3q29 Microduplication syndrome: a new family case of phenotype heterogeneity

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Abstract

Here, we describe a family case carrier with 3q29 microduplication, a 1.56Mb and 1.68 Mb duplicate region, that was identified by CNV-seq. Different from the common clinical traits of previously reported cases, this family of individuals shows an apparently normal phenotype.

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ABSTRACT:

Phenotype heterogeneity of 3q29dup among different patients not only draw the attention of geneticists but also increase the complexity and variability of the relationship between gene and phenotype. We described two family members carrier with 3q29 microduplication, a 1.56Mb and 1.68 Mb duplication region,

(chr3:g.195760000_197320000dup and chr3:g.195720000_197400000dup, GRCh37) that was identified by Copy number variation sequencing (CNV-seq). The precise breakpoints were analyzed by whole-exome sequencing (WES) and the duplication region was validated by real-time PCR. *DLG1* and *BDH1* were not involved in this duplicated area, may provide new clues for the phenotype heterogeneity. More cases needed to detect and apply to this rare syndrome to analyze the relationship between genes-associated and the phenotype of 3q29 duplication syndrome.

Keywords: Microduplication 3q29 Syndrome, Phenotype, Genetic Heterogeneity, Microdeletion 3q29 Syndrome

INTRODUCTION

3q29 microdeletion was firstly described in 2005, the clinical features of 3q29 microdeletion were more severe than those of 3q29 microduplication, now called 3q29 microduplication syndromes[1]. Typical 3q29 microduplication syndrome includes a 1.6MB region with low copy repeats that are flanked with high identity sequences, resulting in increased genomic instability and causing copy number variations[2-4]. The discovery of region-specific low-copy repeats near the breakpoints by Willatt et al. indicated that the arrangement of chromosomes mediated by nonallelic homologous recombination may be the major mechanism of microdeletions[1]. Similar to microdeletions, the underlying mechanism of 3q29 duplications can be interpreted as the rearrangement of LCR on either side of the breakpoints at a frequency equal to that of deletions or reciprocal duplications.

The clinical phenotype of 3q29dup (OMIM:611396) was firstly reported by Lisi et al. in 2008 and was accompanied by mild to moderate intellectual disability and minor dysmorphic features, such as microcephaly, round face, bulbous nose, short or down slanting palpebral fissures, excessive hand creases, and pes planus, associated with a microduplication of chromosome 3q29[2], partially overlap with 3q29 microdeletion syndrome[5-7].

In this work, we reported a family case of 3q29 microduplication. Three family individuals were identified using CNV-seq, and they were diagnosed with a paternal inheritance pattern. We report two family individuals carrying with a typical 3q29 microduplication , with an apparently normal phenotype.

METHODS AND MATERIALS

Editorial Policies and Ethical Considerations

Our study was approved by the medical ethic committee of Taihe Hospital.

Copy number variation sequencing

Amniotic fluid cells were obtained by amniotic fluid puncture. They were first centrifuged at 3500 rpm for 15 minutes and washed with physiological saline solution. Then, the amniotic fluid cells were centrifuged at 3900 rpm for 1 minute. Genomic DNA was extracted from 200 uL of peripheral venous blood using a DNeasy® Blood & Tissue Kit (Qiagen, Germany) according to the manufacturer's protocol. The DNA concentration was measured by Qubit 3.0 (Axygen, USA). The establishment of the DNA library was completed using Genomics DNA Clean & Concentrator kits (Zymo Research, USA). The concentration of the DNA library was quantified by real-time PCR. A total of 3.2 pm of DNA from each library sample was used for CNV-seq analysis following the manufacturer's protocol.

DNA isolation and whole-exome sequencing

Peripheral blood and amniotic fluid cells were collected with the patient's informed consent, and genomic DNA was isolated following the manufacture's protocol (Qiagen, Germany). The concentration and purity of the DNA samples were quantified by Nanodrop 2000. Genomic DNA was prepared as an Illumina sequence library by a KAPA HyperPrep Kit (Roche, Genentech), and the concentration of DNA library was detected by a quantitative approach of real-time PCR. Libraries were enriched by xGen Exome Research Panel v1 (IDT, Coralville, Iowa, USA) and sequenced by Novaseq 6000 (Illumina, San Diego, CA, USA). The raw data

were mapped to the reference sequence of human genomics by Burrows-Wheeler Aligner (BWA). Variant calling was realigned by GATK Haplotypecaller, samtools and FreeBayes. Annotation was completed using ANNOVAR according to the American College of Medical Genetics and Genomics (ACMG) guidelines, and the grade of the variants was evaluated.

CNV validation by real-time quantitative PCR

DNA was isolated following the manufacturer's protocol (Qiagen, Germany), and the concentration and quality of DNA were detected by Nanodrop 2000. *PCYT1A* and *CEP19* genes were chosen, and primers were designed to validate the duplication of 3q29(Chr3:196051942-197297204 dup, GRCh38). qPCR-PCYT1A-F:5' CACGGTGATGAACGAGAATG 3' qPCR-PCYT1A-R:5' AGAGGTAGAAGTAAAACACGGCT 3' qPCR-CEP19-F:5' GAGTGAAATCAAGGGGAAAAT 3' qPCR-CEP19-R:5' GACAGGAGGCAAGATAAGACAT 3' GAPDH-F:5'CCTCTGACTTCAACAGCGACAC 3' GAPDH-R: 5'ATGAGCTTGACAAAGTGGTCGT 3'. *GAPDH* was used as a reference gene to normalize the expression data. A total volume of 20 μL of a PCR system containing 10 μL 2 × ChamQ Universal SYBR qPCR Master Mix and 5 μL DNA template was used. Copy number quantification was evaluated using the 2^{-T} method.

Results:

The CNV-seq results showed that the molecular karyotype of the proband foetus was seq[hg19]dup(3)(q29)chr3:g.195760000_197320000dup(GRCh37)(Figure B). Twenty-eight genes were included in this duplicate region(refseq database), such as *DLG1, BDH1, FBXO45, PIGX, RNF168, NRROS, PIGZ,*and non-coding RNA *MIR4797* and *LINC00885* . The molecular karyotype of the foetus's father was seq[hg19]dup(3)(q29) chr3:g.195720000_197400000dup(GRCh37), a 1.68 Mb length duplicate segement, 33 genes are involved in this area, including *DLG1, BDH1, RUBCN, SLC51A, MIR4797, LINC02012* . Apart from this pathogenic CNV, a 0.34Mb microdeletion (chr14:g.22640000_22980000del) without biological significance gene was discovered and evaluated as a benign CNV(Figure C).

The whole-exome sequencing results revealed that a 1.245Mb duplication was located in chromosome 3(chr3:196051942-197297204, GRch hg38)in the genomes both the proband and her father(Figure A). Q-PCR was used to verify the duplication which WES results shows(Figure D). The left breakpoint was located in exon 19 of *TFRC* , and the right breakpoint was located in exon 2 of *DLG* . This duplication region encompasses 18 protein-coding genes: *ZDHHC19, SLC51A, PCYT1A, TCTEX1D2, TM4SF19, UBXN7, RNF168, SMCO1, WDR53, FBXO45, NRROS, CEP19, PIGX, PAK2, SENP5, NCBP2, PIGZ ,MELTF* . No other pathogenic variants that were highly associated with diseases were found in the whole exome sequence.

CASE PRESENTATION

The proband, a nineteen-week-old foetus was the third child of healthy, nonconsanguineous parents. A 3q29 microduplication(seq[hg19]dup(3)(q29)chr3:g.195760000_197320000dup GRCh37) was identified by CNV-seq in Taihe Hospital when she was 6.5 months old. To evaluate the source of the duplicate segment, the parents agreed to CNV-seq analysis of their peripheral blood after genetic counselling. The result indicated that the prenatal diagnosis of the 3q29dup was of paternal origin based on the molecular karyotype. The corresponding risks and suggestions were given to the proband's mother, and her mother chose to continue the pregnancy. She was born at 40 weeks by spontaneous vaginal delivery. Her birth weight was 3950g, and her birth height was 52 cm. The Apgar score was nine at one minute and ten at 5 minutes. A slightly larger bregma of 3 cm*3 cm was found by physical examination, but no other deformities were detected at birth. On day 4, she was transferred to neonatology and diagnosed with hyperbilirubinemia, neonatal pneumonia, neonatal omphalitis and congenital heart disease. Echocardiography showed moderate tricuspid insufficiency. After symptomatic treatment and based on the doctor's advice, she was discharged on Day nine.

She pronounced the words father and mother when she was five months old, and she subsequently pronounced grandmother and grandfather when she was eight months old. Thereafter, her language development slowed down and simple but complete sentences could not be expressed fluently until now(19 months). No physical

defects were found. She could walk independently without any assistance when she was 17 months old, and there was hardly any struggle.

She was admitted for fever without obvious cause when she was nine months old. Her body temperature reached 39.2, and she experienced cough and conjunctival hyperaemia for four days before admission. She was diagnosed with sepsis, bronchopneumonia, infantile diarrhoea, and suspected Kawasaki disease. Echocardiography revealed that the moderate tricuspid insufficiency had disappeared and returned to normal morphology and function. The left and right ventricular structures were normal, similar to the pulmonary valve and aortic valve both in morphology and function after symptomatic treatment. After approximately six months, she received regular echocardiography reexaminations, and the results revealed a normal heart structure and normal cardiac function as well as no dilatation of bilateral coronary arteries.

The proband's father was another family case of 3q29 microduplication syndrome. He was a healthy individual carrier of 3q29dup, and he did not present the common symptoms of intellectual disability and speech or developmental dalay. In 2019, there was also a foetus identified to have 3q29 microduplicate syndrome, but delivery of this foetus was induced at the doctor's suggestion since the CNV was evaluated as a pathogenic CNV.

DISCUSSION

3q29 duplication syndrome (OMIM 611936) was firstly described by Lisi et al. in 2008[2], for the microduplication in chromosome 3q29 (chr3:197.1-198.9Mb, NCBI35). An increasing number of individuals that carry with 3q29 duplication have been reported, and the clinical phenotypes of mild to moderate cognitive disorder,microcephaly,developintellectual disability,speech delay,optical and neuropsychiatric problems and congenital heart disease were found at a high frequency in these reports.

During the first year of our present case, problems that had been reported at a high frequency in the literature, such as feeding problems, gaining weight disability, hypotonia, and respiratory distress, not appeared in the onset of this disease[8]. These results were consistent with the case reported by IOANA STREATA et al., with an uneventful infancy and early childhood[8].

The present case present with heart disease of moderate tricuspid insufficiency at birth, and a recovery to normal morphology and function at nine months was confirmed by cardiac ultrasonography. Previously, Pollazzon, M., et al. reported a *de novo* 3q27.33q29 deletion in a female patient, approximately 9.3 Mb in size and overlapped with a 1Mb region of a common sites for 1.6Mb 3q29 microdeletion syndrome that is associated with mild tricuspid valve dysplasia[9]. Congenital heart defects were not found in this case which is consistent with previous results, described by five out of ten patients found in previous documents, even though containing the same genes, including *WDR53, TFRC, PIGX, MELTF* and *ZDHHC19* .

As previous reported a nine years old individual carrier with 448.8kb microduplication of 3q29, only two genes are involving in this area,*DLG1* and *BDH1* . Patients suffer from growth restriction,epileptic abnormalities and speech delay[11]. DLG1, discs large MAGUK scaffold protein one, which has multiple transcripts were generated by alternative splicing, play roles in signal transduction, cell proliferation, trafficking and synaptogenesis and lymphocyte activation[2]. By interaction with AMPA and NMDA-type glutamate receptors, DLG1 protein contribute to the ion channels trafficking and surface expression, associated with the clinical feature of intelligence disability and epilepsy. In our presented case,these symptoms were not appear. The whole-exome sequencing results indicates a break point located in the exon two of*DLG1* (The coding sequence are not including in the exon one of*DLG1*) , which demonstrate a normal copy of *DLG1* and*BDH1* in our case, that's may be the caustive factor responsible for the unaffected phenotype .

PAK2 , a subtype of PAK subfamily, ubiquitously expressed in tissues while *PAK1* and *PAK3* are highly expressed in the brain. As previous reported, *PAK2* is a regulator associated with cytoskeletal function and dynamics activated by Rac1 and Cdc42.*PAK2* also participate in maintaining the proliferation and survival of hematopoietic progenitor cells (HPC) in vitro and contribute to the quantity maintenance of peripheral blood leukocytes as the same as granulocyte/monocyte skewing and T and B cell differentiation/maturation[12].

Yan Wang et al. point out that *PAK2* may interaction with other genes, such as*DLG1* , in a synergistic manner by comprehensively considering of their animal results combined with human genetics phenotype[13]. Our case present here may provide some useful information especially when *PAK2* and *DLG1*genes are not involved in 3q29dup, thus may also contribute to the complexity and variability between genotype and phenotype.

In summary, we described a family cases carrier of 3q29dup, who did not present with the common features of the 3q29 microduplication syndrome or other abnormal symptoms. In fact, they display apparently normal phenotype. The dosage effect of *DLG1* and *BDH1* may partially account for this observation. More cases and in-depth studies are required to elucidate the relationship between the genomics and phenotypes of this rare syndrome.

Conflict of interests

The authors declare no conflict of interest.

Consent statement

The participants agree to write and publish the case reports.

Acknowledgements

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Data availability statement

The data that support the findings of this study are available from the corresponding author

upon reasonable request.

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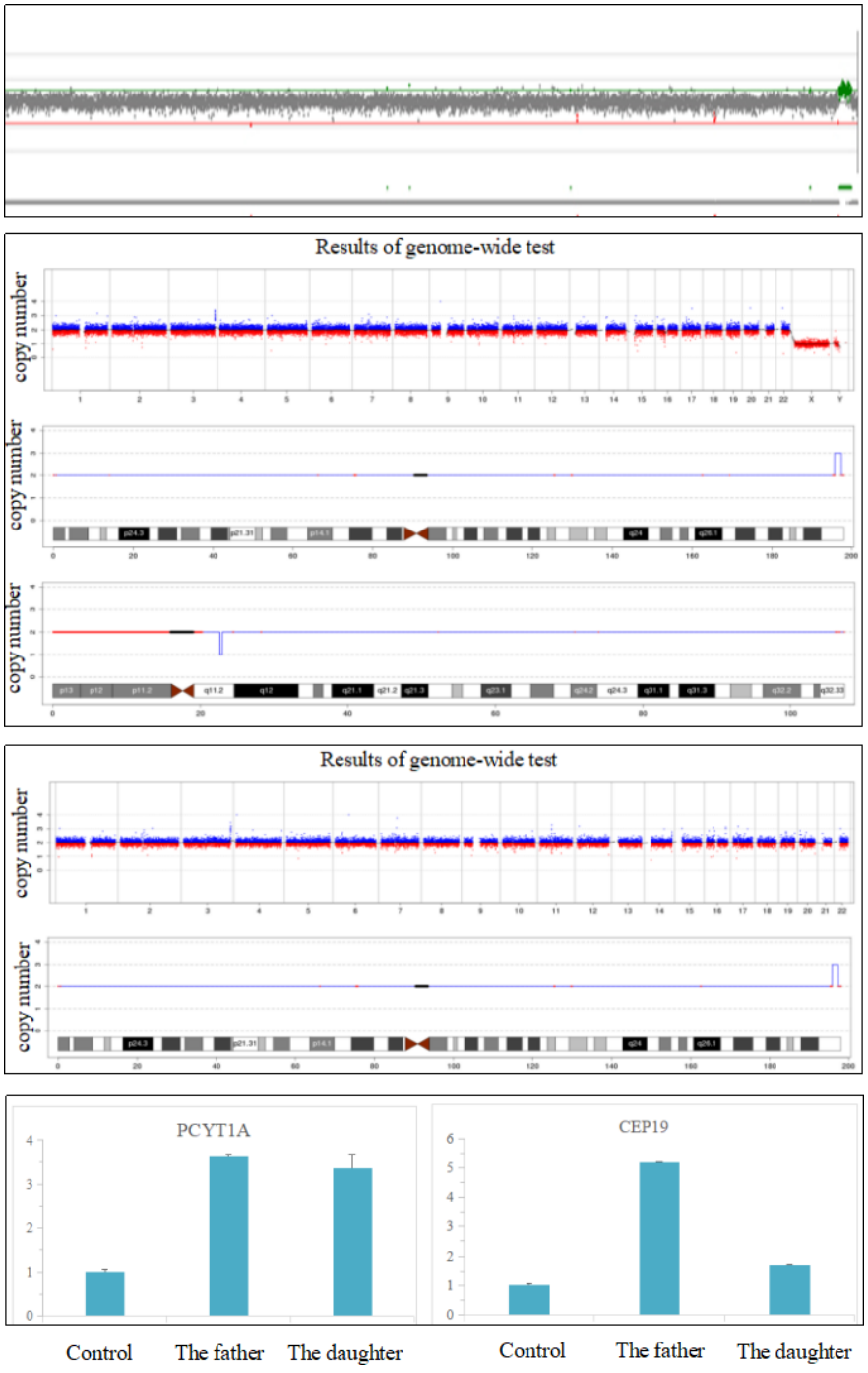
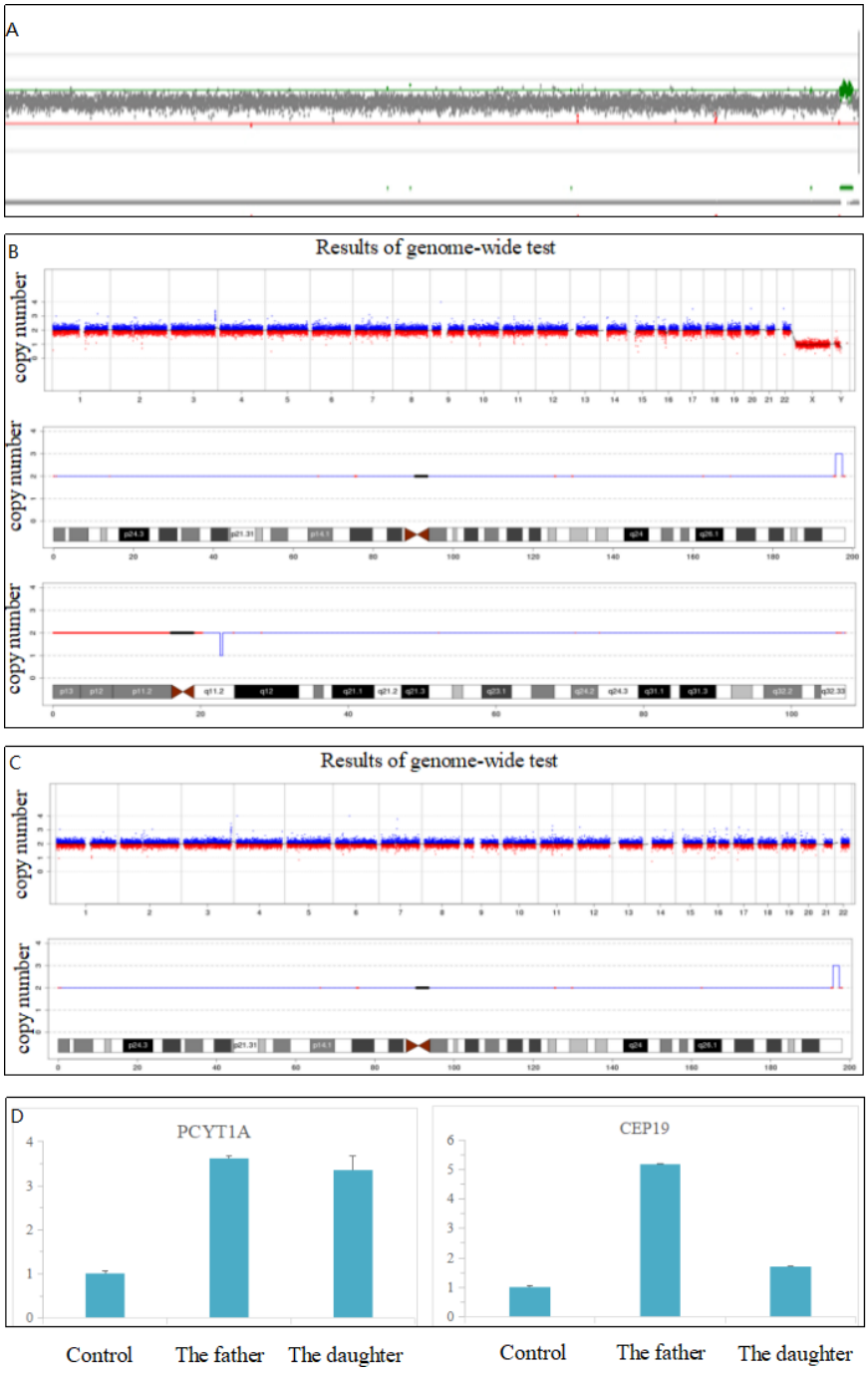


Figure 1. A familial 3q29 microduplication. (A) Thefather and daughter had a 1.245 Mb interstitial duplication of 3q29 detected by WES. (B) The father had an interstitial duplication of 3q29 and deletion of 14q11.2 by CNV-seq. (C) The daughter had an 3q29 microduplication segment detected by CNV-seq. (D) The duplication area validated by qPCR, *PCYT1A* and *CEP19* were genes involved in this duplication area.



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Approval Letter of Medical Ethics Committee of Shiyan Taihe Hospital(Research)

批件号 Reference Number：科研会审（201928）号

审查日期	2019 年 9 月 27 日	审查地点	医务处会议室
研究方案名称	染色体病和染色体拷贝数变异的多组学研究		
承担学科	生殖医学中心	项目负责人	张志军
审批材料清单	☑审查申请表 Application form ☑研究方案 Protocol ☑知情同意书 Informed consent form ☑CRF 表 CRF Table ☐其它 Other_____		*组长单位： 无 Lead Site：
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	其中：同意 8 票，不同意 0 票，作必要修改后再审查 0 票，作必要修改后同意 2 票，暂停或者终止研究 0 票。		

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太和医院医学伦理委员会（盖章）

Medical Ethics Committee of Taihe Hospital

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2、本审查意见仅对本次项目审批有效，如内容有变动需再次审批。

3、伦理委员会在试验过程中将进行年度跟踪审查，请在到期前一个月提出跟踪审查申请。

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