Copy Number Gain at Xq28 in a Child with Global Developmental Delay Associated with a Variant Form of Hoyeraal-Hreidarsson Syndrome

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Keywords

Chromosomal microarray analysis · *DKC1* · Genetic syndrome · Global developmental delay

Abstract

We report the case of a child from Central Brazil with global developmental delay (GDD), syndromic features, and absence of abnormal skin pigmentation, nail dystrophy, and leukoplakia of the oral mucosa, with a rearrangement at Xq28 harboring the *DKC1* gene. GTC-banding revealed a male karyotype (46,XY) with no visible numerical or structural alterations. Chromosomal microarray analysis (CMA) showed a 0.36-Mb gain at Xq28 of maternal origin, encompassing 22 genes, including *DKC1*. Rearrangements and mutations involving this gene have been associated with dyskeratosis congenita, X-linked (OMIM 305000) and Hoyeraal-Hreidarsson syndrome. CMA was a powerful and efficient

approach to identify a gain at Xq28 harboring the *DKC1* gene in our patient with GDD syndromic features and no cutaneous alterations, suggesting that this variant is associated with the Hoyeraal-Hreidarsson syndrome.

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The Hoyeraal-Hreidarsson syndrome (HHS) is a very rare X-linked multisystem recessive genetic disorder first described by Aalfs et al. [1995]. Clinically, HHS is seen as a severe form of dyskeratosis congenital (DKC), characterized by intrauterine growth retardation, global developmental delay (GDD), neurological impairment, microcephaly, cerebellar hypoplasia, aplastic anemia, and immunodeficiency with progressive bone marrow failure [Bakar et al., 2015; Glousker et al., 2015; Dehmel et al., 2016]. Germline mutations in *DKC1*, an important telomere biology gene, cause HHS in males. To date, less than 20 patients have been re-

ported with this syndrome worldwide for which prevalence and incidence rate are unknown, most likely due to its high mortality rate.

DKC1 located in Xq28 encodes for dyskerin pseudouridine synthase 1, a nucleolar protein which is a constituent of H/ACA ribonucleoprotein particles and plays a role in the pseudouridylation of specific residues in nascent rRNA and snRNA molecules [Mochizuki et al., 2004; Gu et al., 2008]. *DKC1* is also active in stabilizing and maintaining the telomerase, a ribonucleoprotein that uses an RNA template and a reverse transcriptase to add a species-dependent telomere repeat sequence to the 3' end of the telomeres [Gu et al., 2008]. Dyskerin plays an additional part in nucleocytoplasmic shuttling, DNA damage response, and cell adhesion [Mochizuki et al., 2004; Gu et al., 2008].

Either numerical or structural chromosomal aberrations are considered to be the main cause of unexplained GDD, intellectual disability (ID), and multiple congenital anomalies. Together, aberrant chromosomes account for about 15–30% of the GDD/ID cases and from this group, 5–10% occurring predominantly in males could be explained by X-chromosomal aberrations [Rafati et al., 2012; Capalbo et al., 2017; Muthusamy et al., 2017]. Herein, we report a case of microduplication at Xq28 associated with a variant form of HHS in a Brazilian boy with GDD and syndromic features, lacking abnormal skin pigmentation, nail dystrophy, and leukoplakia of the oral mucosa.

Patient and Methods

Case Presentation

The patient is a 1-year-old boy born by cesarean delivery at 34 weeks of gestation to a nonconsanguineous couple, a 21-year-old mother and 30-year-old father. His birth weight was 1,640 g, crown-heel length was 39 cm, head circumference 32 cm, and Apgar score 4 at 1 min and 8 at 5 min. After birth, the newborn went into cardiorespiratory arrest and remained at an intensive care unit for 36 days. Physical examination of the child at 1 year of age revealed GDD, with a height of 64 cm and weight of 5,450 g. His craniofacial dysmorphisms included a large nasal bridge, hypertelorism, long philtrum, thin upper and lower lips, and retrognathism. He also had low-set ears, enlarged eyebrows, progeria, foramen ovale, and secondary adrenal insufficiency. On the other hand, the child lacked abnormal skin pigmentation, nail dystrophy, and leukoplakia of the oral mucosa. Assistant physicians from the public health system referred the patient to the genetic services (Laboratory of Human Cytogenetics and Molecular Genetics) of the State Health Secretary and the Replicon Research Group of the School of Agricultural and Biological Sciences, Pontifical Catholic University in Central Brazil.

Materials and Methods

Peripheral blood from the child and his parents were collected. Genomic DNA was isolated from whole blood samples using the Illustra Blood Genomic Prep Mini Spin Kit (GE Healthcare Life Sciences, USA), following the manufacturer's instructions. Quality and quantity of genomic DNA was assessed by spectrophotometer readings.

Conventional cell cultures, harvesting, and G-banding at the level of >550 bands were performed for the entire family, following modified procedures [Verma and Babu, 1995]. Chromosome analyses were performed using a Zeiss Axioscope® (Göttingen, Germany) with the aid of IKAROS® software (Metasystems Corporation, Altlußheim, Germany). The analyses were carried out on the child and his parents to establish whether the DNA rearrangements were de novo or inherited.

A total 250 ng of DNA for each sample was digested with NspI, ligated, PCR amplified and purified, fragmented, biotin-labeled, and hybridized for use in a GeneChip®, CytoScan™ HD (Affymetrix, Santa Clara, CA, USA). The array was specifically designed for cytogenetic research, including 2.7 million copy number variation markers. CEL files obtained by scanning the arrays were analyzed using the Chromosome Analysis Suite® software (Affymetrix). Gains and losses were set at a minimum of 50 and 25 markers, respectively, and 100 kb length.

Results

Karyotyping showed a male karyotype (46,XY). However, CMA detected a 356.77-kb gain – maternally inherited – at Xq28, arr[hg19] Xq28(153,792,708–154,149,474) ×2 mat, harboring the following genes: *IKBKG*, *LINC00204B*, *LINC00204A*, *CTAG1A*, *CTAG1B*, *CTAG2*, *GAB3*, *DKC1*, *SNORA36A*, *MIR644B*, *SNORA56*, *MPP1*, *CXorf68*, *F8*, *H2AFB3*, *H2AFB1*, *F8A1*, *F8A3*, *F8A2*, *MIR1184-3*, *MIR1184-2*, and *MIR1184-1* (Fig. 1). Among these, the *DKC1* gene is related to DKC, X-linked (OMIM 305000) and HHS.

Discussion

Herein, we report the first case of a copy number gain at Xq28 affecting the *DKC1* gene in a child from Goiás State in Central Brazil. The proband exhibited GDD, short stature, craniofacial dysmorphisms, including a large nasal bridge, hypertelorism, long philtrum, thin upper and lower lips, and retrognathism. The clinical description of our patient was similar to the patients described by Hoyer et al. [1970], Hreidarsson et al. [1988], and Aalfs et al. [1995], but abnormal skin pigmentation, nail dystrophy, and leukoplakia of the oral mucosa were absent.

Fig. 1. A Chromosomal microarray analysis of chromosome X from the child and his mother with a copy number gain at Xq28. **B** Zoom into the CNV region Xq28. Genes are shown in pink.

The classical phenotype of HHS is commonly associated with the lack of function of *DKC1* mostly due to mutations that inactivate the gene. Our proband showed a gain at Xq28; thus, he most likely had 2 copies of the gene, which led to the deviation of the frequently described phenotype of HHS in males who are hemizygous for the gene. Similar to genomic variations observed in other syndromes, such as in developmental neuropsychiatric disorders [Hiroi et al., 2013], duplications and deletions may coincidentally affect genes that might induce the same phenotypes despite gene-dose deviation in either direction. Thus, here we report a variant form of HHS due to a gain in *DKC1* at Xq28, reinforcing CNV-associated traits that are yet to be clearly understood.

Both HH and DKC syndromes are X-linked, and X chromosome inactivation is an important process to understand the potential outcomes in heterozygous female carriers of *DKC1* mutations. A study by Alder et al. [2013] showed 100% skewing in X-inactivation in female carriers of heterozygous mutations in *DKC1*. Thus, heterozygous females may show DKC-like phenotype/genotype correlation, pending the inactivation of the unaffected chromosome [Xu et al., 2016]. Although, in our report, the microduplication seen in the child was maternally inherited, the mother had no clinical manifestation of DKC or HHS, most likely due to the inactivation of the X chromosome harboring the 2 copies of *DKC1*. Thus, the mendelian recurrence risk estimates for our family are 50 and 25% for female carriers and affected males, respectively. The number of cases of HHS and DKC are scarce, preventing us from any inferences regarding the potential contribution of incomplete penetrance or variable expressivity in HHS patients.

The biological mechanism subjacent to HHS progression remains unknown. Nonetheless, some studies have shown that nonfunctional telomeres play an important etiological role in the development of DKC, HHS, and several other genomic disorders [Glousker et al., 2015; Dehmel et al., 2016; Xu et al., 2016]. Finally, our findings suggest that imbalances in gene dosage in addition to mutations in the *DKC1* gene can lead to telomere-related phenotypes.

Conclusion

CMA identified a copy number gain at Xq28 involving the *DKC1* gene in a boy from Central Brazil, with GDD and syndromic features. This genotype-phenotype correlation enabled the diagnosis of a variant form of HHS,

contributing to adequate clinical management, genetic counseling, and a better follow-up of the proband and his family.

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Statement of Ethics

The parents of the proband gave written informed consent approved by the Ethics Committee on Human Research at the Pontifical Catholic University of Goiás (CEPPUC/GO), under the protocol number CAAE 0051.0.168.000-11.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

L.L. Gonçalves Ramos, I. Plaza Pinto, and A.D. da Cruz contributed to conception and design of the research. Formal analysis was done by I. Plaza Pinto. L.L. Gonçalves Ramos, I. Plaza Pinto, and L. Bernardes Minasi performed the investigations. C. Luiz Ribeiro, D. Mírian da Cruz e Cunha, I. Plaza Pinto, L. Bernardes Minasi, and R. Deb developed the methodology. A.D. da Cruz and A.M.T. Cordeiro Silva supervised the study. L.L. Gonçalves Ramos, I. Plaza Pinto, R. Deb, and A.D. da Cruz wrote the manuscript, and the last 2 authors reviewed and edited the work.

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