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A novel case of global developmental delay syndrome with microdeletion at 10p14–p15.3 and microduplication at 18p11.31–p11.32

Danyan Zhang, MS[a,b], Yijian Zhu, MD[b], Xuefei Feng, BS[c], Limeng Dai, PhD[a], Mingfu Ma, BS[b], Lianbing Li, BS[b], Hong Guo, PhD[a], Letian Zhao, MS[b], Yuping Zhang, MS[c,*], Yun Bai, PhD[a,*]

Abstract

To characterize the etiology underlying a novel case of global developmental delay syndrome (GDDS) identified in a female child, aged 3 years old. This syndrome is a common pediatric presentation estimated to affect 3.65% of children aged 3 to 17 years.

The proband's detailed family history was used to infer a likely mode of inheritance for the GDDS. Genomic DNA samples collected from the proband and her parents were evaluated using conventional karyotyping, multiplex ligation-dependent probe amplification (MLPA), comparative genomic hybridization microarray (aCGH), and fluorescent in situ hybridization (FISH) analysis techniques.

An analysis of the proband's family history suggested that she inherited the GDDS from her father. The conducted conventional karyotyping and MLPA methods failed to identify a causative defect for the GDDS; however, the aCGH analysis revealed both a 6.6-Mb deletion at p14–p15.3 of chromosome 10 (arr[hg19]; 100,026–6,710,183), and a 6.3-Mb duplication at p11.31–p11.32 of chromosome 18 (arr[hg19]; 136,226–6,406,733) in the proband. The conducted FISH analysis subsequently determined that these mutations resulted from a balanced translocation t(10;18)(p15.3; p11.32) carried by the proband's father. Finally, a bioinformatic analysis of the proband's mutations revealed *ZMYND11* as a promising candidate causative gene for this case of GDDS.

The present study demonstrates that the aCGH method can be used to effectively identify the location and approximate size of microdeletions and/or microduplications, but not balanced reciprocal translocations. The nonconventional analysis methods used in the present study may be applicable to other GDDS cases with elusive etiology, and likewise, *ZMYND11* should be considered as a potential causative gene during the investigation of future GDDS cases.

Abbreviations: aCGH = comparative genomic hybridization, CMA = chromosomal microarray analysis, CNVs = copy number variations, DD = developmental delay, FISH = fluorescent in situ hybridization, GDDS = global developmental delay syndrome, MLPA = multiplex ligation-dependent probe amplification, MR = mental retardation, OMIM = Online Mendelian Inheritance in Man.

Keywords: comparative genomic hybridization, developmental delay, microdeletion, microduplication

1. Introduction

Global developmental delay syndrome (GDDS) is a common pediatric presentation estimated to affect approximately 3.65% of children aged 3 to 17 years.[1] For children aged less than 5 years, it is characterized as the exhibition of a significant delay in 2 or more developmental domains (ie, intelligence, language, social communication, cognition, and/or daily motor activities).[2] Currently, there is no consensus neuroimaging method used to study and/or diagnose this condition, and furthermore, causes of developmental delay (DD) are difficult to elucidate

[a] Department of Medical Genetics, College of Basic Medical Science, Army Medical University (Third Military Medical University), [b] Population and Family Planning Science and Technology Research Institute/Key Laboratory of Birth Defects and Reproductive Health of The National Health and Family Planning Commission, [c] Department of Pediatrics, Xinqiao Hospital, Army Medical University (Third Military Medical University), Chongqing, PR China.

* Correspondence: Yun Bai, Department of Medical Genetics, College of Basic Medical Science, Army Medical University (Third Military Medical University), Chongqing, PR China (e-mail: geneby@126.com); Yuping Zhang, Department of Pediatrics, Xinqiao Hospital, Army Medical University (Third Military Medical University), Chongqing, PR China (e-mail: liaoq375@gmail.com).

using only routine diagnostic techniques and detailed clinical information. The chromosomal microarray analysis (CMA) technique facilitates the detection of small chromosome imbalances that are unable to be unidentified via microscope-guided karyotyping. In fact, CMA is already established as a major platform for the identification of copy number variations (CNVs) in patients with autism spectrum disorder and/or mental retardation (MR).[3,4]

In the present study, comparative genomic hybridization array (aCGH) and fluorescence in situ hybridization (FISH) techniques were used to investigate the etiology and pathogenesis of GDDS in a female child aged 3 years old.

2. Materials and methods

2.1. Proband family history

The study participants comprised members of a Chinese-Han family, who were identified and enrolled at the Department of Pediatrics at the Xinqiao Hospital (Third Military Medical University). The proband was a female child aged 8 months, who was diagnosed with GDDS. She was unable to either sit or crawl without assistance. The conducted physical examination of the proband identified no cortex thumb syndrome; however, she was found to exhibit bilateral ankle clonus, a poor active-conscious grip in both hands, grade-IV lower-limb muscle tension and strength, and the ability to support a prone position. Her bilateral knee-jerk and Achilles tendon reflex were found to be normal, and she was also assessed for Babinski (+), Kernig (−), Brudzinski (−), and Auspitz (−) signs. The proband was calculated to have a mental developmental index of 70, and a psychomotor developmental index of 63. The results of the generated electroencephalogram report were abnormal, comprising a small number of sharp waves, and slow spike waves in the central region.

The proband's mother reported a history of 3 spontaneous miscarriages. A chromosomal karyotype analysis did not reveal any positive findings for either the proband or her parents.

2.2. Ethics statement

A written statement of informed consent was obtained from the proband's guardians for her and their participation in the study, which was approved by the Ethics Committee of the Third Military Medical University (Chongqing, China), and by the Population and Family Planning Science and Technology Research Institute.

2.3. DNA extraction

Venous blood samples were collected in vacutainer tubes containing EDTA, and genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, WI), according to the manufacturer's instructions. The quantity and quality of the extracted DNA were determined using a NanoDrop 1000 spectrophotometer (Thermo, MA).

2.4. Multiplex ligation-dependent probe amplification

Multiplex ligation-dependent probe amplification (MLPA) was performed at numerous sites of the proband's genome using the SALSA MS-MLPA kit P245-B1 (MRC-Holland), according to the manufacturer's instructions. This kit includes 40 probes that target chromosomal regions known to be altered in 23 multiple-microdeletion syndromes (including Prader-Willi/Angelman,

Cri-du-chat, DiGeorge, Langer-Giedion, and Miller-Dieker syndrome, among others).

2.5. Array-CGH

The proband's extracted DNA was screened via an aCGH analysis conducted by the KingMed Diagnostics Corporation (Guangzhou, China), using the Affymetrix Genome-Wide CGH CytoScan HD array (ThermoFisher Scientific), according to the manufacturer's instructions. This array includes more than 2,000,000 copy-number and 750,000 SNP probes. Genotype and CNV identification, and an assessment of genotyping integrity were conducted using Affymetrix Chromosome Analysis Suite software (ThermoFisher Scientific).

2.6. FISH

A blood sample was accordingly collected from the proband's father, and the extracted DNA was used to conduct a FISH analysis of chromosomes 10 and 18. This analysis used 2 probe pairs, one of which comprised an Agilent SureFISH 18p11.32 red fluorescent (R) and a Chr18 CEP green fluorescent (G) label, and the second of which comprised an Agilent SureFISH 10p15.3 red fluorescent (R) and a Chr10 CEP green fluorescent (G) label.

3. Results

The conducted MLPA analysis of the proband's genomic DNA did not identify any genetic abnormalities. Similarly, while the conducted aCGH analysis detected the proband to harbor a 6.6-Mb deletion between p14–p15.3 of chromosome 10 (arr[hg19]; 100,026–6,710,183), and a 6.3-Mb duplication at p11.31–p11.32 of chromosome 18 (arr[hg19]; 136,226–6,406,733), both mutations were not identified in her healthy parents (Fig. 1).

By analysis of the provided family history, we determined that proband's paternal aunt had a child who exhibited similar symptoms to those displayed by the proband, and that the proband's paternal grandmother reported several spontaneous miscarriages. Taken together, these observations suggest that the proband's genetic disorder was likely paternally inherited. The proband's father was conducted a FISH analysis of chromosomes 10 and 18 (Fig. 2). The results in 10 middle split like cells indicated that a translocation event happened. Furthermore, this translocation was shown to be balanced, as evidenced by the normal aCGH analysis result and phenotype exhibited by the proband's father. Thus, the proband's father was determined to harbor a t(10;18)(p15.3; p11.32) balanced translocation, from which the proband inherited her chromosome 10 p14–p15.3 deletion and chromosome 18 p11.31–p11.31 duplication.

4. Discussion

Balanced reciprocal translocations are the most common chromosomal rearrangements affecting humans, and are estimated to occur in 0.16% to 0.20% (1/625–1/500) of live births.[5] The great majority of cases with apparently balanced structural rearrangements exhibit a normal phenotype; however, 0.6% of patients with MR harbor these balanced structural rearrangements, which likely cause a deleterious phenotype by inducing gene disruption/dysregulation, microdeletion/duplication, and/or position effects at the chromosome breakpoint. Theoretically, balanced reciprocal translocation-carriers can
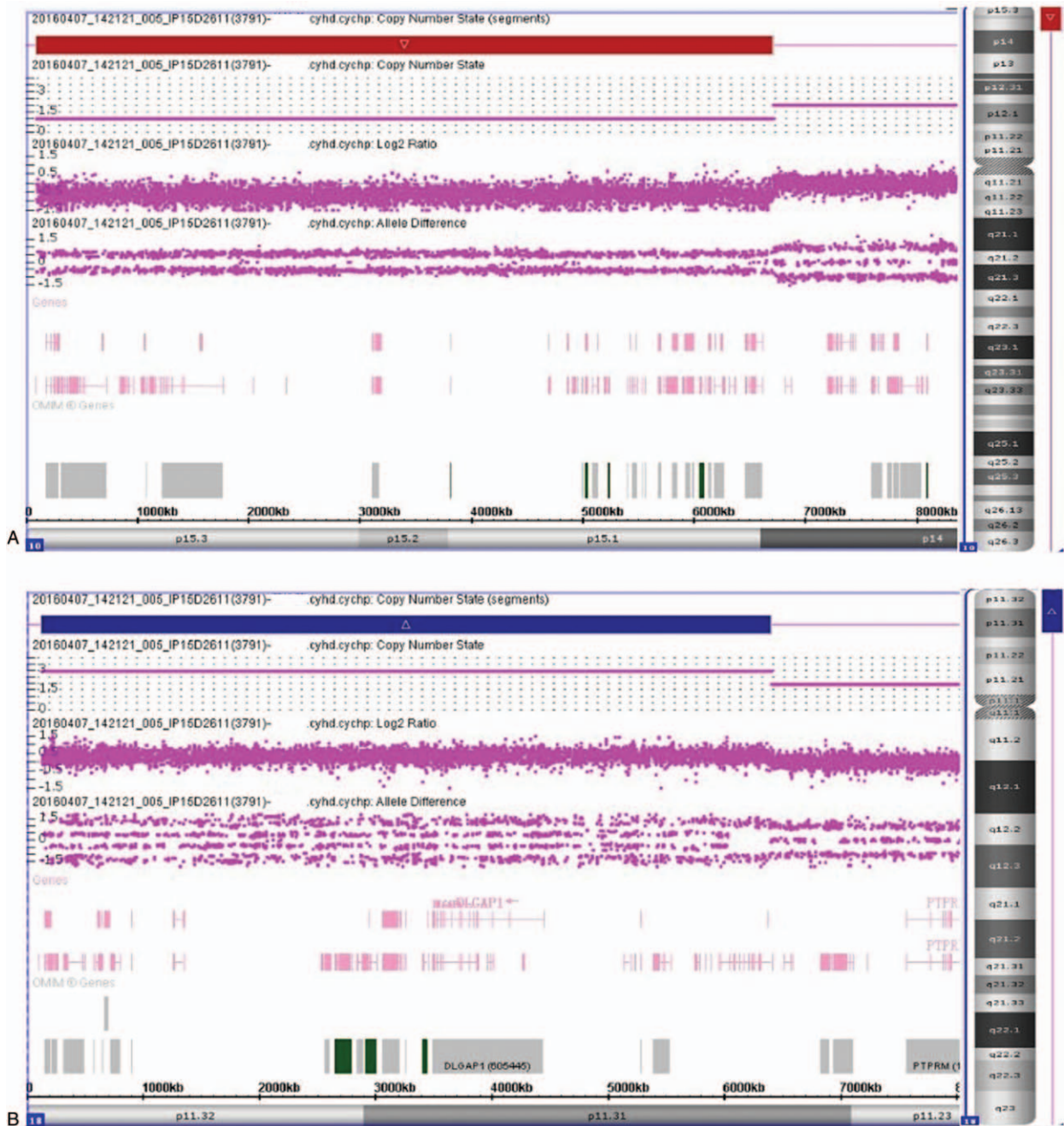
Figure 1. aCGH result of proband. (A) The deletion of chr10 p14–p15.3 in the aCGH result; (B) The duplication of chr18 p11.31–p11.32 in the aCGH result. aCGH = comparative genomic hybridization.

produce 18 types of gametes, including only 1 normal and 1 balanced reciprocal chromosomal translocation, but 16 cytogenetically abnormal gamete types. As a result, the probability of such carriers producing healthy offspring is relatively low, and many carriers are clinically infertile, experience a high rate of miscarriage, and/or produce offspring affected by chromosomal disease. In the present study, the proband's father was identified to carry the balanced translocation t(10;18)(p15.3; p11.32), which was likely the cause of the multiple spontaneous miscarriages reported by his wife.

The proband was identified to harbor a 6.6-Mb deletion at p14–p15.3 of chromosome 10 (arr[hg19]; 100,026–6,710,183) and a 6.3-Mb duplication at p11.31–p11.32 of chromosome 18 (arr[hg19]; 136,2266,406,733), via the conducted aCGH analysis. A literature search using the University of Santa Cruz genome browser (http://genome.ucsc.edu/) and Online Mendelian Inheritance in Man (OMIM) database (http://www.omim. org/) identified *ZMYND11* and *TGIF1* (located at 10p15.3 and 18p11.3, respectively) as potential causative genes for the proband's observed GDDS phenotype. Coe et al recently reported
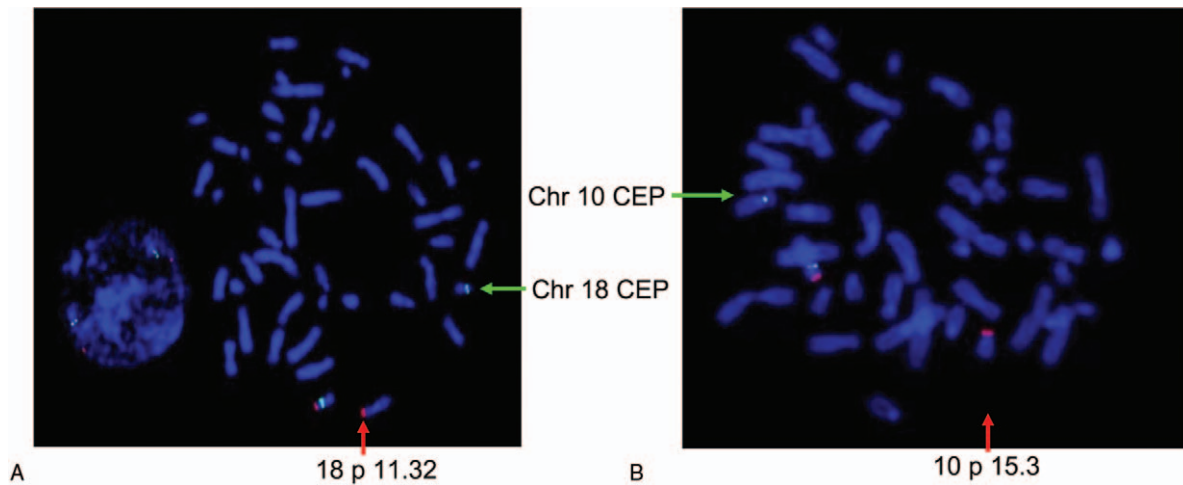
Figure 2. The FISH result of proband's father. (A) The FISH result of Chr18, the 18p11.32 region was showed by Red fluorescent label, Chr18 CEP was showed by Green fluorescent label; (B) The FISH result of Chr10, the 10p15.3 region was shown by red fluorescent label, Chr10 CEP was shown by green fluorescent label. FISH = fluorescent in situ hybridization.

loss-of-function mutations in *ZMYND11* in 7 individuals from 6 families.[6] One of these familial cases comprised a male individual observed to exhibit GDDS, as well as delayed speech, social and behavioral difficulties, and dysmorphic facial features. Moreover, his father exhibited a milder version of this phenotype, comprising GDDS, and behavioral difficulties including aggressive childhood behavior and mood swings. In general, patients with mutations in *ZMYND11* exhibit a mild intellectual disability, and subtle facial malformations that may include hypertelorism, ptosis, and/or a wide mouth. Both of the females studied by Coe et al were described as having autistic tendencies, and 3 of the 4 studied males exhibited increased aggression. Taken together, these results support those of the present study, and suggest that *ZMYND11* is a promising candidate causative gene for GDDS. In contrast, *TGIF1* is a dosage-sensitive gene, and *TGIF1* haploinsufficiency is established to induce various human disorders (OMIM: 142946).[7] However, the proband in the present study harbors a chromosome 18 duplication that includes *TGIF1*, while she was not observed to exhibit any related clinical phenotypes.

The aCGH technique is routinely used to detect chromosomal imbalances since it enables researchers to achieve a very high level of resolution without requiring specific probes for target subregions. It is well established to be effective in detecting CNVs, long-term continuous homozygosity, and chimeras (at a rate of greater than 20%), but it is unable to detect balanced chromosomal translocations such as reciprocal and/or Robertson translocations, inversions, and balanced insertions.[8] It is also unable to detect point mutations, and/or pathogenic tandem repeats (as observed in Fragile-X Syndrome). In the present study, the proband's father was identified as a chr18p–10p-balanced translocation carrier; however, the results of his karyotype and aCGH analyses showed no cytogenetic abnormalities. This is because while large balanced translocations can be identified via a conventional karyotype analysis, small balanced translocations must be detected via more sensitive methods than aCGH. Importantly, this emphasizes the fact that failure of these techniques to detect chromosomal lesion sites in the clinical setting should not be considered sufficient to exclude the possibility of their contribution to disease pathogenesis.

The American College of Medical Genetics (ACMG) has made a guideline on the cytogenetic evaluation of the individual with DD or MR in 2005. And it also made guidance for constitutional cytogenomic microarray analysis to explain CNV. For any child with unexplained MR/DD, even in the absence of dysmorphic facial features, other clinical features or positive family history, routine chromosome analysis is indicated according to these advices of ACMG. FISH or other molecular techniques should be performed before or at the same time as with chromosome analysis for children with clinical features suggestive of a particular microdeletion/microduplication syndrome.[9] In general, unaffected parent carried the detected CNV in patient with MR/DD, which it may be taken as evidence that supports the CNV as unrelated to the clinical features and likely benign in the patient.[10–12] In our study, no abnormal findings were present in karyotype analysis for all individuals, but a microdeletion of chromosome 10 with a microduplication of chromosome 18 was found in patient. For this situation, some doctors may regard the parents as normal individuals, while the patient carried a de novo variation of CNV. Notably, minor balanced translocation between chromosome 10 and 18 may be present in proband's parents, and the results of FISH confirmed our speculation in proband's father. Although our study only involved a rare case, it was important supplementary information to the current guidelines, especially in some special families similar to our case, where the results of FISH in proband's parents will help us identify the genetic pathogenesis.

The present study also demonstrates that the efficacy of genetic counseling in advising patients and their relatives of the risks and consequences associated with an inherited disorder, (particularly with regards to fertility management and family planning), is highly dependent upon the provision of an accurate patient medical history. The present study was initially hampered because the proband's parents did not disclose their full family medical history until a potential genetic basis of the observed GDDS was identified.

Ensuring that accurate genetic counseling is available to the families of patients with GDDS is essential, since the identification of the underlying disease pathogenesis in each GDDS case may facilitate the provision of tailored symptomatic treatment and/or rehabilitation services, thus ensuring that affected individuals are adequately supported.[13] In fact, children with GDDS are usually able to learn in a similar way to most children unaffected by the disorder, but take longer, and require additional support to acquire and develop new skills. Effective genetic counseling may allow the families of patients with GDDS to anticipate their current and future needs, and to thus to psychologically and financially prepare for the provision of future treatments and rehabilitation. This may, in turn, reduce the familial stress caused by the high level of care required to support patients in daily activities (such as eating, dressing, communicating, etc), and by parental anxiety for the future wellbeing of patients with this disorder.

Author contributions

Conceptualization: Danyan Zhang.
Data curation: Yijian Zhu, Xuefei Feng, Letian Zhao, Yuping Zhang.
Formal analysis: Limeng Dai.
Investigation: Xuefei Feng.
Methodology: Danyan Zhang, Mingfu Ma, Lianbing Li.
Project administration: Yijian Zhu, Hong Guo.
Resources: Limeng Dai, Hong Guo, Yuping Zhang.
Supervision: Danyan Zhang, Xuefei Feng, Limeng Dai, Mingfu Ma, Yun Bai.
Validation: Yijian Zhu, Xuefei Feng, Letian Zhao.
Visualization: Lianbing Li.
Writing – original draft: Danyan Zhang, Yun Bai.
Writing – review and editing: Danyan Zhang.

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