome. *Eur J Hum Genet* 2005; 13(01):52–58
- Prontera P, Buldrini B, Aiello V, et al. Familial pericentric inversion of chromosome 18: intrafamilial variability of the recombinant dup(18q). *Genet Couns* 2010;21(01):91–97
- Kariminejad A, Kariminejad R, Moshtagh A, et al. Pericentric inversion of chromosome 18 in parents leading to a phenotypically normal child with segmental uniparental disomy 18. *Eur J Hum Genet* 2011;19(05):555–560
- Zamani AG, Acar A, Durakbasi-Dursun G, Yildirim MS, Ceylaner S, Tuncez E. Recurrent proximal 18p monosomy and 18q trisomy in a family due to a pericentric inversion. *Am J Med Genet A* 2014; 164A(05):1239–1244
- Quiroga R, Monfort S, Oltra S, et al. Partial duplication of 18q including a distal critical region for Edwards Syndrome in a patient with normal phenotype and oligoasthenospermia: case report. *Cytogenet Genome Res* 2011;133(01):78–83
- Cereda A, Carey JC. The trisomy 18 syndrome. *Orphanet J Rare Dis* 2012;7:81
- Boghosian-Sell L, Mewar R, Harrison W, et al. Molecular mapping of the Edwards syndrome phenotype to two noncontiguous regions on chromosome 18. *Am J Hum Genet* 1994;55(03): 476–483
- Cody JD, Hale DE. Making chromosome abnormalities treatable conditions. *Am J Med Genet C Semin Med Genet* 2015;169(03): 209–215
- Skinner MK, Guerrero-Bosagna C, Haque MM. Environmentally induced epigenetic transgenerational inheritance of sperm epimutations promote genetic mutations. *Epigenetics* 2015; 10(08):762–771
- Ning L, Kurihara H, de Vega S, et al. Laminin $\alpha 1$ regulates age-related mesangial cell proliferation and mesangial matrix accumulation through the TGF- β pathway. *Am J Pathol* 2014;184(06): 1683–1694