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Mutation of glucose-6-phosphate dehydrogenase deficiency in Chinese Han children in eastern Fujian

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Abstract

We aim to investigate the mutation types of glucose-6-phosphate dehydrogenase (G6PD) deficiency in Chinese Han children in eastern Fujian Province.

A total of 904 Chinese Han neonates (male: 733 with positive G6PD deficiency and 28 with weakly positive deficiency; female: 73 with positive G6PD deficiency and 70 with weakly positive deficiency) received G6PD screening in our center from January 2014 to December 2016 were included in this study. Additionally, 904 age-matched normal Chinese Han individuals (male: 761; female: 143) were selected as control. Neonatal G6PD deficiency screening was performed through blood sample collection from the heels, using the commercial kits. Multicolor melting curve analysis (MMCA) method was used to determine the *G6PD* mutation type in the 904 cases. If it failed to detect mutations in the cases with abnormal enzyme activity, the polymerase chain reaction (PCR) and gene sequencing were used to determine the mutation sites. PCR and gene sequencing were used to determine the mutation sites in the 904 individuals with normal enzyme activity. Three most common mutation types in Chinese population were compared between Fujian and other provinces.

Among the 904 neonates with abnormal G6PD enzyme activity, 17 mutation types were detected including 15 single point mutations and 7 complex mutations. Three most common mutation types were *c.1376G>T*, *c.1388G>A*, and *c.95A>G* accounted for 72.6% of the total mutations in eastern Fujian.

The proportion of mutational types in *G6PD* and the degree of enzyme activity change in various mutational types were found in the neonates of Fujian Province. Our study may enrich the molecular diagnosis of *G6PD* deficiency meaning Fujian Province.

Abbreviations: G6PD = glucose-6-phosphate dehydrogenase, MMCA = multicolor melting curve analysis, PCR = polymerase chain reaction.

Keywords: gene mutation, gene sequencing, glucose-6-phosphate dehydrogenase, multicolor melting curve analysis, polymerase chain reaction

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency, is an X-linked incomplete dominant genetic disease mainly characterized by hemolysis caused by the decreased G6PD activity or the changed enzyme properties in erythrocytes.[1] Males are hemizygous for having only one X chromosome. Females, with 2 X chromosomes, may be heterozygous or homozygous having less severe clinical manifestations. To date, more than 400 *G6PD* biochemical mutants have been reported worldwide, and more than 140 kinds of *G6PD* mutations have been identified with at least 31 mutations identified in Chinese population.[2,3] Nowadays, rare studies have been focused on *G6PD* mutations in Han children in China mainland. In this study, we aim to investigate the mutation type of *G6PD* gene deficiency in Chinese Han children in eastern Fujian Pronvince.

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2. Patients and methods

2.1. Patients

From January 2014 to December 2016, we screened 200,260 G6PD samples. In total, 1813 samples (male: 1489; female: 324) with decrease in G6PD activity were included. Using the random digits table, a total of 904 Chinese Han neonates (male: 733 with positive *G6PD* deficiency and 28 with weakly positive deficiency; female: 73 with positive *G6PD* deficiency and 70 with weakly positive deficiency) received *G6PD* screening using fluorimetric method in Fujian Province were randomly selected in this study. Their parents are Chinese Han in Fujian confirmed by telephone. Among these positive cases, 524 males and 15 females showed a G6PD concentration of 0 to 1.0 U/g Hb, while 209 males and 58 females showed a concentration of 1.0 to 2.1 U/g Hb. Among

the patients with weakly positive deficiency, 28 males and 70 females showed a G6PD concentration of 2.1 to 2.6 U/g Hb. Besides, 904 age-matched normal Chinese Han individuals (male: 761; female: 143) were selected as control. The G6PD activity was > 2.6 U/g Hb. Written consent was obtained from the patients' guardians. The study protocols were approved by the Ethical Committee of the Fujian Provincial Maternity and Children's Hospital, Affiliated Hospital of Fujian Medical University (No. 2015069).

Blood specimens were collected from the heels, and then were dripped on special filter paper (Schleicher and Schull 903, Wortmann Co., Britain). Three blood spots with diameters of 10 to 12 mm were used for multicolor melting curve analysis (MMCA) detection. Two tubes of EDTA-K2 anticoagulant vein blood (1 mL) were used for G6PD activity determination, genomic DNA extraction, polymerase chain reaction (PCR), and gene sequencing. All the G6PD deficiency cases showed no following aspects after a face-to-face conversation with their parents: eating fava bean; contacting the mothballs; and drug-induced hemolysis.

Subjects were divided into 2 groups according to the screening results, including G6PD deficiency group and normal control. They were numbered according to the birth time, and selected according to the random number table. Blood samples were collected for laboratory tests.

Table 1

Primers sequence, amplification length, and temperature for G6PD.

Exon	Primer sequences	Tm	Product length, bp
2	2F-TCTAACCCATCAACCACTCC 2R-AGGCACTTCCTGGCTTTT	60	336
3+4	3F-TTGAATCTCGGGGCTCTT 4R-TCACGGGGGCTGGTAATG	60	573
5	5F-TGTCTGTGCTGCCTGCTTT 5R-AAAGGCGGTGTTTCGTGGA	61	599
6	6F-AGGTGTTGAGCCAGAGGGTC 6R-GAGGCAGTGGGCCAGGTG	67	399
7	7F-GGCAAGGAGATGGTGCAGAA 7R-TTCACCTGGTTCAAGGGCAT	60	659
8	8F-AAGACAAGGGGGATCAGGAA 8R-TGTGCTCAGAGGTGGTGACTT	62	351
9	9F-TATGTGACCAGGGAAGGCCA 9R-CCTCAGCACCAGCTCTCTCA	63	551
10	10F-GGAGCTCCCACTGAGACACT 10R-TGCTGATGCCACTGCCTG	63	399
11+12	11F-GCAGTGGCATCAGCAAGA 12R-AGTGACGGGTGGAGGAGA	63	401
13	13F-TATGGCAGGTGAGGAAAGG 13R-AGGTCAATGGTCCCGGAGT	63	285

G6PD = glucose-6-phosphate dehydrogenase.

2.2. Determination of G6PD enzymatic activity

G6PD enzymatic activity was measured with G6P/6PG ratio method using the commercial kit (Mickey Co. Ltd, Guangzhou, China) according to the manufacturer's instructions. DNA was extracted from dried blood spots. Three blood spots with a diameter of 3.2 mm were made with a punch. DNA was extracted from the dried blood specimens obtained from 904 cases with aberrant G6PD activity based on the Lab-Aid 820 Nucleic Acid Extraction System (Zeesan Biotech, Xiamen, China).

2.3. PCR amplification

DNA samples isolated from cases with G6PD deficiency were detected using commercial kit (Access Bio, NJ) according to the manufacturer's instructions. This kit could be used to qualitatively detect the 12 common *G6PD* mutations in human peripheral blood genomic DNA in China: *c.95A > G*, *c.383T > C*, *c.392G > T*, *c.487G > A*, *c.517T > C*, *c.592C > T*, *c.871G > A*, *c.1004C > A*, *c.1024C > T*, *c.1360C > T*, *c.1376G > T*, and *c.1388G > A*.

Ten pairs of PCR primers (Table 1) were designed for amplifying exon 2 to 13 (exon 1 does not encode protein) and partial introns using Primer Premier 5.0 according to the NCBI data (G6PD: NG_009015) and the previous description.[4] PCR amplification was carried out in a 25 μL volume containing 1 μL each primer, 2 μL DNA template, 8.5 μL double distilled water, and 12.5 μL TIANGEN PCR Master Mix, based on the following reaction conditions: 94°C for 5 minutes, followed by 35 cycles of 94°C for 1 minute, 60–67°C for 1 minute and 72°C for 1 minute. A final extension of 10 minutes at 72°C completed the reaction. PCR products were sent to Sangon (Shanghai, China) for DNA sequencing.

2.4. Statistical analysis

All data were analyzed by using SPSS 17.0. As the number of homozygous and heterozygous mutations in females was small, the G6P/6PGD was compared between patients in the 6 kinds of common G6PD hemizygous mutation in Fujian. Wicoxon rank sum test was used for the comparison between patients as the above results presented non-normal distribution. Chi-square test was used for the comparison of the most common 3 G6PD mutations between eastern Fujian and other provinces in China. $P < .05$ was considered to be statistically significant.

3. Results

3.1. Determination of G6PD enzymatic activity

G6P/6PGD enzyme activity ratio was < 0.95 in 748 males and 104 females. A range of 0.95 to 1.05 was detected in 13 males and 39 females. According to the manufacturer's instructions, it was considered as positive in the presence of G6P/6PGD enzyme activity ratio of < 0.95, and suspicious positivity in a range of 0.95 to 1.05. A value of 1.1 was detected in normal individuals. Results of G6PD enzymatic activity determination were consistent with the results of neonatal screening for G6PD activity in males. G6P/6PGD enzyme activity ratio was greater than 1.1 in the 904 normal individuals (Table 2).

3.2. Sequencing results

PCR and gene sequencing results showed that there was no mutation in the exon and intron of G6PD in the 904 normal individuals. Results of MMCA showed that of the 904 patients with G6PD enzyme abnormality, 11 G6PD mutations including *c.1376G > T*, *c.1388G > A*, *c.95A > G*, *c.1024C > T*, *c.392G > T*, *c.871G > A*, *c.1360C > T*, *c.487G > A*, *c.517T > C*, *c.383T > C*, and *c.1004C > A* were detected in the 883 patients. Hemizygous mutation was identified in 742 cases, and homozygous mutation was identified in 20 cases. Single heterozygous mutation was detected in 113 cases, and 8 showed compound heterozygous mutation. No *G6PD* mutation was identified in the

Table 2

Relation between G6PD screening value and G6PD activity value in 1808 children.

N	G6PD screening value, U/g Hb	G6P/6PGD enzyme activity ratio
904 (normal controls)	>2.6	>1.1
539	0–1.0	0.01–0.85
267	1.0–2.1	0.04–1.05
98	2.1–2.6	0.45–1.05

G6PD = glucose-6-phosphate dehydrogenase.

rest 21 patients with G6PD enzyme abnormality. Then whole blood DNA was extracted from the 21 cases, and 6 mutations including *c.202G > A*, *c.697G > C*, *c.406C > T*, *c.493A > G*, *1365–13T >* C and *c.1311C > T* were detected by PCR amplification and gene sequencing. Among these patients, there were 19 hemizygous mutations and 2 homozygous mutations (Table 3). The proportion of various mutations was counted as follows: hemizygous mutation in males was defined 1, homozygous mutation in females was defined as 2, and compound heterozygous mutation was defined as 1 (Table 4).

3.3. Results of Wilcoxon rank sum test

The extent of reduced enzyme activity caused by hemizygous mutation in the 6 *G6PD* mutations in Fujian Chinese Han children was compared. Statistical difference was noticed in the comparison between *c.1376G > T* and *c.1388G > A*. No statistical differences were observed in the other mutation types. The 3 most common mutation types in eastern Fujian were compared with the *G6PD* mutation in other provinces in China.[5–15] The numbers of mutation was 933 although the positive individuals were 904 (Table 5). Chi-square test and Calibration test were used for the comparison of the 3 most common *G6PD* mutations between Fujian and other provinces in China. Results were shown in Table 6.

3.4. Sequencing map

The mutation sites of G6PD were listed in Figures 1 and 2. The *c. DNA202G > A* was noticed in exon 4, and *c.DNA406C > T* was notice in exon 5 (Fig. 1A and B). Additionally, *c.DNA697G > C* was noticed in exon 7 and *c.DNA1311C > T* was observed in exon 11 (Fig. 1C and D). Furthermore, *1365-13T > C* site was identified in intron 11 (Fig. 2A) and *493A > G* was noticed in intron 6 (Fig. 2B).

4. Discussion

From January 2014 to December 2016, a total of 200,260 G6PD samples were screened. Among these samples, 1813 (0.91%) samples (male: 1,489; female: 324) with decreased activity of G6PD were identified. G6PD deficiency, the most common enzyme defect, has a high prevalence in China especially in boys. The prevalence of G6PD deficiency in northern China was comparatively lower than that of southern China. The south regions of the Yangtze River Valley including Guangdong, Guangxi, Guizhou, Hainan, Yunnan, and Taiwan had a high risk of G6PD deficiency.[6–11] Incidence of G6PD deficiency in the north of Chongqing and Wuhan showed gradual decline and was rarely reported in Shandong Province, which was similar with that of the studies in China mainland [16] and abroad.[17–20] There are indeed limitations in this study. We cannot confirm the association between the malaria and G6PD deficiency. In males, *G6PD* gene was in a nonmethylation status, while it showed

Table 3

***G6PD* mutation types and range of enzyme specific activity value.**

Mutation type	Hemizygous mutation	Specific activity value	Homozygous mutation	Specific activity value	Heterozygous mutation	Specific activity value
*c.1376G>*T	313	0.01–1.05	9	0.01–0.48	56	0.39–1.05
*c.1388G>*A	168	0.01–1.02	5	0.07–0.69	17	0.56–1.03
*c.95A>*G	71	0.01–0.99	3	0.03–0.66	9	0.40–1.01
*c.1024C>*T	67	0.04–1.01	1	0.25	9	0.55–0.96
*c.392G>*T	41	0.01–1.01	2	0.14–0.77	6	0.03–0.97
*c.871G>*A	38	0.06–0.76	0	–	6	0.45–1.01
*c.1360C>*T	20	0.01–0.54	0	–	5	0.11–1.03
*c.487G>*A	15	0.05–0.93	0	–	3	0.11–0.91
*c.517T>*C	6	0.10–0.85	0	–	0	–
*c.406C>*T	3	0.21–0.25	0	–	0	–
*c.493A>*G	2	0.41–0.66	0	–	1	0.77
*c.202G>*A	2	0.03–0.38	1	0.28	0	–
*c.697G>*C	2	0.22–0.64	0	–	0	–
*c.1004C>*A	1	0.5	0	–	0	–
*c.383T>*C	0	–	0	–	1	0.65
1365–13T>C/c.1311C>T	12	0.03–0.54	1	0.47	0	–
*c.95A>*G/*c.1376G>*T	0	–	0	–	2	0.17–0.96
*c.1388G>*A/*c.392G>*T	0	–	0	–	2	0.38–0.78
*c.871G>*A/*c.1376G>*T	0	–	0	–	1	0.83
*c.1360C>*T/*c.392G>*T	0	–	0	–	1	1.05
*c.392G>*T/*c.1376G>*T	0	–	0	–	1	0.85
*c.95A>*G/*c.1388G>*A	0	–	0	–	1	0.35

G6PD = glucose-6-phosphate dehydrogenase.

Table 4

Proportion of various *G6PD* mutation types.

Mutation type	Proportion
c.1376G>T	41.9%
c.1388G>A	21.2%
c.95A>G	9.5%
c.1024C>T	8.4%
c.392G>T	5.9%
c.871G>A	4.8%
c.1360C>T	2.8%
c.487G>A	1.9%
c.517T>C	0.6%
c.406C>T	0.3%
c.493A>G	0.3%
c.202G>A	0.4%
c.697G>C	0.2%
c.1004C>A	0.1%
c.383T>C	0.1%
1365–13T>*C*/*c.1311C*>T	1.5%

G6PD = glucose-6-phosphate dehydrogenase.

partial methylation in females. The *G6PD* gene in males was in non-methylation status, while it showed partial methylation in females.[21] Males have only one X chromosome, while females have 2 X chromosomes. Random inactivation phenomenon namely "Lyon hypothesis" existed in the females. To ensure the equality of transcription levels between the sexes in mammalian, females will random inactivate one X chromosome, and maintain and transfer during cell proliferation and differentiation. Maintenance of X chromosome inactivation mainly depends on the methylation of the promoter. Range of G6PD enzyme activity fluctuates greatly in female heterozygote, which may be responsible for this.

For the comparison in the 6 most common *G6PD* mutations in eastern Fujian, statistical difference was observed between *c.1376G > T* and *c.1388G > A*. This may be related to the fact that *c.1376G > T* and *c.1388G > A* were located in exon 12 with a very close distance (only 12 bases), which resulted in the Arg in 459 and 463 sites were substituted by Leu and His, respectively. Arg of 459 and 463 sites are located in the regions containing more than 10 highly conserved amine acids. These 2 Arg are very close to the location of the first binding site of NADP (386–387) in space, which play an important role in the binding process between enzyme and NADP. Moreover, *c.1376G > T* is a conservative amino acid substitution (Arg-Leu) and it may affect the G6PD enzyme activity.[22] Additionally, *c.487G > A* and *c.493A > G* were located in exon 6, and their distance is only 6 bases. Gly in the 163 site was replaced by Ser, the Asp in the 165 site was replaced by aminosuccinic acid. The accuracy of the statistics may be hampered by the small case number with mutations. The mutations may cause different amino acid changes because of different location, which may lead to different changes of protein secondary structure and enzyme activity.

Table 5

The 3 most common *G6PD* mutations in China (n, %).

Area	G1376T	G1388A	A95G
Eastern Fujian (n = 933)	391 (41.9)	198 (21.2)	89 (9.5)
Chinese She Population[5] (n = 54)	38 (70.4)	10 (18.5)	6 (11.1)
Guangdong[6] (n = 168)	72 (42.8)	35 (20.8)	30 (17.9)
Guangxi[7] (n = 42)	13 (31)	13 (31)	
Chinese Yao Population[8] (n = 34)	14 (41.2)	9 (26.5)	5 (14.7)
Taiwan[9] (n = 94)	47 (50)	20 (21.3)	7 (7.4)
Guizhou[10] (n = 34)	9 (26.5)	8 (23.5)	10 (29.4)
Yunnan[11] (n = 28)	9 (32.1)	12 (42.9)	1 (3.6)
Chinese Li Population[12] (n = 32)	18 (56.2)	6 (18.8)	2 (9.4)
Chinese Dong Population[13] (n = 34)		4 (11.8)	
Chinese Miao Population[14] (n = 32)		1 (3.1)	7 (21.9)
Tianjin[15] (n = 10)	3 (30.0)	6 (60.0)	

G6PD = glucose-6-phosphate dehydrogenase.

For the comparison of the *G6PD* mutation between eastern Fujian and other areas, there were no significant differences in the sum of *c.1376G > T*, *c.1388G > A*, and *c.95A > G* between Chinese Han population in eastern Fujian (72.67%) and other provinces including Taiwan (78.7%), Guangdong (81.5%), Chinese Han population in Guangxi (62%), Chinese Yao population in Guangxi (82.4%), Yunnan (78.6%), and Guizhou (79.4%). In contrast, there were differences in the proportion of *c.1376G >* T, *c.1388G >* A, and *c.95A >* G in different provinces.

G6PD mutation types and proportion varied in different geographical locations. For example, *c.1376G > T* was the main mutation type in Taiwan, Guangdong, Chinese Yao population in Guangxi, Chinese She population in Fujian Province, Chinese Han population in eastern Fujian. No statistical differences were noticed in the constituent ratio of *c.1376G > T*, *c.1388G > A*, and *c.95A > G* between Chinese Han population in Fujian and other areas including nonindigenous people in Taiwan, Guangdong, Chinese Han population in Guangxi, and Chinese Yao population in Guangxi. There were significant differences in the *G6PD* mutations between Chinese Han population in Fujian and other areas including Guizhou, Yunnan, and Tianjin. *c.1388G > A* was the main mutation type in Guizhou, Yunnan, and Tianjin, *c.1376G > T* and *c.1388G > A* have the same proportion in these areas.[10,11,15] For the Dong population, *c.592C > T* was the most common mutant which accounted for more than 50% of the *G6PD* gene mutation,[13] and the mutation type in other ethnic groups were relatively rare, which indicated that every region or nation had its own characteristics.

Mutation in G6PD deficiency was almost single nucleotide substitution. In this study, 17 *G6PD* mutation types were detected in Chinese Han population in Fujian. These mutations include 15 single point mutation and 7 compound mutations. These were all single nucleotide substitutions, and no frameshift mutation was found.

Among the 17 mutation types, *c.1376G > T* and *c.1388G > A* showed the largest enzyme activity fluctuation range, which indicated that there existed difference between phenotype and genotype. The same *G6PD* mutation would lead to different enzyme activity loss in different individuals. The most frequent mutation associated with *c.1311C > T* was *T1365-13C* in intron 11. The frequency of *c.1311C > T* was significantly higher in cases with abnormal G6PD enzyme activity than that in normal individuals, which demonstrated the presence of genetic linkage disequilibrium.

In conclusion, this study has confirmed that G6PD *c.1376G > T* was the most common G6PD deficiency mutation type, followed by G6PD *c.1388G > A* and *c.95A > G* in Chinese Han children in eastern Fujian. In this study, we did not find any novel G6PD deficiency mutation type. Further studies are required to identify novel G6PD mutation types, and functional trials are required to confirm the novel mutations.

Table 6

Comparison of the 3 most common G6PD mutations between Fujian and other provinces in China.

Comparison	G1376T		G1388A		A95G	
	χ^2	*P*	χ^2	*P*	χ^2	*P*
Taiwan	1.288	.256	0.000	1.000	0.546	.460
Chinese She Population in Fujian	15.909	.000*	0.151	.698	0.065	.799
Guangdong	0.020	.886	0.000	1.000	2.749	.097
Chinese Han Population in Guangxi	2.610	.106	2.599	.107	10.419	.001*
Chinese Yao Population in Guangxi	0.021	.886	0.908	.341	1.200	.273
Guizhou	5.195	.023*	0.221	.639	11.721	.001*
Yunnan	2.145	.143	11.121	.001*	2.700	.100
Chinese Li Population in Hainan	3.922	.048*	0.125	.724	0.048	.827
Chinese Dong Population in Guizhou	53.165	.000*	2.940	.086	10.419	.001*
Chinese Miao Population in Xijiang	53.165	.000*	15.341	.000*	5.495	.019*
Tianjin	3.125	.077	31.559	.000*	10.419	.001*

G6PD = glucose-6-phosphate dehydrogenase.
* $P < .05$ was considered to be statistically significant.

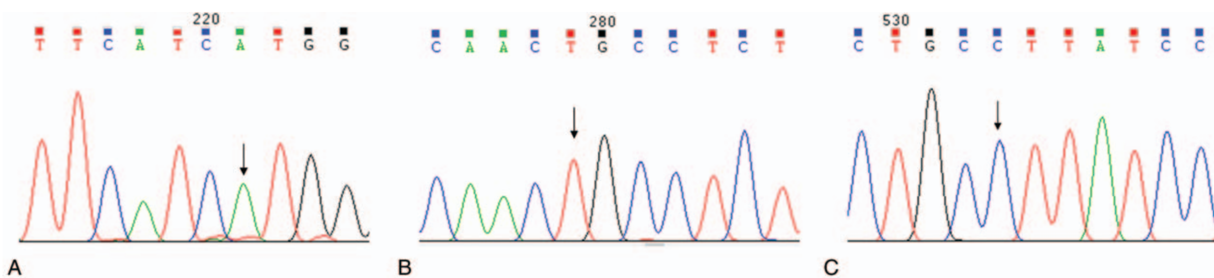

Figure 1. Mutation analysis of exon 4, 5, and 7. (A) The hemizygous and homozygous mutations in the *c.DNA202G>A* site in exon 4. (B) The hemizygous and homozygous mutation in the *c.DNA406C>T* site in exon 5. (C) The hemizygous and homozygous mutation in the *c.DNA697G>C* site in exon 7.

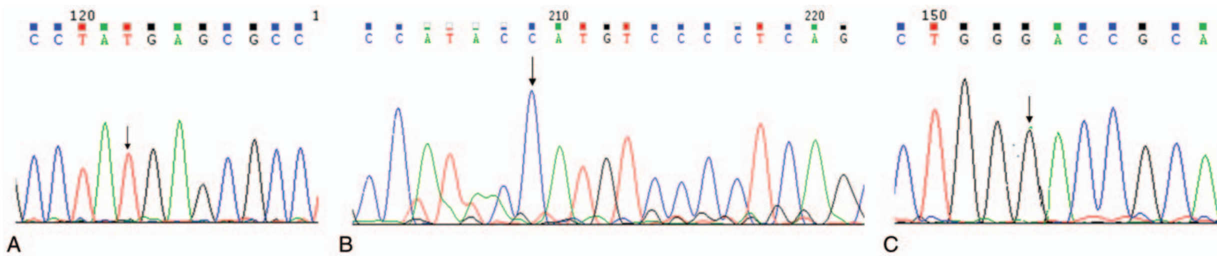

Figure 2. Mutation of extron 6, 11, and intron 11. (A) The hemizygous and homozygous samesense mutation in the *c.DNA1311C>T* site in extron11. (B) The hemizygous and homozygous mutation in the *1365-13T>C* site in intron (C) The hemizygous/homozygous mutation in the *c.DNA493A>G* site in extron 6.

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