Copy number variations in a Chinese series of patients with DiGeorge syndrome-related hypoparathyroidism

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Abstract

Purpose: Large genic copy number variations (CNVs) that are rare in the general population have been identified as pathogenic variations in many human diseases. Microdeletion of chromosome 22 leads to DiGeorge syndrome-1 (DGS-1), however, research on the influence of CNVs on the phenotype of DGS-1 related hypoparathyroidism (HP) is still lacking. To understand the CNV profiles in whole genome and their correlation with HP related phenotype in a series of DGS-1 related HP patients by CNV-sequencing.

Methods: CNVs were detected by low-depth whole genome sequencing. The clinical data were collected retrospectively. The HP related phenotype were compared between DGS-1 patients with and without CNV other than 22q11 deletion. Meanwhile, the incidence of CNVs and phenotype were also compared between patients with DGS-1 and idiopathic hypoparathyroidism (IHP) matched in their gender and age.

Results: A total of 34 DGS-1 patients were enrolled in this CNV analysis, of whom 4 were adult-onset. The pathogenic CNV in 22q11 was confirmed in 32 (94.1%) cases. Moreover, 15 (44.1%) patients carried 22 CNVs other than 22q11.2. There was no significant difference in phenotype between patients with and without CNVs (≥100kb) other than 22q11, as well as the incidence of CNVs between DGS-1 and IHP patients.

Conclusion: In our study, there seemed to be a relatively high percentage (44.1%) of patients who carried CNVs (≥100kb) other than 22q11.2, which may be related to the phenotype of DGS-1. Further analyses on larger DGS-1 related HP series, especially with normal controls from different races should be performed.

Background

Hypoparathyroidism (HP) is an uncommon disorder characterized by hypocalcemia, hyperphosphatemia caused by deficiency in production or secretion of parathyroid hormone (PTH)[1, 2]. The prevalence of HP is estimated 37 per 100000 person-years in the United States and 22 per 100000 person-years in Denmark. The incidence in Denmark is approximately 0.8 per 100000 person-years. According to different causes, HP can be divided into hereditary, acquired, and idiopathic HP (IHP)[1, 3]. Hereditary HP is one of the main causes of non-surgical HP, among which DiGeorge syndrome-1 (DGS-1) related HP is the most common type. Previous studies have shown that DGS-1 has a prevalence of approximately 1/2000-1/9700 in infants and children and accounts for a large proportion of patients with a genetic form of HP[4–7].

DGS-1 is one of the most common chromosomal microdeletion syndrome, affecting multiple systems, usually caused by a deletion in chromosome (Chr) 22q11.2[8]. The clinical manifestations of DGS-1 are highly heterogeneous. The typical manifestations include special facial features, congenital heart abnormalities (~ 60%-75%), repeated infections caused by immune deficiency, parathyroid hypoplasia, growth and development retardation, low learning ability, mental disorders, nephro-eye-bone malformations[5]. Targeted deletion studies in animals and case reports of mutations in humans have implicated that *TBX1* located in this region of microdeletion is the key gene responsible for DGS-1, and most mutations in *TBX1* are haploid deletion, a few point mutations or small deletions[9, 10].

Copy number variants (CNVs) refer to the deletion or duplication of DNA fragments with a length more than 1 kilobase (kb), which can affect the dose of genes expression and lead to the occurrence of diseases[11]. Large genic CNVs that are rare in the general population have been identified as pathogenic in a variety of human diseases and disorders. Mlynarski EE et al analyzed the relationship between rare CNVs and congenital heart defect (CHD), which is one of the main components of DGS-1. They found that an overabundance of CNVs affecting cardiac-related genes was detected in 22q11DS individuals with CHD, suggesting that CNVs outside the 22q11.2 region may contain genes that modify risk for CHD in some 22q11DS patients[12]. As another common manifestations of DGS1, studies about impacts of genetic factors including CNV on the clinical phenotype of DGS-1 related HP are still lacking.

The purpose of this study is to explore the CNV profiles in DGS-1 related HP patients and its correlation with HP related clinical phenotype.

Patients And Methods

Patients

This study included non-surgical HP (ns-HP) patients who visited the endocrinology department of Peking Union Medical College Hospital (PUMCH) from 1975 to 2021. All patients met the diagnostic criteria of HP, hypocalcemia with low or inappropriate normal PTH level. Among 183 HP patients who agreed to conduct gene screening, 36 cases of DGS-1 were detected through TBX1-multiplex ligation dependent probe amplification (TBX1-MLPA) combined with targeted-next generation sequencing (T-NGS), of which 34 cases were available for analysis of CNV. Among the patients who fulfilled the forementioned criteria, those with no rare variants or rare variants classified as benign or likely benign of candidate genes for HP were defined as IHP[9]. Gender and age matched IHP patients were included and analyzed for large CNV, and then the incidence of large CNV and clinical characteristics were compared between patients with DGS-1 and patients with IHP.

Clinical Data

Demographic characteristics, clinical manifestations and treatments of the patients were collected, including gender, age of onset, epileptic seizures, visual impairment, memory impairment, mental impairment, cataract, urinary calculus or calcification, intracranial calcification, Trousseau's sign, Chvostek's sign, and the treatment for HP (elemental calcium, active vitamin D and/or high dose of plain vitamin D). Extra-parathyroid manifestations included unusual facial features, slurred speech, repeated infections, arthralgia, renal malformation, hearing impairment, cardiac abnormalities, cleft palate, abnormalities of other endocrine glands, and family history, were also obtained by a chart review of medical records of clinically driven data collection at the clinic of metabolic bone diseases in our center. For patients with suggestive symptoms/signs or family history, it was suggested to the patients or their parents for further evaluation by specialists of other departments (such as slit-lamp by an ophthalmologist, hearing evaluation by otolaryngologist and cardiac evaluation by cardiologist).

Biochemical results at the first and last visit were recorded. Serum total calcium (TCa), ionized calcium (iCa), phosphorus (P), creatinine (Cr), alkaline phosphatase (ALP), potassium (K), magnesium (Mg), total 25-hydroxy-vitamin D (T-25OHD), creatine kinase (CK), total procollagen type 1 N-peptide (TP1NP), β-C-terminal telopeptide of type collagen (β-CTX) were measured with the automatic biochemical analyzer (Beckman Coulter, Indianapolis, IN, USA; AU5800). Twenty-four-hour urine calcium (24hUCa) and phosphate (24hUP) were measured with the automatic biochemical analyzer (Beckman Coulter; AU2700). Serum PTH was measured by chemiluminescence (Siemens ADVIA Centaur, Munich, Germany).

This study has been reviewed by the Research Ethics Committee of PUMCH (JS-3312). All subjects provided informed consent for genetic analysis signed by the patients or his/her parents.

DNA extraction

Genomic DNA was extracted from peripheral blood lymphocytes of subjects with HP using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany; LOT 169019868) according to the manufacturer's protocol.

TBX1-MLPA combined with T-NGS

To screen for large deletions of the *TBX1* gene in patients with HP, TBX1-MLPA was performed, using the SALSA® MLPA® Probemix P463-A2 MRKH kit (MRC-Holland, Amsterdam, Netherlands; Lot A2-0520) according to the manufacturer's instructions as previously described[13].

A gene panel (Agilent Technologies SureSelect™ Target Enrichment System, Santa Clara, CA, USA) was designed to capture all exons and 10-basepair (bp) flanking intron sequences of the 15 candidate genes before 2018[13], including *TBX1, GATA3, GCM2, FAM111A, SOX3, TBCE, CHD7, PTH, CASR, GNA11, AP2S1, TRPM6, CLDN16, CLDN19*, and *AIRE*, and 20 genes (5 new genes been added to the list, including *NEBL, SEMA3E, DHCR7, HADHA*, and *HADHB*) since 2019, which had been selected from previous studies and the Online Mendelian Inheritance in Man (OMIM) database. T-NGS was performed according to the manufacturer's instructions.

CNV detection with low-depth Whole Genome Sequencing (WGS)

CNVs (≥ 100kb) was detected by low-depth WGS. The genome DNA of 50 ng was fragmented and DNA libraries were constructed by end fulfilling, adapter ligation, and polymerase chain reaction (PCR) amplification. DNA libraries were subjected to massively parallel sequencing on the NextSeq 500 platform (Illumina, San Diego, CA) to generate approximately 5 million raw sequencing reads with genomic DNA sequences of 36-bp in length. Using the hg19 genomic sequence as reference, a total of 2.8–3.2 million reads were uniquely and precisely mapped using the Burrows-Wheeler algorithm. Mapped reads were allocated progressively to 20-kb bin sizes from the p to q arms of the 24 chromosomes. Counts in each bin were then compared between all test samples run in the same flow cell to evaluate copy number (CN) changes using previously described algorithms. Plots of log2 [mean CN ratio] per bin (Y-axis) versus each 20-kb bin (X-axis) were generated for each of the 24 chromosomes using a blue line to track the mean CNV. For reference, a contiguous blue line running at log2 [0] indicates a CN of 2.0 (disomy) and the dotted lines at position log2 [1.5] and log2 [0.5] indicate the theoretical position of the blue line for a CN of 3.0 (duplication) and a CN of 1.0 (deletion), respectively. For reporting CNV, we applied stringent CN ranges of 2.9–3.1 for a duplication and 0.9–1.1 for a deletion[14].

CNVs identified and mapped are queried and interpreted using publicly available databases, including Decipher, Database of Genomic Variants (DGV), 1000 Genomes and OMIM, such as size, gene content and CNV type (deletion or duplication), and each detected CNV was classified to one of five known categories: pathogenic (P), likely pathogenic (LP), variants of uncertain clinical significance (VUS), likely benign (LB), and benign (B), in accordance with the 2011 recommended guidelines of the International Standard Cytogenomic Array and American College of Medical Genetics and Genomics (ACMG)[15–17]. When necessary, classifications were assessed and revised according to the updated 2020 ACMG guidelines[18]. In cases where variable penetrance and expressivity of a certain CNV has been reported, the CNV was scored as a LP variant.

Statistical analysis

The Kolmogorov-Smirnov test was used to determine the distribution of continuous variables. Normally distributed variables were expressed as mean ± standard deviation (SD) and compared by a Student's t test. Non-normally distributed variables were expressed as median (interquartile range) and compared by a Mann-Whitney test. Categorical variables were compared by a Pearson $\chi 2$ test, Fisher's exact test, or continuity-adjusted $\chi 2$ test. A p value < 0.05 was considered statistically significant. Patients with and without extra-22q11 CNV (≥ 100kb) other than 22q11 deletion

were define as CNV positive and negative group, respectively, to compare the clinical data related to HP. The correlation between the size of second CNV burden and clinical phenotype was further analyzed in the group with CNV (≥ 100kb) outside 22q11. In patients with second CNV burden group, the size of CNV was calculated by the sum of base number of CNV other than 22q11. According to gender and age at the first visit to our center, DGS-1 and IHP patients were matched in a 1:1 ratio to DGS-1 patients based on the proportion score with a standard caliper width of 0.2. CNV incidence and clinical characteristics were compared between DGS-1 and IHP patients. All statistical analyses were performed with SPSS 26.0 software (IBM Corp, Armonk, NY, USA).

Results

General characteristics of 34 DGS-1 subjects

A total of 34 DGS-1 patients were enrolled in this study for CNV analysis. Through TBX1-MLPA, Final Ratio (FR) at multiple probe locations in the *TBX1* exon region was significantly decreased in 32 patients compared with normal controls, suggesting *TBX1* haploinsufficiency (as shown in Table 1). Pt.33 carried *TBX1* missense mutation (NM_080647, exon9: C.A1469G: P.Y490C), whose clinical manifestation had been reported before[9]. A frameshift deletion of *TBX1* (NM_080647, exon3: C.161_186del: P. A54Afs*105) was found in Pt.34 with an onset age of 50 years(as shown in Fig. 1). When he was 52 years old, he came to our hospital for " intermittent tetany for 2 years and aggravation with seizures for nearly half a year". In addition to HP, the patient had other DGS-1 related manifestations, including speech confusion and intellectual decline and memory loss. No special medical history, personal history or family history was reported. At the initial visit, the serum TCa was 1.27mmol/L, and the serum PTH was 1.07pmol/L (10.1pg/ml). The patient had intracranial calcification and cataract, while there was no abnormality in urinary system and cardiac ultrasound.

Table 1
The 22q11-CNV status in 34 DGS-1 patients

Patient ID	Gender	Pathogenicity	CNV Type	Chr	Start	End	cytoband	Size (kb)	LCRs
1	F	P	del	chr22	18880000	21460000	22q11.21	2580	A-D
2	F	P	del	chr22	18900000	21480000	22q11.21	2580	A-D
3	M	P	del	chr22	18880000	21460000	22q11.21	2580	A-D
4	F	P	del	chr22	18880000	21480000	22q11.21	2600	A-D
5	M	P	del	chr22	18880000	21040000	22q11.21	2160	A-B
6	M	P	del	chr22	18880000	21480000	22q11.21	2600	A-D
7	F	P	del	chr22	18880000	20320000	22q11.21	1440	A-B
8	F	P	del	chr22	18880000	21480000	22q11.21	2600	A-D
9	M	P	del	chr22	18940000	21480000	22q11.21	2540	A-D
10	M	P	del	chr22	18880000	21440000	22q11.21	2560	A-D
11	F	P	del	chr22	18920000	21480000	22q11.21	2560	A-D
12	M	P	del	chr22	18880000	20280000	22q11.21	1400	A-B
13	M	P	del	chr22	19060000	21480000	22q11.21	2420	A^{+}-D
14	M	P	del	chr22	18960000	21480000	22q11.21	2520	A-D
15	F	P	del	chr22	18600000	21480000	22q11.21	2880	A-D
16	M	P	del	chr22	18940000	21480000	22q11.21	2540	A-D
17	M	P	del	chr22	18880000	21480000	22q11.21	2600	A-D
18	M	P	del	chr22	18880000	21480000	22q11.21	2600	A-D
19	M	P	del	chr22	18880000	21480000	22q11.21	2600	A-D
20	F	P	del	chr22	18960000	21480000	22q11.21	2520	A-D
21	M	P	del	chr22	18960000	21460000	22q11.21	2500	A-D
22	M	P	del	chr22	18880000	21480000	22q11.21	2600	A-D
23	F	P	del	chr22	18880000	21040000	22q11.21	2160	A-B
24	M	P	del	chr22	18880000	21480000	22q11.21	2600	A-D
25	F	P	del	chr22	18880000	21480000	22q11.21	2600	A-D
26	M	P	del	chr22	18880000	21480000	22q11.21	2600	A-D
27	F	P	del	chr22	18880000	21480000	22q11.21	2600	A-D
28	M	P	del	chr22	18880000	21460000	22q11.21	2580	A-D
29	F	P	del	chr22	18880000	21480000	22q11.21	2600	A-D
30	M	P	del	chr22	18900000	21460000	22q11.21	2560	A-D
31	M	P	del	chr22	18900000	21480000	22q11.21	2580	A-D
32	F	P	del	chr22	18960000	21480000	22q11.21	2520	A-D
33#	M	-	-	-	-	-	-	-	-
34#	M	-	-	-	-	-	-	-	-

Abbreviation: CNV: copy number variant; DGS-1: DiGeorge syndrome type-1; Chr: chromosome; kb: kilobase; LCRs: low copy repeats; F: female; M: male; P: pathogenic; del: deletion.

33# *TBX1* missense mutation (NM_080647, exon9: C.A1469G: P.Y490C)

34[#] *TBX1* frameshift deletion (NM_080647, exon3: C.161_186del: P. A54fs)

Of these 34 DGS-1 patients, the onset age was 7.0[0.7,13.3] years, and the course of disease was 3.2[0.0,12.2] years. Among them, 4 patients developed symptoms in adulthood (age ≥ 18 years), as 34 (female, Pt.7), 28 (female, Pt.20), 18 (male, Pt.33), and 50 (male, Pt.34) years, respectively. Among them, Pt.33 and Pt.34 are patients with *TBX1* missense mutation and frameshift mutation mentioned above respectively. Pt.7 had a three-year history of intermittent tetany, and no family history of HP. At the initial visit, serum TCa was 1.6 mmol/L and serum PTH was 1.09 pmol/L (10.3 pg/ml). Pt.20 also suffered from intermittent tetany with hypocalcemia (plasma iCa is about 0.8 mmol/L). She also had other DGS-1 related manifestations, including cardiac abnormalities (aortic and tricuspid regurgitation), dysphonia, facial dysplasia, and susceptibility to infection. At the initial visit to our hospital, her serum TCa was 2.19 mmol/L and serum PTH was 1.35 pmol/L (12.7pg/ml) on the treatment of calcitriol of 0.25μg tid and calcium orally. In addition, the patient had intracranial calcification, but no abnormality was found in the urinary system and fundus.

When the 34 DGS-1 patients first came to our hospital, 25 (73.5%) patients had received treatment for hypocalcemia, while only 16 (47.1%) patients received regular treatment (calcium supplements combined with active vitamin D and/or high dose of plain vitamin D ≥ 3 months). At baseline, the serum PTH was 1.0[0.3,1.5] pmol/L (9.6[3.0,13.7]pg/ml), and serum TCa was 1.7 ± 0.3 mmol/L. Only 2 patients had a family history of HP. These patients had been followed at our center for 6.5[2.1,10.1] years. At the most recent visit, the prevalence of cataract was 35.3% (6/17), 14.8% (4/27) of patients had nephrocalcinosis/urolithiasis, and 90.6% (29/32) of patients had intracranial calcification. During follow-up in our center, all patients received calcium supplements of 1.2[1.0,1.2] g/d. Among them, 2 patients received high dose of plain vitamin D only, 18 received active vitamin D (calcitriol in 15 cases, and alfacalcidol in 3 cases) only, and 13 received high dose of plain vitamin D combined with active vitamin D (calcitriol in 5 cases, and alfacalcidol in 8 cases).

For the extra-parathyroid manifestations, 27 of 34 patients (79.4%) had intellectual problems. Dysmorphic facies (26 cases, 76.5%) were the most common anomalies in these patients, followed by kidney anomalies (7 cases, 20.6%), and congenital heart disease (6 cases, 17.6%). Fifteen cases (44.1%) had slurred speech. Ten patients (29.4%) had recurrent upper respiratory-tract or lung infections. Four patients (11.8%) had hearing loss.

CNVs (≥100kb) tested by low-depth WGS

The pathogenic CNV in 22q11 was confirmed in 32 (94.1%) cases of DGS-1 by low-depth WGS. Of them, 27 (84.4%) had low copy repeats (LCRs) A-D, 4 (12.5%) had LCRs A-B, and 1 (3.1%) had atypical absence of A^{+}-D (Table 1).

Moreover, 15 (44.1%) patients carried 22 CNVs outside 22q11.2, including 19 VUS (86.4%) and 3 benign variants (13.6%). The loci of 16 CNVs (72.7%) overlapped with known genes, which did not include known HP causative genes, and only 6 (27.3%) loci were located in non-coding regions. The 22 variants included 16 (72.7%) duplications with a median size of 270.0 kb and 6 (27.3%) deletions with a median size of 300.0 kb, respectively. As shown in Table 2, the median length of CNVs found in this study is 280.0 kb (ranged from 120.0 to 1840.0 kb), distributed on Chr1, 2, 3, 7, 8, 9, 14, 15, 16, 17, 22 and X. Among them, the most frequently involved chromosomes were Chr8 (4/21). The largest CNV was a duplication spanning 1840.0 kb in Chr 1q42.3.

Table 2
The features of CNVs other than 22q11 deletion found in DGS-1 patients

Patient ID	Gender	pathogenicity	CNV Type	Chr	Start	End	cytoband	Size (kb)	Population frequency (%) #	refseq gene
1	F	VUS	dup	chr7	118700000	118840000	7q31.31q31.31	140	0.0092–0.0096	-
2	F	VUS	del	chr2	209380000	209660000	2q34q34	280	-	LOC101927960
2	F	VUS	dup	chr8	79700000	80520000	8q21.12q21.13	820	-	IL7
2	F	VUS	dup	chrX	56260000	56460000	Xp11.21p11.21	200	-	KLF8
3	F	VUS	dup	chr1	235920000	237760000	1q42.3q43	1840	-	MIR1537, ACTN2, HEATR1, RYR2, EDARADD, GPR137B, MTR, MIR4428, LYST, LGALS8, LGALS8-AS1, MT1HL1, ERO1B, NID1
3	F	VUS	dup	chrX	94280000	94400000	Xq21.33q21.33	120	0.0092	MIR548M
4	M	VUS	dup	chr8	18540000	19840000	8p22p21.3	1300	-	LPL, INTS10, CSGALNACT1, SH2D4A, LOC100128993, PSD3
5	F	VUS	dup	chr8	160000	540000	8p23.3p23.3	380	0.0034–0.0363	FAM87A, RPL23AP53, TDRP, ZNF596, FBXO25
6	M	B	dup	chr14	106060000	106380000	14q32.33q32.33	320	-	MIR8071-2, MIR4539, MIR4538, MIR8071-1, ELK2AP, MIR4507, MIR4537
7	M	VUS	dup	chr7	153400000	153660000	7q36.2q36.2	260	0.0494–0.5525	DPP6
8	M	VUS	dup	chr17	200000	620000	17p13.3p13.3	420	-	VPS53, RFL NB, LOC105371430, C17orf97, RPH3AL
8	M	B	dup	chr15	32020000	32440000	15q13.3q13.3	420	-	OTUD7A, CHRNA7
9	M	VUS	dup	chr8	143560000	143760000	8q24.3q24.3	200	-	LOC101928087, ADGRB1, ARC, JRK, PSCA
10	F	VUS	dup	chr22	22320000	22600000	22q11.22	280	3.6364	VPREB1, TOP3B, PRAMENP
10	F	B	del	chr14	22540000	22920000	14q11.2	380	-	-
11	M	VUS	del	chr4	66580000	66860000	4q13.1q13.2	280	-	-
12	M	VUS	del	chrX	143280000	143580000	Xq27.3q27.3	300	-	-
12	M	VUS	dup	chr9	32100000	32360000	9p21.1p21.1	260	-	-

Abbreviation: CNVs: copy number variants; DGS-1: DiGeorge syndrome type-1; Chr: chromosome; kb: kilobase; RefSeq: reference sequence; F: female; M: male; VUS: variants of uncertain clinical significance; B: benign; dup: duplication: del: deletion.

#: The rate of natural population carrying fragments in this region queried by DGV and gnomAD databases.

Patient ID	Gender	pathogenicity	CNV Type	Chr	Start	End	cytoband	Size (kb)	Population frequency (%) #	refseq gene
12	M	VUS	dup	chr17	80560000	80800000	17q25.3q25.3	240	-	FOXK2, TBCD, RAB40B, FN3K, WDR45B, FN3KRP, MIR4525, LOC101929552, ZNF750
13	F	VUS	dup	chr3	63000000	63240000	3p14.2p14.2	240	0.0034–2.1053	LINC00698
14	M	VUS	del	chr3	162360000	162660000	3q26.1q26.1	300	0.0034–1.6129	-
15	M	VUS	del	chr16	76600000	76720000	16q23.1q23.1	120	0.0138–0.0494	LINC02125

Abbreviation: CNVs: copy number variants; DGS-1: DiGeorge syndrome type-1; Chr: chromosome; kb: kilobase; RefSeq: reference sequence; F: female; M: male; VUS: variants of uncertain clinical significance; B: benign; dup: duplication: del: deletion.

#: The rate of natural population carrying fragments in this region queried by DGV and gnomAD databases.

Comparison of DGS-1 patients with and without CNVs (≥100kb) other than 22q11 deletion

The clinical data were compared between extra-22q11 CNV positive and negative group (Tables 3 and 4). There was no significant difference in age of onset between the two groups (7.0[3.0,12.0] years vs 2.0[0.1,14.0] years, P = 0.690). At the first visit to our center, the 24hUCa in adults in the extra-22q11 CNV positive group was lower than those without (1.5 ± 0.6 mmol vs 4.7 ± 2.9 mmol, P = 0.043). There were no significant differences in other biochemical indexes, HP related symptoms or signs, as well as chronic complications of HP between the two groups. In addition, there was no difference in the proportion of extra-parathyroid manifestations between the two groups (Table 3). At the most recent visit, patients in CNV positive group had lower serum Mg compared with negative group (0.7 ± 0.03 mmol/L vs 0.8 ± 0.06 mmol/L, P = 0.016) (Table 4). For patients treated with calcitriol alone (7 cases in CNV positive group; 8 cases in CNV negative group), no differences in the doses were observed between the two groups when obtaining the same target level of serum Ca in both groups (0.5 ± 0.4 μg/d vs. 0.7 ± 0.4 μg/d, P = 0.506).

Table 3
Comparison of clinical characteristics between DGS-1 patients with and without CNVs (≥ 100kb) other than 22q11 deletion at first visit at our center

	Total (n = 34)	With CNVs other than 22q11 (n = 15)	Without CNVs other than 22q11 (n = 19)	P value
Female, n (%)	13(38.2)	6(40.0)	7(36.8)	0.851
Onset age (years)	7.0[0.7,13.3]	7.0[3.0,12.0]	2.0[0.1,14.0]	0.690
Serum PTH (pmol/L)	1.0[0.3,1.5]	0.7[0.3,1.5]	1.0[0.3,1.3]	0.861
Serum TCa (mmol/L)	1.7 ± 0.3	1.6 ± 0.3	1.7 ± 0.3	0.720
Plasma iCa (mmol/L), n	0.9 ± 0.1,7	0.8 ± 0.2,3	0.9 ± 0.1,4	0.427
Serum P (mmol/L)	2.3 ± 0.7	2.4 ± 0.7	2.3 ± 0.8	0.928
Serum ALP (U/L)	152.0[73.0,247.0]	130.5[73.8,264.3]	173.0[73.0,237.0]	0.677
Serum Cr (μmol/L)	63.0 ± 20.5	69.6 ± 25.5	56.8 ± 12.4	0.095
Serum Mg (mmol/L)	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.376
24hUCa[a]				
Adults (mmol), n	2.9 ± 2.5,9	1.5 ± 0.6,5	4.7 ± 2.9,4	0.043
Children(mmol/kg), n	0.041 ± 0.027,11	0.042 ± 0.031,5	0.039 ± 0.027,6	0.860
24hUP (mmol)	14.5 ± 7.1	13.5 ± 6.8	15.4 ± 7.6	0.602
Epileptic seizure, n/N (%)	25/34(73.5)	11/15(73.3)	14/19(73.7)	0.982
Visual impairment, n/N (%)	6/25(24.0)	2/13(15.4)	4/12(33.3)	0.378
Memory impairment, n/N (%)	9/17(52.9)	6/10(60.0)	3/7(42.9)	0.637
Trousseau's sign, n/N (%)	19/34(55.9)	11/15(73.3)	8/19(42.1)	0.069
Chvostek's sign, n/N (%)	15/32(46.9)	7/14(50.0)	8/18(44.4)	0.755
Cataract, n/N (%)	6/19(31.6)	3/8(37.5)	3/11(27.3)	1.000
Nephrocalcinosis/Urolithiasis, n/N (%)	2/30(6.7)	1/15(6.7)	1/15(6.7)	1.000
Intracranial calcification, n/N (%)	29/33(87.9)	13/15(86.7)	16/18(88.9)	1.000
Proportion of patients receiving regular HP treatment at first visit, n/N (%)	16/34(47.1)	8/15(53.3)	8/19(42.1)	0.760
Extra-parathyroid manifestations				
Mental impairment, n/N (%)	27/31(87.1)	12/14(85.7)	15/17(88.2)	1.000
Dysmorphic facies, n/N (%)	26/34(76.5)	13/15(86.7)	13/19(68.4)	0.257
Kidney anomalies, n/N (%)	7/26(26.9)	4/14(28.6)	3/12(25.0)	1.000
Congenital heart disease, n/N (%)	6/15(40.0)	2/7(28.6)	4/8(50.0)	0.608

Note: Data represent the mean (SD), median [P25, P75].

P value for comparison between with and without extra-22q11 CNVs (≥ 100kb).

Adults refer to age ≥ 18 years, children refer to the age 18 years.

[a]Urinary calcium is expressed by mmol/24h in adults, and mmol/kg/24h in children.

Normal reference ranges for indexes: PTH: 1.6–6.9 pmol/L; Serum Ca: 2.13–2.70 mmol/L; Plasma iCa: 1.13–1.23 mmol/L; Serum P: <11 years: 0.97–1.87 mmol/L, 11–18 years: 0.81–1.53 mmol/L, > 18 years: 0.81–1.45 mmol/L; Serum ALP: <15 years: 42–390 U/L, 16–18 years: 52–171 U/L, > 18 years: 35–100 U/L; Serum Cr: 59–104 μmol/L; Serum Mg: 0.70–1.10 mmol/L.

The normal reference range of 24hUCa was < 8.75 mmol (350mg) /24h for adults and < 0.1 mmol (4mg) /kg/24h for children.

Abbreviation: CNV: copy number variation; PTH: parathyroid hormone; TCa: total serum calcium; iCa: ionized calcium; P: serum phosphorus; ALP: alkaline phosphatase; Cr: creatinine; Mg: magnesium; 24hUCa: twenty-four-hour urine calcium; 24hUP: 24-hour urine phosphate; kg: kilogram; HP: hypoparathyroidism; n: sample size for test examinations; N, sample size.

	Total (n = 34)	With CNVs other than 22q11 (n = 15)	Without CNVs other than 22q11 (n = 19)	P value
Slurred speech, n/N (%)	15/34(44.1)	7/15(46.7)	8/19(42.1)	0.432
Recurrent infections, n/N (%)	10/34(29.4)	3/15(20.0)	7/19(36.8)	0.332
Hearing loss, n/N (%)	4/16(25.0)	1/7(14.3)	3/9(33.3)	0.585
Note: Data represent the mean (SD), median [P25, P75].				
P value for comparison between with and without extra-22q11 CNVs (≥ 100kb).				
Adults refer to age ≥ 18 years, children refer to the age 18 years.				
[a]Urinary calcium is expressed by mmol/24h in adults, and mmol/kg/24h in children.				
Normal reference ranges for indexes: PTH: 1.6–6.9 pmol/L; Serum Ca: 2.13–2.70 mmol/L; Plasma iCa: 1.13–1.23 mmol/L; Serum P: <11 years: 0.97–1.87 mmol/L, 11–18 years: 0.81–1.53 mmol/L, > 18 years: 0.81–1.45 mmol/L; Serum ALP: <15 years: 42–390 U/L, 16–18 years: 52–171 U/L, > 18 years: 35–100 U/L; Serum Cr: 59–104 μmol/L; Serum Mg: 0.70–1.10 mmol/L.				
The normal reference range of 24hUCa was < 8.75 mmol (350mg) /24h for adults and < 0.1 mmol (4mg) /kg/24h for children.				
Abbreviation: CNV: copy number variation; PTH: parathyroid hormone; TCa: total serum calcium; iCa: ionized calcium; P: serum phosphorus; ALP: alkaline phosphatase; Cr: creatinine; Mg: magnesium; 24hUCa: twenty-four-hour urine calcium; 24hUP: 24-hour urine phosphate; kg: kilogram; HP: hypoparathyroidism; n: sample size for test examinations; N, sample size.				

Table 4
Comparison of clinical characteristics between DGS-1 patients with and without CNVs (≥ 100kb) other than 22q11 deletion at the last visit of follow-up

	Total (n = 34)	With CNVs other than 22q11 (n = 15)	Without CNVs other than 22q11 (n = 19)	P value
Serum TCa (mmol/L)	2.0 ± 0.2	2.0 ± 0.3	2.0 ± 0.2	0.732
Serum P (mmol/L)	1.7 ± 0.4	1.8 ± 0.4	1.7 ± 0.3	0.792
Serum ALP (U/L)	86.0[69.0,118.8]	89.0[83.5,135.0]	98.5[72.5,121.5]	0.862
Serum Cr (μmol/L)	70.0[65.0,84.8]	70.0[69.5,71.0]	72.5[65.0,84.5]	0.608
Serum K (mmol/L)	4.1 ± 0.3	4.1 ± 0.4	4.2 ± 0.2	0.831
Serum Mg (mmol/L)	0.8 ± 0.1	0.7 ± 0.03	0.8 ± 0.06	0.016
Serum T-25OHD (ng/ml)	38.8[27.4,79.0]	66.8[53.5,118.4]	31.8[21.7,39.3]	0.114
TP1NP (ng/ml)	151.6 ± 187.1	190.2 ± 269.2	112.9 ± 103.0	0.667
Serum β-CTX (ng/ml)	0.5[0.1,0.7]	0.5[0.3,1.6]	0.4[0.2,0.7]	0.806
24hUCa[a]				
Adults (mmol), n	4.9 ± 3.3,20	4.8 ± 2.5,10	5.0 ± 4.1,10	0.875
Children(mmol/kg), n	0.055 ± 0.021,7	0.052 ± 0.004,2	0.057 ± 0.025,5	0.790
24hUP (mmol)	19.0 ± 7.8	16.7 ± 4.7	22.7 ± 8.3	0.060
Epileptic seizure, n/N (%)	2/33(6.1)	1/14(7.1)	1/19(5.3)	1.000
Visual impairment, n/N (%)	10/25(40.0)	5/11(45.5)	5/14(35.7)	0.934
Memory impairment, n/N (%)	12/22(54.5)	7/12(58.3)	5/10(50.0)	1.000
Mental impairment, n/N (%)	28/30(93.3)	13/13(100.0)	15/17(88.2)	0.492
Hearing loss, n/N (%)	7/13(53.8)	2/4(50.0)	5/9(55.6)	1.000
Trousseau's sign, n/N (%)	6/30(20.0)	1/12(8.3)	5/18(27.8)	0.358
Chvostek's sign, n/N (%)	2/33(6.1)	1/14(7.1)	1/19(5.3)	1.000
Cataract, n/N (%)	6/17(35.3)	3/7(42.9)	3/10(30.0)	0.644
Nephrocalcinosis/Urolithiasis, n/N (%)	4/27(14.8)	1/121(8.3)	3/15(20.0)	0.605
Intracranial calcification, n/N (%)	29/32(90.6)	13/15(86.7)	16/17(94.1)	0.589
Note: Data represent the mean (SD), median [P25, P75].				
P value for comparison between with and without extra-22q11 CNVs (≥ 100kb).				
Adults refer to age ≥ 18 years, children refer to the age 18 years.				
[a]Urinary calcium is expressed by mmol/24h in adults, and mmol/kg/24h in children.				
Normal reference ranges for indexes: Serum Ca: 2.13–2.70 mmol/L; Serum P: <11 years: 0.97–1.87 mmol/L, 11–18 years: 0.81–1.53 mmol/L, > 18 years: 0.81–1.45 mmol/L; Serum ALP: <15 years: 42–390 U/L, 16–18 years: 52–171 U/L, > 18 years: 35–100 U/L; Serum Cr: 59–104 μmol/L; Serum K: 3.5–5.5 mmol/L; Serum Mg: 0.70–1.10 mmol/L; Serum T-25OHD: 8–50 ng/ml; Serum TP1NP: 15.1–58.6 ng/ml; Serum β-CTX: 0.26–0.51 ng/ml.				
The normal reference range of 24hUCa was < 8.75 mmol (350mg) /24h for adults and < 0.1 mmol (4mg) /kg/24h for children.				
Abbreviation: CNV: copy number variation; TCa: total serum calcium; P: serum phosphorus; ALP: alkaline phosphatase; Cr: creatinine; K: potassium; Mg: magnesium; T-25OHD: total 25-hydroxy-vitamin D; TP1NP: total procollagen type 1 N-peptide; β-CTX: β-C-terminal telopeptide of type collagen; 24hUCa: twenty-four-hour urine calcium; 24hUP: twenty-four-hour urine phosphate; kg: kilogram; n: sample size for test examinations; N, sample size.				

Further analysis showed that there is on relationship between the size of CNV other than 22q11 deletion and clinical characteristics in CNV positive group (data not shown).

Comparison of CNV incidence and clinical characteristics between DGS-1 and IHP patients

As shown in Table 5 and Table 6, there was no significant difference in the incidence of CNVs between the two groups except 22q11 (15/34 vs 16/34, P = 0.808) (Table 5). The onset age of DGS-1 patients was younger than that of IHP group (7.0[0.7,13.3] years vs 12.5[8.0,15.0] years, P = 0.005). At the first visit to our center, serum PTH, plasma iCa and serum ALP of DGS-1 patients were higher than those of IHP patients (1.0[0.3,1.5] pmol/L vs 0.3[0.3,0.7] pmol/L, P = 0.011; 0.9 ± 1.0 mmol/L vs 0.7 ± 0.2 mmol/L, P = 0.023; 164.6 ± 98.5 U/L vs 100.8 ± 63.4 U/L, P = 0.013, respectively). In addition, the incidence of mental impairment in DGS-1 group was higher than that in IHP group (27/31 (87.1) vs 4/14 (28.6), P = 0.000). No significant difference was found in other biochemical indicators, HP related symptoms or signs, as well as chronic complications of HP between the two groups (Table 5). At the most recent visit, the serum ALP of DGS-1 group was still higher than that of IHP group (104.6 ± 56.8 U/L vs 74.8 ± 32.2 U/L, P = 0.005). In addition, the incidence of mental impairment in DGS-1 group was still higher than that in IHP group (28/30 (93.3) vs 5/12 (41.7), P = 0.001) (Table 6).

Table 5
Comparison of CNVs incidence and clinical characteristics between DGS-1 and IHP patients at first visit at our center

	DGS-1 patients (n = 34)	IHP patients (n = 34)	P value
CNV incidence			
22q11 CNV, n/N (%)	32/34(94.1)	0/34(0.0)	0.000
22q11 CNV size (kb)	2520.0[2580.0,2600.0]	-	-
Extra-22q11 CNV, n/N (%)	15/34(44.1)	16/34(47.1)	0.808
Extra-22q11 CNV size (kb)	240.0[320.0,840.0]	320.0[165.0,435.0]	0.890
Clinical characteristics			
Onset age (years)	7.0[0.7,13.3]	12.5[8.0,15.0]	0.005
Serum PTH (pmol/L)	1.0[0.3,1.5]	0.3[0.3,0.7]	0.011
Serum TCa (mmol/L)	1.7 ± 0.3	1.5 ± 0.2	0.116
Plasma iCa (mmol/L), n	0.9 ± 0.1,7	0.7 ± 0.2,9	0.023
Serum P (mmol/L)	2.3 ± 0.7	2.3 ± 0.6	0.970
Serum ALP (U/L)	164.6 ± 98.5	100.8 ± 63.4	0.013
Serum Cr (μmol/L)	63.0 ± 20.5	73.8 ± 45.8	0.679
Serum K (mmol/L)	3.9 ± 0.5	3.9 ± 0.5	0.827
Serum Mg (mmol/L)	0.7 ± 0.1	0.7 ± 0.1	0.301
Serum T-25OHD (ng/ml)	15.2 ± 8.4	19.7 ± 7.2	0.242
Serum CK(U/L)	775.0[527.0,809.0]	309.0[221.5,1110.0]	0.827
24hUCa[a]			
Adults (mmol), n	2.9 ± 2.5,9	1.6 ± 1.1,13	0.367
Children(mmol/kg), n	0.041 ± 0.027,11	0.037 ± 0.014,3	0.815
24hUP (mmol)	14.5 ± 7.1	12.9 ± 10.0	0.339
Epileptic seizure, n/N (%)	25/34(73.5)	20/30(66.7)	0.549
Visual impairment, n/N (%)	6/25(24.0)	6/24(25.0)	0.935
Memory impairment, n/N (%)	9/17(52.9)	5/10(50.0)	0.883
Mental impairment, n/N (%)	27/31(87.1)	4/14(28.6)	0.000
Hearing loss, n/N (%)	4/16(25.0)	0/4(0.0)	0.538
Trousseau's sign, n/N (%)	19/34(55.9)	26/34(76.5)	0.073
Chvostek's sign, n/N (%)	15/32(46.9)	27/34(79.4)	0.006
Cataract, n/N (%)	6/19(31.6)	6/12(50.0)	0.452

Note: Data represent the mean (SD), median [P25, P75].

Adults refer to age ≥ 18 years, children refer to the age 18 years.

[a]Urinary calcium is expressed by mmol/24h in adults, and mmol/kg/24h in children.

Normal reference ranges for indexes: PTH: 1.6–6.9 pmol/L; Serum Ca: 2.13–2.70 mmol/L; Plasma iCa: 1.13–1.23 mmol/L; Serum P: <11 years: 0.97–1.87 mmol/L, 11–18 years: 0.81–1.53 mmol/L, > 18 years: 0.81–1.45 mmol/L; Serum ALP: <15 years: 42–390 U/L, 16–18 years: 52–171 U/L, > 18 years: 35–100 U/L; Serum Cr: 59–104 μmol/L; Serum K: 3.5–5.5 mmol/L; Serum Mg: 0.70–1.10 mmol/L; Serum T-25OHD: 8–50 ng/ml; Serum CK: 24–170 U/L.

The normal reference range of 24hUCa was < 8.75 mmol (350mg) /24h for adults and < 0.1 mmol (4mg) /kg/24h for children.

Abbreviation: CNV: copy number variation; DGS-1: DiGeorge syndrome-1; IHP: idiopathic hypoparathyroidism; kb: kilobase; PTH: parathyroid hormone; TCa: total serum calcium; iCa: ionized calcium; P: serum phosphorus; ALP: alkaline phosphatase; Cr: creatinine; K: potassium; Mg: magnesium; T-25OHD: total 25-hydroxy-vitamin D; CK: creatine kinase; 24hUCa: twenty-four-hour urine calcium; 24hUP: 24-hour urine phosphate; kg: kilogram; n: sample size for test examinations; N, sample size.

	DGS-1 patients (n = 34)	IHP patients (n = 34)	P value
Nephrocalcinosis/Urolithiasis, n/N (%)	2/30(6.7)	1/17(5.9)	1.000
Intracranial calcification, n/N (%)	29/33(87.9)	23/28(82.1)	0.720
Proportion of patients receiving regular HP treatment at first visit, n/N (%)	25/34(73.5)	20/34(58.8)	0.200
Note: Data represent the mean (SD), median [P25, P75].			
Adults refer to age ≥ 18 years, children refer to the age 18 years.			
[a]Urinary calcium is expressed by mmol/24h in adults, and mmol/kg/24h in children.			
Normal reference ranges for indexes: PTH: 1.6–6.9 pmol/L; Serum Ca: 2.13–2.70 mmol/L; Plasma iCa: 1.13–1.23 mmol/L; Serum P: <11 years: 0.97–1.87 mmol/L, 11–18 years: 0.81–1.53 mmol/L, > 18 years: 0.81–1.45 mmol/L; Serum ALP: <15 years: 42–390 U/L, 16–18 years: 52–171 U/L, > 18 years: 35–100 U/L; Serum Cr: 59–104 μmol/L; Serum K: 3.5–5.5 mmol/L; Serum Mg: 0.70–1.10 mmol/L; Serum T-25OHD: 8–50 ng/ml; Serum CK: 24–170 U/L.			
The normal reference range of 24hUCa was < 8.75 mmol (350mg) /24h for adults and < 0.1 mmol (4mg) /kg/24h for children.			
Abbreviation: CNV: copy number variation; DGS-1: DiGeorge syndrome-1; IHP: idiopathic hypoparathyroidism; kb: kilobase; PTH: parathyroid hormone; TCa: total serum calcium; iCa: ionized calcium; P: serum phosphorus; ALP: alkaline phosphatase; Cr: creatinine; K: potassium; Mg: magnesium; T-25OHD: total 25-hydroxy-vitamin D; CK: creatine kinase; 24hUCa: twenty-four-hour urine calcium; 24hUP: 24-hour urine phosphate; kg: kilogram; n: sample size for test examinations; N, sample size.			

Table 6
Comparison of clinical characteristics between DGS-1 and IHP patients at the last visit of follow-up

	DGS-1 patients (n = 34)	IHP patients (n = 34)	P value
Serum TCa (mmol/L)	2.0 ± 0.2	1.9 ± 0.3	0.082
Serum P (mmol/L)	1.7 ± 0.4	1.9 ± 0.4	0.054
Serum ALP (U/L)	104.6 ± 56.8	74.8 ± 32.2	0.005
Serum Cr (μmol/L)	88.6 ± 73.2	71.9 ± 14.9	0.472
Serum K (mmol/L)	4.1 ± 0.3	4.1 ± 0.4	0.540
Serum Mg (mmol/L)	0.8 ± 0.1	0.8 ± 0.1	0.092
Serum T-25OHD (ng/ml)	38.8[27.4,79.0]	44.8[18.6,103.8]	0.884
TP1NP (ng/ml)	68.0[27.6,292.5]	35.3[30.4,40.9]	0.439
Serum β-CTX (ng/ml)	0.5[0.1,0.7]	0.2[0.2,0.3]	0.186
24hUCa[a]			
Adults (mmol), n	4.0 ± 3.3,20	4.0 ± 2.4,24	0.390
Children(mmol/kg), n	0.055 ± 0.021,7	0.041 ± 0.032,2	0.558
24hUP (mmol)	19.0 ± 7.8	14.5 ± 7.1	0.065
Epileptic seizure, n/N (%)	2/33(6.1)	2/33(6.1)	1.000
Visual impairment, n/N (%)	10/25(40.0)	8/24(33.3)	0.628
Memory impairment, n/N (%)	12/22(54.5)	12/16(75.0)	0.197
Mental impairment, n/N (%)	28/30(93.3)	5/12(41.7)	0.001
Hearing loss, n/N (%)	7/13(53.8)	0/2(0.0)	0.467
Trousseau's sign, n/N (%)	6/30(20.0)	17/34(50.0)	0.013
Chvostek's sign, n/N (%)	2/33(6.1)	8/34(23.5)	0.083
Cataract, n/N (%)	6/17(35.3)	8/18(44.4)	0.733
Nephrocalcinosis/Urolithiasis, n/N (%)	4/27(14.8)	3/29(10.3)	0.700
Intracranial calcification, n/N (%)	29/32(90.6)	27/32(84.4)	0.708
Note: Data represent the mean (SD), median [P25, P75].			
Adults refer to age ≥ 18 years, children refer to the age 18 years.			
[a]Urinary calcium is expressed by mmol/24h in adults, and mmol/kg/24h in children.			
Normal reference ranges for indexes: Serum Ca: 2.13–2.70 mmol/L; Serum P: <11 years: 0.97–1.87 mmol/L, 11–18 years: 0.81–1.53 mmol/L, > 18 years: 0.81–1.45 mmol/L; Serum ALP: <15 years: 42–390 U/L, 16–18 years: 52–171 U/L, > 18 years: 35–100 U/L; Serum Cr: 59–104 μmol/L; Serum K: 3.5–5.5 mmol/L; Serum Mg: 0.70–1.10 mmol/L; Serum T-25OHD: 8–50 ng/ml; Serum TP1NP: 15.1–58.6 ng/ml; Serum β-CTX: 0.26–0.51 ng/ml.			
The normal reference range of 24hUCa was < 8.75 mmol (350mg) /24h for adults and < 0.1 mmol (4mg) /kg/24h for children.			
Abbreviation: DGS-1: DiGeorge syndrome-1; IHP: idiopathic hypoparathyroidism; TCa: total serum calcium; P: serum phosphorus; ALP: alkaline phosphatase; Cr: creatinine; K: potassium; Mg: magnesium; T-25OHD: total 25-hydroxy-vitamin D; TP1NP: total procollagen type 1 N-peptide; β-CTX: β-C-terminal telopeptide of type collagen; 24hUCa: twenty-four-hour urine calcium; 24hUP: twenty-four-hour urine phosphate; kg: kilogram; n: sample size for test examinations; N, sample size.			

Discussion

The present study analyzed the frequency of CNVs (≥ 100kb) in a Chinese series of DGS-1 related HP patients, and explored the correlation between CNV and DGS-1 related HP clinical phenotype.

In 34 patients with DGS-1, 15 (44.1%) patients carried 22 large CNVs (≥ 100kb) outside 22q11.2. There was no significant difference in the incidence of CNVs between DGS-1 and IHP patients (15/34 vs 16/34, P = 0.808) except 22q11. Jensen M et al. analyzed CNV and clinical data from 66 individuals with 22q11.2 deletion syndrome (22q11DS), and found that 77% (51/66) of individuals with the 22q11DS also carry additional rare

CNVs (< 0.1% frequency, > 50kb)[19]. Mlynarski EE et al used two algorithms to analyze CNV in 946 patients with 22q11DS[12]. A total of 13,310 autosomal CNVs (12095 deletions and 1215 duplications) outside of the 22q11.2 deleted region were detected by both algorithms. The frequency of 22q11DS CNV in the general population was determined by using the control cohort of 11256 phenotypically normal individuals[20]. The results showed that, of the 13310 autosomal CNVs detected outside the 22q11.2 deleted region, 7217 CNVs (54.22%, average size of 47.21 ± 71.81 in CHD group and 45.43 ± 65.91 kb in no-CHD group) occurred at a frequency < 1.0% in the control population, and were categorized as rare. A greater number of rare deletions (n = 6489) were identified than rare duplications (n = 728). Our results show that the frequency of CNV outside 22q11.2 is slightly lower than that in these two previous studies. It may be due to the small simple size in our study, differences in the size of CNVs and methods used for CNV detection. However, our study still confirmed that there were a relatively high percentage of DGS-1 patients who carried large or rare CNVs besides the known pathogenic chromosomal microdeletion, which may be related to the phenotype of this disease.

In this study, there was no significant difference of clinical significance in HP related phenotype between DGS-1 patients with and without extra-22q11 CNVs (≥ 100kb). Mlynarski EE et al. used Affymetrix SNP Array 6.0 to genotype 22q11DS subjects in two groups. CNV analysis was completed on a total of 949 subjects (cohort 1, n = 562; cohort 2, n = 387), 603 with CHDs (cohort 1, n = 363; cohort 2, n = 240) and 346 with normal cardiac anatomy (cohort 1, n = 199; cohort 2, n = 147). It was revealed that the *SLC2A3* duplication was the most frequent CNV detected and the only significant finding in their combined analysis ($P = 2.68*10^4$, two-tailed Fisher's exact test), indicating that the *SLC2A3* duplication might serve as a genetic modifier of CHDs and/or aortic arch anomalies in individuals with 22q11DS[21]. However, in our study, no known HP related genes were found in the CNVs outside of 22q11.2 region among the 15 DGS-1 patients. The reasons for no differences in clinical phenotype in our study may include: (1) The number of patients studied was too small to compare the differences in clinical phenotype of the patients. (2) Some patients had received drug treatment at the first visit in our center, which may affect the analysis of baseline biochemical indicators. (3) It is also possible that second site CNV burden are not sufficient on their own to cause the observed phenotypic variability in DGS-1. Because the information collected from the clinical data of these patients is not very complete, there is no comparison of clinical phenotypes other than HP.

In our study, there were 4 adult-onset DGS-1 related HP patients. Of note, although the Pt.34 was found to have hypocalcemia at the age of 52 years, a detailed history collection at one follow-up visit found that he had febrile convulsion at childhood when he took calcium and cod liver oil for a short period to relieve the occasional muscle cramp. Therefore, the possibility of childhood onset cannot be completely excluded in this patient. A previous multicenter Italian retrospective study[22] identified 436 adult-onset chronic HP in clinical electronic databases between 1980–2015. Among them, hereditary HP patients included 11 DGS-1, 14 autoimmune poly-endocrinopathy syndrome type 1 (APS1), 2 autosomal dominant hypocalcemia type 1 (ADH1), and 1 familial isolated hypoparathyroidism (FIH). The results of this study suggests that hereditary HP does not always occur in childhood. A few previous studies have shown that DGS-1 patients mostly present with hypocalcemia in infancy or childhood but spontaneous remission has been observed in a subset of patients[23, 24], the reason for this may be due to hypertrophy of the remaining parathyroid cells to increase the amount of secreted PTH to compensate for the deficiency in its own function[25], while the delayed onset afterwards may be because the level of PTH secretion does not meet the demand of the body to maintain calcium homeostasis in adolescence and adulthood[26]. On the other hand, two of the four adult-onset patients had small deletions of *TBX1* gene. This indicates that the difference in clinical manifestations may be related to the severity of *TBX1* gene deletion. And point mutations or small deletions in *TBX1* might cause less severe loss of function, resulting in a less severe clinical phenotype, whereas large deletions or single copy complete deletions of the *TBX1* gene often involve multiple systems, and the onset time is earlier[27].

Of the 32 patients we detected 22q11.2 microdeletion with low-depth WGS, 27 (84.4%) were LCRA-D, 4 (12.5%) were LCRA-B, and 1 (3.1%) were atypical absence of $LCRA^+$-D. The region of Chr 22q11.2 contains 8 LCRs with high sequence homology, LCRs are prone to non-allelic homologous recombination (NAHR) during meiosis, resulting in loss of heterozygous large segments. These LCR22s were named LCR22A-H from the centromere to telomere. LCR22A-D with a total loss of 3Mb has been reported in more than 85% of 22q11.2DGS-1 patients, while in other cases ~ 5% are LCR22A-B (1.5Mb), ~ 4% are LCR22B-D (1.5Mb), ~ 2% are LCR22A-C deletion (2Mb), ~ 1% is LCR22C-D deletion (1Mb)[13, 28–34]. The overall structure of these rearranged alleles remains elusive. It has been reported that the breaking point of the missing fragment is located at LCRs in the vast majority of cases (98%) so far. However, about 2% of 22q11.2DGS-1 patients have at least one end of the missing fragment breaking point outside the LCR22s region, which is called atypical deletion[28, 35]. Due to the small sample size in the present study, the types of LCRs were somewhat different from previous studies and the exact location also requires PCR confirming. So far, the genetic mechanism driving atypical deletions has not been fully explained.

The first limitation of this study is the small sample size which might lead to some statistical bias of the result. Next, low-depth WGS does not detect CNV accurately in regions of low sequence coverage[36]. What's more, because clinical features were collected by chart review, not all patients were fully examined for HP-related complications and extra-parathyroid manifestations, which would bring about some bias in the prevalence of clinical phenotype. And finally, although the treatment was similar between the two groups, some patients had received drug treatment at the first visit in our center, which may affect the analysis of baseline biochemical indicators. Thus, it is possible that a larger cohort of patients with DGS-1 would provide more information to identify a significant association between second CNV burden and DGS-1 phenotype.

Conclusion

In summary, CNVs (≥ 100kb) detected by low-depth WGS, similar to MLPA or comparative genomic hybridization (CGH), can also be used for genetic diagnosis of most DGS-1 patients; It further confirmed that nearly half of DGS-1 patients carry extra-22q11.2 CNV besides pathogenic CNV in 22q11.2. However, the association between these CNV outside 22q11 and phenotype of DGS-1 still need further studies in larger samples.

Declarations

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Data Availability: Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Ethics approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Peking Union Medical College Hospital (JS-3312).

Consent to participate: Informed consent was obtained from all individual participants or their parents included in the study.

Consent to publish: Not applicable.

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Figures

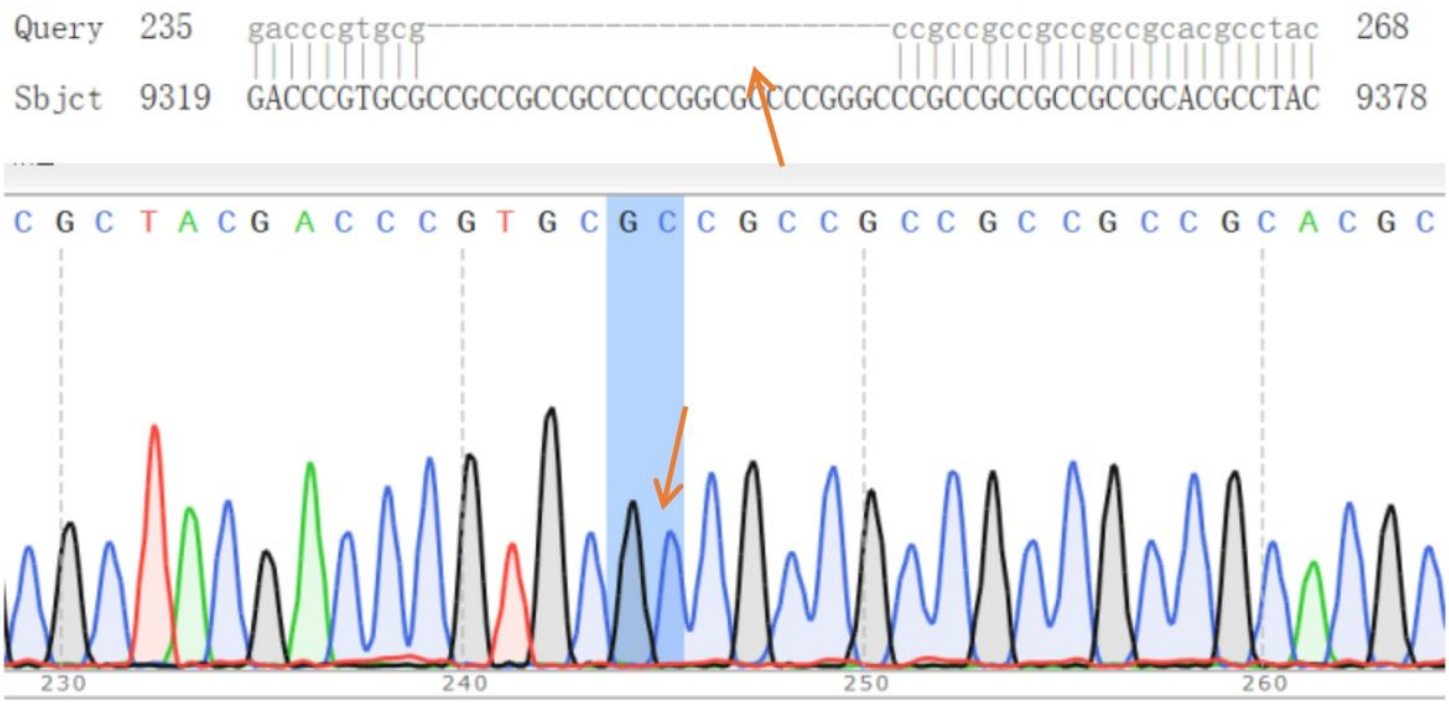

Figure 1

(TA cloning sequencing) *TBX1*: exon3: c.161_186del/p. A54Afs*105 in patient 1 (arrow)