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Case report

Concurrent copy number variations on chromosome 8 and 22 combined with mutation at FGA locus revealed in a parentage testing case

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ABSTRACT

Copy number variations (CNVs) are one of the major sources of human genetic diversity and are associated with rare genomic disorders as well as complex traits and diseases. A copy number variation was observed at the D8S1179 locus during routine STR based parentage testing, in which the child exhibited three alleles, "13, 15, 16", with the putative father a homozygous "15" and the mother homozygous "13". In addition, in the same testing case, there was a one-step mutation at the STR locus FGA, in which the putative father was a "22, 24", the mother was a "22, 25", and the child was a "22, 23". After further investigations by re-amplified with different primer sets, clone-based sequencing, karyotype analysis and whole-genome SNP analysis, the results showed that the child had the CNVs at chromosome 8q24.3 and 22q11.21. In conclusion, for parentage testing cases encountered with tri-allele patterns, more testings, such as cloning sequencing, karyotyping, or even whole genome analysis, as well as more appropriate statistical estimations might be conducted to further confirm or exclude the relationship.

1. Introduction

The human genome shows extensive copy number variations (CNVs) and the presence of variable numbers may range from about one kilobase to several megabases in size [1]. CNVs can be caused by structural rearrangements of the genome such as deletions, duplications, inversions, and translocations. Since CNVs correspond to relatively large regions of the genome that have been deleted (fewer than the normal number) or duplicated (more than the normal number) on certain chromosomes, the genotyping results of the genetic markers located in these regions may be affected and lead to null allele, tri-allele and peak height imbalance in short tandem repeats (STRs) markers routinely used in parentage testing. Therefore, false exclusion and incorrect interpretations may occur in these scenarios.

In this study, we report a paternity testing case with two concurrent CNVs at Chromosome 8q24.3 and 22q11.21 from the child and a one-step mutation of FGA between the father and the child. These genetic variables caused abnormal genotyping results for the corresponding STR loci.

2. Materials and methods

2.1. STRs analysis and clone-based sequencing confirmation

Blood samples from the putative father, the child (male) and the mother were collected with informed consent. The research protocol was approved by the ethical review committees of the Beijing Institute of Genomics (Protocol name: a study on the copy number variations revealed in a parentage testing case no. 2014032, date adopted: September 15, 2014). DNA was extracted by the Chelex-100 resin method [2] for all the STR amplifications.

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Genomic DNA for SNPs Chip was extracted from peripheral blood samples by QIAamp DNA Blood Midi kit (QIAGEN), according to the manufacturer's protocol. The quantity of the recovered DNA was determined by Qubit® Quantitation System (Invitrogen, CA, USA), according to the manufacturer's specifications.

First, 1 ng DNA was amplified with commercial STR typing kit GoldenEye™ 20A (Peoplespot Incorporation, China) which included 19 autosomal STR loci [3]. The same amount of DNA was also re-amplified with another commercial STR typing kit AmpFlSTR Identifiler® (Life Technologies, USA), which had 15 overlapped autosomal STR loci with GoldenEye™ 20A, including the loci D8S1179 and FGA. Polymerase chain reaction (PCR) was performed according to the manufacturer's recommendations. The amplified PCR products were separated by capillary electrophoresis on a genetic analyzer (ABI PRISM 3130XL, Applied Biosystems, USA) and analyzed with GeneMapper ID-X (Applied Biosystems, USA).

Sequencing reactions were performed to analyze the alleles of the loci D8S1179, FGA and D22GATA198B05. The primer sequences of loci D8S1179 and FGA were obtained from the STRbase website (http://www.cstl.nist.gov/biotech/strbase/) and the loci D22GATA198B05 was designed using Primer5 software according to the databases UCSC Genome Browser (http://genome.ucsc.edu/), and were as follows: D8S1179 Forward – ATTGCAACTTATATGTATTTTTGTATTTCATG, D8S1179 Reverse – ACCAAATTGTGTTCATGAGTATAGTTTC, FGA Forward – GGCTGCAGGGCATAACATTA, FGA Reverse – ATTCTATGACTTTGCGCTTCAGGA, D22GATA198B05 Forward – GGTCTCCAGGCGGCCTGTCGT, D22GATA198B05 Reverse – TTGATAAGATTTAGGATTGAT. PCR products were cloned and sequenced using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) using M13 forward and reverse primers according to the manufacturer's recommendations. Capillary electrophoresis was performed on a genetic analyzer (ABI PRISM 3130XL, Applied Biosystems, Foster City, CA) with Data Collection Software V3.0. The sequence data was analyzed using DNA sequencing analysis software V5.2 (Applied Biosystems).

2.2. Karyotype analysis

Karyotype analysis was performed on conventional GTG-banded metaphases prepared from peripheral blood samples obtained from the child and his parents using standard protocols [4].

2.3. CNV analysis with whole genome SNPs Chip

250 ng genomic DNA for each sample was used to performe the CNV analysis with Illumina HumnaOmni ZhongHua-8 BeadChip (Illumina, USA) according to the manufacturer's protocols. The 900,015 markers on this chip captured 81% variation with minor allele frequency >5% in East Asians. The genotyping data, B allele frequency (BAF) and log R ratio (LRR) of each marker were collected, and the copy number of each genome loci was calculated using GISTIC 2.0 software package [5].

3. Results

The 19 autosomal STR genotypes of the putative father, the mother, and the child were showed in Table 1. At the D8S1179 locus, the putative father, the mother, and the child had genotypes "15", "13", and "13, 15, 16", respectively. The same results were obtained from both Identifiler® and GoldenEye™ 20A (Fig. 1). In addition, there was a mutation at the STR locus FGA, in which the putative father was a "22, 24", the mother was a "22, 25", and the child was a "22, 23". The Combined Paternity Index (CPI) for trios paternity excluding D8S1179 was 3,226,464.

Table 1
Nineteen autosomal STR genotyping of the father, mother and the child. A Tri-allele was found in D8S1179 (underlines) and one-step mutation was found in FGA (italic).

Loci	Father	Child	Mother	Paternity index
D19S433	13,15.2	13,14	14	1.871
D5S818	10,12	10,11	11	2.203
D21S11	31.2,32.2	28,32.2	28,32.2	1.779
D18S51	13,15	13,14	12,14	4.878
D6S1043	14,20	16,20	16	9.259
D3S1358	15,17	15	15,17	1.219
D13S317	11,12	11,12	8,12	2.1
D7S820	11,12	10,11	10	1.233
D16S539	12	9,12	9	5.195
CSF1PO	10,11	11	11	2.433
Penta D	9,14	9,14	8,9	12.755
vWA	16,18	16,19	17,19	3.253
TPOX	8	8,9	8,9	3.516
Penta E	9,10	10,20	20	12.5
TH01	7,9	9	9	0.973
D12S391	17,19	17,19	17,20	2.033
D2S1338	20,22	22,23	23,24	9.671
FGA	*22,24*	*22,23*	*22,25*	0.007
D8S1179	15	13,15,16	13	–
CPI				32,26,464

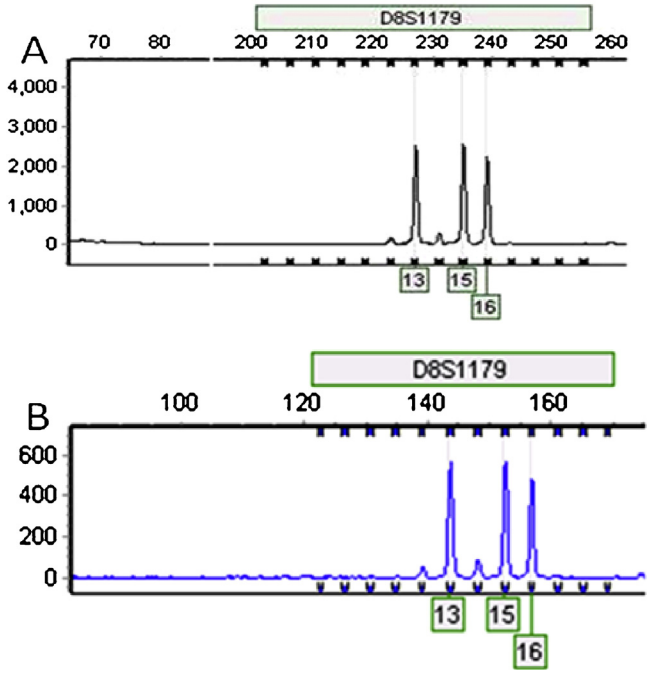

Fig. 1. Tri-allelic patterns observed at locus D8S1179. (A) Electropherogram profile amplified with AmpFlSTR Identifiler®. (B) Electropherogram profile amplified with GoldenEye™ 20A.

The clone-based sequencing results of the D8S1179 locus showed that sequence heterogeneity was observed between the two 15 alleles of the putative father, with one repeat structure was $[TCTA]_2[TCTG]\,[TCTA]_{12}$ and the other was $[TCTA]_1[TCTG]\,[TCTA]_{13}$. Sequence heterogeneity was also observed between the two 13 alleles of the mother, in which the repeat structures were $[TCTA]_1[TCTG]\,[TCTA]_{11}$ and $[TCTA]_{13}$, respectively. The repeat structures of the alleles 13, 15 and 16 of the child were $[TCTA]_1[TCTG]\,[TCTA]_{11}$, $[TCTA]_1[TCTG]\,[TCTA]_{13}$ and $[TCTA]_2[TCTG]\,[TCTA]_{13}$, respectively. The sequencing result confirmed that the allele 13 and allele 15 of the child was maternal and paternal, respectively. But the source of the allele 16 of child was not clear.