A Balanced Reciprocal Translocation t(2;9) (p25;q13) Disrupting the *LINC00299* Gene in a Patient with Intellectual Disability

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Established Facts

- Long intergenic noncoding RNAs (lincRNAs) are involved in a range of biological processes including gene regulation and development.
- These molecules have also been involved in the pathogenesis of different types of cancer.

Novel Insights

- We confirm that *LINC00299* may be a candidate gene for intellectual disability and therefore plays a role in the development and maintenance of the central nervous system.

Keywords

Intellectual disability · LincRNA · *LINC00299*

Abstract

Long intergenic noncoding RNAs (lincRNAs) are a class of noncoding RNAs implicated in several biological processes. LincRNA 299 (*LINC00299*) maps to 2p25.1 and its function is still unknown. However, this gene has been proposed as a candidate for intellectual disability (ID) in a patient with a balanced translocation where the breakpoint disrupted its ORF.

Here, we describe a new case of *LINC00299* disruption associated with ID. The individual, a 42-year-old woman, was referred to the clinical geneticist because of her son who had severe syndromic ID. G-banding and chromosomal microarray analysis were performed. Karyotyping of the boy revealed an extranumerary derivative chromosome identified as an unbalanced translocation between chromosomes 2 and 9 of

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maternal origin. The mother's karyotype showed a balanced translocation 46,XX,t(2;9)(p25;q13). Chromosomal microarray indicated a disruption of *LINC00299*. These data corroborate the role of *LINC00299* as a causative gene for ID and broadens the spectrum of *LINC00299*-related phenotypes.

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Long intergenic noncoding RNAs (lincRNAs) are a class of noncoding RNAs characterized by their size of more than 200 nucleotides and their location, up to 10 kb apart from protein-coding genes [Bhan et al., 2014]. These genes are involved in a range of biological processes such as X-chromosome inactivation, gene expression regulation, particularly in stem and cancer cells, and the development of several tissues, including testis, myocardium, and the central nervous system [Derrien et al., 2011; Kurihara et al., 2014; Sun and Kraus, 2015; Hou et al., 2016, 2017]. They also seem to play a role in the pathogenesis of different types of cancer such as glioblastomas, gastric and lung cancer as well as acute myeloid leukemia [Wang et al., 2014; Liu et al., 2016].

LincRNA 299 (*LINC00299*) maps to 2p25.1 (8,147,901–8,468,549; hg19), and its function is still unknown. This gene has been proposed as a candidate for intellectual disability (ID) since it was the single gene disrupted in a patient with a balanced translocation t(2;11), displaying severe ID [Talkowski et al., 2012]. Disruption of a single locus from a de novo balanced translocation enables the association with a specific phenotype, notably ID in this case. Its role in ID was further corroborated by identification of 4 patients with a single CNV (>10 Mb) that disrupted the *LINC00299* gene in CNV databases (Signature Genomics, Lab Corp, DECIPHER, and ISCA) [Talkowski et al., 2012]. In addition, in vitro studies using pluripotent stem cells derived from neural progenitors, suggested that *LINC00299* expression increases during the differentiation of these neural progenitors, hence during brain development, and therefore could have an important role in central nervous system development [Talkowski et al., 2012].

Here, we present a new case of *LINC00299* disruption in a patient with ID.

Case Report

The patient is a 42-year-old woman with moderate ID. She left school when she was 8 years old due to learning difficulties. She can count to 10, but she is illiterate. Her mother, brother, and maternal grandfather also have ID but were unavailable for study. On

physical examination, the patient had low-set ears, a prominent nasal bridge, a bulbous nose, and a short smooth philtrum. She also had short stature (<3rd centile), a single palmar crease, and a short 5th finger of the left hand. No other significant dysmorphisms were observed (Fig. 1B).

The patient's 11-year-old son presented with severe ID, short stature, microcephaly, and patent ductus arteriosus (Fig. 1A). He also displayed dysmorphic features such as a long face, low-set ears with large lobes, a prominent nasal bridge, a broad nose, a short smooth philtrum, sparse eyebrows, deep set eyes, a single palmar crease, thoracic asymmetry, sandal gap toes, and deep set toenails. Furthermore, the boy has a speech impairment and can only say a few words. He has no sphincter control and is unable to perform daily living activities without assistance.

Materials and Methods

G-banding was performed on peripheral blood lymphocytes following standard procedures. Genomic DNA was extracted from peripheral blood using a Qiagen Kit following the manufacturer's protocol. In order to further delimit the segments involved in the rearrangement, chromosomal microarray analysis was performed using Affymetrix CytoScan 750k Array in accordance with the manufacturer's protocol.

Results

Karyotyping of the boy revealed an extranumerary derivative chromosome identified as an unbalanced translocation between chromosomes 2 and 9 of maternal origin: 47,XY,+der(9)t(2;9)(p25;q13)mat. The mother's karyotype showed a balanced translocation: 46,XX,t(2;9)(p25;q13) (Fig. 2A).

This study confirmed that the whole short arm of chromosome 9 was duplicated with a partial duplication of the short arm of chromosome 2, with the proximal breakpoint lying between the last 2 exons of *LINC00299*. The boy's karyotype was defined as 47,XY,+der(9)t(2;9)(p25;q13).arr 2p25.3p25.1(12,770–8,187,008)×3,9p24.3q13 (208,454–68,358,120)×3 mat (Fig. 2B).

Chromosomal microarray analysis of the mother did not identify any pathogenic CNVs. However, we can postulate that the breakpoint of chromosome 2 in the mother should be similar to the boy's, thus disrupting *LINC00299* (Fig. 3).

Discussion

This report presents clinical and molecular data from a mother and her son who carry chromosomal rearrangements that explain their observed phenotypes. The moth-

er has a balanced translocation between chromosomes 2 and 9, and her son carries an unbalanced translocation between chromosome 2 and 9 with an extranumerary derivative chromosome maternally inherited.

The mother has moderate ID and minor dysmorphic features, whereas her son presents with a more severe phenotype, probably due to the inheritance of the derivative chromosome. The breakpoint regions in both chromosomes 2 and 9 were identified by comparing the microarray analysis of both patients. In chromosome 2, the

LINC00299 gene was disrupted between the last 2 exons, whereas in chromosome 9, the breakpoint lies in the centromeric region. No other genes are involved in this rearrangement.

LINC00299 belongs to a new class of RNA molecules, which are essential in different biological processes including: X-chromosome inactivation [Brown et al., 1992] and the regulation of gene expression in both stem cells and cancer cells as well as their development [Ji et al., 2003; Petruk et al., 2006].

Fig. 1. A Son at 11 years old. **B** Mother at age 42.

Recently, the role of *LINC00299* in breast cancer has been supported as Bermejo et al. [2019] showed that the hypermethylation of *LINC00299* in peripheral blood may constitute a useful circulating biomarker for triple negative breast cancer. In addition, an important effect of

LINC00299 in regulating cell proliferation and migration in atherosclerosis was also recently reported [Liu et al., 2019].

Although lincRNAs may play a role in development, until recently, none have been directly implicated in human developmental abnormalities.

In 2012, Talkowski et al. were the first to propose that *LINC00299* could be a causative gene for ID as it was the only gene disrupted in a 16-year-old (DGAP 162) female patient with significant neurological delays, severe learning difficulties, and nonverbal communication among other symptoms. The search for similar genomic lesions in order to associate the disruption of *LINC00299* with abnormal neurodevelopment led to the identification of 4 individuals. Patient L1 was particularly interesting with a single 60-kb deletion which was identified in the 2 final exons of *LINC00299*. L1 was a 43-year-old female with speech delay, mild-moderate ID, bouts of confusion, and abnormal behavior. Our patient has a similar phenotype as described in the L1 patient, but has a milder set of symptoms than those reported in the abovementioned 16-year-old.

Fig. 2. Translocations in the mother and son. **A** Balanced translocation between chromosomes 2 and 9 and their ideograms identified in the mother. **B** The extranumerary derivative chromosome found in the boy is shown.

Fig. 3. Chromosomal microarray analysis of the mother (P2) and son (P1) showing the segments duplicated in the boy on chromosomes 2 and 9 as depicted by blue bars. The breakpoint on chromosome 2 is located between the last 2 exons of *LINC00299* (box).

In addition, Blake et al. [2014] also reported a case whereby balanced chromosome abnormality was identified in a patient with neurocognitive disorder. Although the lesion was more complex than the patient described here and also by Talkowski et al. [2012], and disrupted at least 6 genes, one of them was *LINC00299*, strengthening the possible role of this gene as a cause of abnormal neurodevelopment and ID.

The variability and severity of the neurological phenotypes of our case, L1, and the DGAP162 patient described by Talkowski et al. [2012], who all had a single genomic lesion, notably disruption of the *LINC00299* gene, may be linked to the diversity of isoforms and/or the temporal and spatial expression of RNA among other causes. Therefore, further investigation into the role of *LINC00299* in neurological development is needed in order to better understand its pathological effects.

It is clear that de novo balanced chromosome translocations, very frequent structural variations that occur in approximately 0.2% of the newborns and in most cases bear no clinical phenotype [Currall et al., 2013], could in many instances disrupt genes with important roles in development, notably in brain functions. As the methods for detecting gene disruptions in balanced translocations

develop, it is likely that further confirmation of this mechanism as an important cause of ID and other genetic disorders will arise.

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Statement of Ethics

The study was approved by the institutional ethics committee, and informed consent was obtained.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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