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Low-Pass Genome Sequencing

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Validation and Diagnostic Utility from 409 Clinical Cases of Low-Pass Genome Sequencing for the Detection of Copy Number Variants to Replace Constitutional Microarray

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DNA copy number variants (CNVs) account for approximately 300 Mb of sequence variation in the normal human genome. Significant numbers of pathogenic CNVs contribute toward human genetic disorders. Recent studies suggest a higher diagnostic and clinical significance of low-pass genome sequencing (LP-GS) compared with chromosomal microarrays (CMAs). The performance metrics of the 5X LP-GS was compared with CMA to validate a low-cost and high-throughput method. LP-GS test performed on 409 samples (including 78 validation and 331 clinical) was evaluated using American College of Medical Genetics and Genomics guidelines. The CNV accuracy, precision, specificity, and sensitivity were calculated to be 100% for all previously characterized CNVs by CMA. Samples ($n = 6$) run at both approximately 30X GS and approximately 5X GS (LP-GS) average depth detected a concordance of 89.43% to 91.8% and 77.42% to 89.86% for overall single-nucleotide variants and insertions/deletions, respectively. In the 331 clinical samples, 17.2% each were classified as pathogenic/likely pathogenic and uncertain clinical significance. In addition, several cases with pathogenic CNVs were detected that were missed by CMA. This study demonstrates that LP-GS (5X GS) was able to reliably detect absence of heterozygosity, microdeletion/microduplication syndromes, and intragenic CNVs with higher coverage and resolution over the genome. Because of lower cost, higher resolution, and greater sensitivity of this test, our study in combination with other reports could be used in an evidence-based review by professional societies to recommend replacing CMAs. *(J Mol Diagn 2020, 22: 823–840; https://doi.org/10.1016/j.jmoldx.2020.03.008)*

In the past decade, next-generation sequencing (NGS)–based methods, such as exome sequencing and genome sequencing (GS), have proved to be cost-effective and time efficient in identifying sequence variants for the diagnosis and management of human genetic (mendelian) disorders.[1] The landmark article on the 1000 Genomes project consortium demonstrated that low-coverage genome sequencing (approximately 7.4X depth) could be a useful tool for accurate genotyping of sequencing variants and assessment of copy number variations.[2] The American College of Medical Genetics and Genomics and the Association for Molecular Pathology jointly issued standards and guidelines for the interpretation of sequence variants using NGS techniques as a laboratory-developed test.[3] However, identification of genomic copy number variants (CNVs) from NGS-based methods remains a challenge for most laboratories and consequently microarrays are currently still the first-line test for constitutional disorders.[4] DNA CNVs represent a major class contributing toward significant genomic diversity between two different human individuals. An evolution of CNV detection from visible cytogenetic

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deletions and duplications (by karyotyping techniques) to cryptic microdeletion and microduplication syndromes employing array comparative genomic hybridization or whole genome microarray occurred from mid-1970s to early 2000s. Chromosomal microarray (CMA) became the gold standard first tier test in individuals with developmental disabilities and congenital anomalies after Miller et al[5] published a consensus statement making the recommendations. Although microarray techniques have been universally adopted by molecular and cytogenetic laboratories as a laboratory-developed test, it becomes cost prohibitive for patients to be tested by both CMA and NGS-based methods (exome sequencing/GS) to obtain a genetic diagnosis and allow for effective therapeutic and clinical management. In addition, numerous studies have recommended GS as an alternative to CMA because of its power of enhanced resolution and improved detection of genomic aberrations, including both sequencing and copy number variants.[6–10] However, Wang et al[10] have demonstrated the utility of low-pass GS (LP-GS) compared with that of CMA in a prenatal setting in a cost-effective way. Also, LP-GS has been reported by Dong et al[11] as a genomic tool for identifying chromosomal aberrations in couples with recurrent miscarriages. Recently, the American College of Medical Genetics and Genomics and the Clinical Genome Resource committee published the technical standards for the interpretation and reporting of constitutional copy number variants irrespective of the technology used for reporting them.[12]

Considering the latest low-pass GS studies affirming Moore's law that with decreasing cost of GS methods, an increasing level of throughput will occur, we validated a low-pass GS method [copy number genome (alias LP-GS test)] as a cost-effective and higher-resolution alternative to CMA for CNV detection. The low-pass GS method employed provided an average of 5X depth to evaluate the specificity, sensitivity, accuracy, and precision in the identification and detection of genomic numerical and structural abnormalities. Seventy-eight ($N = 78$) previously well-characterized specimens by karyotype, fluorescence *in situ* hybridization, and CMA were used as part of this exhaustive validation. Of these, three haplotype map samples were run in triplicate to assess the concordance of CNVs published in literature from these samples.[13] The implications from this study immensely impact the cost-effectiveness of this 5X GS method, which is a significant reduction (approximately 20% to 30%) compared with the standard high-resolution GS methods.

Subsequent to the exhaustive validation performed for developing the LP-GS test, 331 clinical specimens have been tested by this method in our laboratory and have led to the detection of microdeletion/microduplication syndromes, intragenic deletions and intragenic duplications, uniparental isodisomy, triploidy, and whole chromosome aneuploidies. Several cases of dual CNV diagnosis were identified, demonstrating the power and potential of the LP-GS test.

Materials and Methods

DNA was extracted with the use of a commercial Chemagic DNA CS200 DNA extraction kit (PerkinElmer, Waltham, MA) with the instrument Chemagic 360 (PerkinElmer). DNA was quantified with the PicoGreen reagent (ThermoFisher, Waltham, MA) and Enspire plate reader (PerkinElmer) for DNA quality control measurement. All samples passing quality control were subjected to library preparation using a KAPA HyperPlus Kit PCR-free library preparation kit following the manufacturer's instructions (KAPA Biosystems/Roche, Wilmington, MA). In brief, 100 ng of genomic DNA was enzymatically digested into an average fragment size of 600 bp. After end repair, the library fragments were adenylated to facilitate adapter ligation. After library purification, DNA was quantified via quantitative PCR using the KAPA library quantitation kit. Libraries were normalized manually by adding different volumes of the diluent and pooled in an equimolar manner to 2 nmol/L concentration. The samples were sequenced on an S2 flow cell (150-base paired-end sequencing, approximately 5X per sample) using the NovaSeq 6000 platform (Illumina, San Diego, CA).

The raw sequence reads were converted to FASTQ format using Illumina bcl2fastq2 Conversion Software version 2.19.1 (Illumina), and then mapped to and analyzed in comparison with human genome nuclear GRCh Build 37 (hg19) and mitochondrial genome reference sequences using Illumina's Bio-IT DRAGEN Platform. Variant calls were annotated using the SnpEff software version 4.3t (*http://snpeff.sourceforge.net*; last accessed November 24, 2017). The average alignment coverage for the whole genome was assessed for the depth of coverage and data quality threshold values. Variants with >5X or more coverage were included in the analyses.

Copy number analysis was performed using NxClinical version 5.0 software (BioDiscovery, Inc., El Segundo, CA). First, a reference profile was generated from whole blood–derived genomic DNA of 20 healthy controls (10 male and 10 female individuals) sequenced at approximately 5X to 10X coverage using the Binary Alignment Map Multi Scale Reference Builder module (BioDiscovery, Inc.). The Multi Scale Reference algorithm uses a dynamic bin size approach with the goal of maintaining a roughly stable number of reads per bin. Herein, we used 500 reads per bin as our target. The number of reads per bin for each test sample was then compared with the expected number of reads followed by a number of systematic correction steps adjusting the ratio for various sources of bias, such as GC content. The procedure generated a log2 profile across the genome centered on zero (no copy number change). CNVs were then detected using the hidden Markov model based fast adaptive states segmentation technique algorithm. This algorithm generates hidden state for each CNV value based on expected log2 ratios as well as B-allele frequency.

Default cutoffs were applied, with high copy number calls expected to have >0.85, copy number gain between 0.35 and 0.85, copy number loss between −0.5 and −1.25, and high copy number loss <−1.25. NxClinical 4.3 or 5.0 software (BioDiscovery, Inc.) was used to determine and visualize the CNVs of statistically significant high frequency.

Results

Clinical Validation of LP-GS Test

In four runs, 78 unique samples (26 of which were run as replicates) were used to obtain global metrics and performance parameters. These included three well-characterized Coriell haplotype map samples (NA12878, NA12891, and NA12892) run in triplicate in three independent runs. Forty positive control samples had a diverse type and size of clinically relevant CNVs and absence of heterozygosity (AOH), such as trisomies ($n = 2$), interstitial and terminal deletions and duplications ($n = 21$), mosaic deletions and duplications ($n = 7$), absence of heterozygosity ($n = 7$), and unbalanced translocations ($n = 3$) (Supplemental Tables S1 and S2). Thirty-eight control samples without clinically significant CNVs were also tested by the LP-GS assay and did not detect any reportable copy number gains or losses.

The coverage histogram showed the number of genomic locations having a given coverage rate for the LP-GS test at approximately 5X (Figure 1A) compared with approximately 30X for GS (Figure 1D). The genome fraction coverage showed that the LP-GS test had approximately 82% at 5X (Figure 1B), whereas GS had 92% at 5X and 60% at 30X (Figure 1E). The coverage across the reference demonstrated no major difference in the covered area between the 5X LP-GS (Figure 1C) and 30X GS (Figure 1F) and the occurrence of areas that were not covered because of the correlation to low GC percentage or high AT percentage.

Six samples were subjected to both GS (approximately 30X) and LP-GS (approximately 5X), and the percentage concordance of single-nucleotide variant (SNV) distribution over the genome was compared as total SNVs and insertions/deletions detected in all genes and genes associated with/without disease (genes causing disease/genes of uncertain significance). The percentage of SNV concordance across the entire genome between 30X and 5X coverage was strikingly similar (Supplemental Figure S1 and Supplemental Table S3). Further characterizing the SNV and insertion/deletion concordance into exonic, intronic, untranslated, intragenic, and intergenic regions among the six samples demonstrated an average concordance range between 77.4% and 91.8% (Supplemental Figure S1 and Supplemental Table S3).

Three haplotype map samples (NA12878, NA12891, and NA12892) were subjected to accuracy and precision studies and were run in three independent runs at >5X GS. The CNV data were compared with the published data[13] from these samples and the global CNV concordance was detected at an average concordance of 88.82%, 94.52%, and 90.77% for NA12878, NA12891, and NA12892, respectively (Supplemental Figure S2 and Supplemental Table S4).

The clinical specificity [true negatives/(true negatives + false positives)] and clinical sensitivity [true positives/(true positives + false negatives)] of the clinically relevant CNVs by 5X LP-GS was calculated to be 100% from 33 samples (positive controls in runs 3 and 4) that had previously identified CNVs by CMA. This approach accurately detected clinically significant deletions (19 kb to 159 Mb) and duplications (24 kb to 154 Mb) of varying sizes (Supplemental Table S1). For instance, a 2.9-Mb clinically significant duplication on 11q, arr (hg18) 11q25 (131,542,057 to 134,434,130) × 3 with Jacobsen syndrome was detected. In contrast, an intragenic single-exon Duchenne muscular dystrophy (*DMD*; exon 55) deletion was detected in a male with Duchenne muscular dystrophy, not previously detected by CMA analysis (because of lack of exonic probe coverage on the array platform). The limit of resolution of CMA and 5X LP-GS was 20 to 50 Kb (with a clinical sensitivity of approximately 15% to 18%) and <25 Kb (with a clinical sensitivity >21%), respectively. The precision, sensitivity, and specificity were each at 100% for all clinically significant CNVs in specimens used for this study. More important, this test detected pathogenic CNVs of clinical relevance that were missed by the CMA platform. Seven samples with AOH/long contiguous stretches of homozygosity regions previously detected by CMA were also concordant with the LP-GS test (Supplemental Table S2). Our attempt to go lower than 5X LP-GS coverage (eg, approximately 1.5X and approximately 2X LP-GS) in 17 cases resulted in detection of whole chromosome aneuploidies; however, the CNV data for microdeletions and microduplications were discordant and the ability to reliably detect AOH was lost (data not shown).

Diagnostic Yield of LP-GS Test in a Global Diagnostic Laboratory

A total of 331 samples were received at the global PerkinElmer Genomics Laboratory (Branford, CT) for 5X LP-GS testing. Of these samples, 180 were from males and 151 were from females. These samples were referred with variable phenotypic presentations such as global developmental delay, autism, intellectual disability, congenital anomalies, and dysmorphic features. Clinical information for case number 31, which harbored a pathogenic CNV, was not provided. Pathogenic and likely pathogenic CNVs (with or without VOUS and AOH findings) were detected in 57 cases (17.2%) (Table 1). Of these, 49 cases had diagnostic findings related to phenotype (33 cases with known microdeletion/microduplication syndromes, 10 cases with intragenic deletions and duplications, 4 cases of a trisomy,

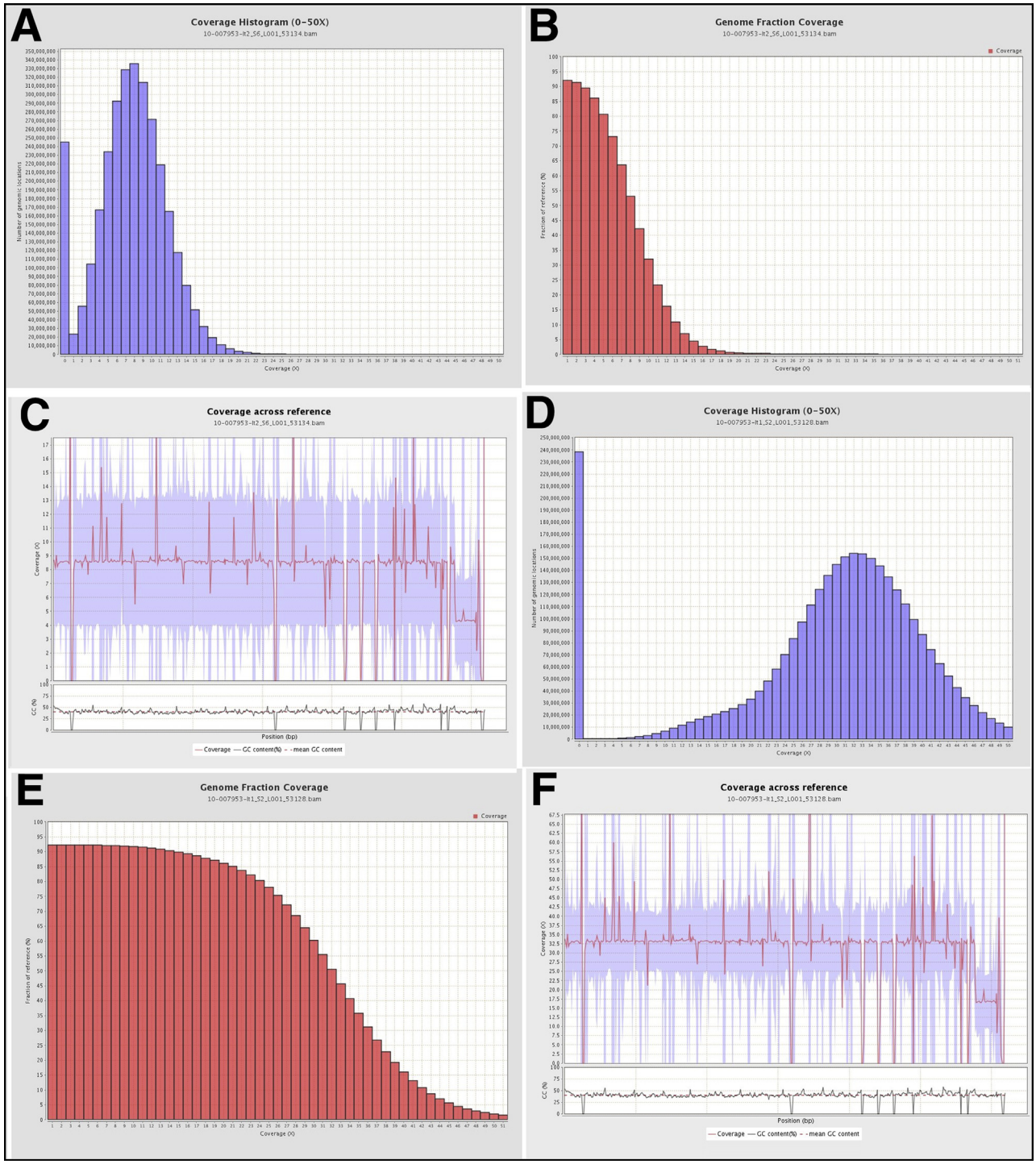

Figure 1 **A** and **D:** Histograms (0 to 50X) for low-pass genome sequencing (LP-GS) (**A**) and 30X GS (**D**). Histograms of the number of genomic locations having a given coverage rate. In these graphs, genome locations with a coverage >50X are grouped into the last bin. By doing so, a higher resolution of the most common values for the coverage rate is obtained. **B** and **E:** The genome fraction coverage for the LP-GS (**B**) and GS (**E**). These graphs provide a visual way of knowing how much reference has been sequenced with at least a given coverage rate. **C** and **F:** The coverage across reference for LP-GS (**C**) and GS (**F**): The coverage distribution (**red line**) and coverage deviation across the reference sequence are shown (**top panels**). GC content across reference (**black line**) together with its average value (**red dotted line**) are shown (**bottom panels**).

and 1 case each of a triploidy and uniparental isodisomy) (Table 2). Fifty-seven cases (17.2%) harbored CNVs classified as variants of uncertain significance, and 27 cases (8.2%) had a reportable AOH finding. There were 190 cases (57.4%) that did not have a reportable CNV finding. CNV analysis and interpretation was performed as per the American College of Medical Genetics and Genomics guidelines by board-certified clinical geneticists (A.C. and

Table 1 Pathogenic and Likely Pathogenic CNVs ($n = 57$) Detected by 5X LP-GS Test in Clinical Samples

No.	Sample ID	Sex	Nomenclature	Size, bp	OMIM genes, *n*	OMIM morbid genes	Classification	Clinical features
1	DS183397	Female	Seq (hg19) 22q13.33 (51122267_ 51304566) × 1 dn	CNV: 182,300	CNV: 3	***CNV:*** *SHANK3, ACR*	P	Epilepsy, autism spectrum disorder, ADHD, nonverbal, delayed motor and language development
2	DS187589	Female	Seq (hg19) 7p22.1 (5789131_ 5900230) × 3, 13q21.1q32.1 (56866739_ 98070738) × 1	CNV1:111,100; CNV2:41,204,000	CNV1: 1 CNV2: 54	***CNV1:*** *RNF216;* ***CNV2:*** *DIAPH3, ATXN8OS, PIBF1, TBC1D4, CLN5, FBXL3, EDNRB, SPRY2, SLITRK1, SLITRK6, MIR17HG, GPC6, TGDS, CLDN10, DNAJC3*	VOUS; P	Hemolytic uremic syndrome and small stature
3	DS187606	Female	Seq (hg19) 9p24.3p24.1 (42115_ 8214109) × 1, 9p24.1p21.1 (8214110_ 30253840) × 3, 9p21.1p13.1 (30402040_ 38858214) × 3	CNV1: 8,171,995; CNV2:22,039,731; CNV3:8,456,175	CNV1: 36 CNV2: 63 CNV3: 71	***CNV1:*** *DOCK8, KANK1, SMARCA2, VLDLR, KCNV2, GLIS3, SLC1A1, PLPP6, JAK2, GLDC;* ***CNV2:*** *TYRP, MPDZ, FREM, IFN, MTAP, CDKN2, PLAA, IFT74, TEK, C9orf72;* ***CNV3:*** *DDX58, TOPORS, APTX, B4GALT1, AQP7, AQP3, DNAI1, SIGMAR1, GALT, IL11RA, VCP, FANCG, PIGO, RUSC2, RMRP, TPM2, GBA2, NPR2, GNE, NM, PAX5, GRHPR, EXOSC3*	P	Delayed motor and language development, developmental regression, intellectual disability, multiple congenital anomalies, amenorrea, macrocephaly, almond palpebral fissures, dysplastic ears, overlapping fingers, autism, cardiac disorder, recurrent vomit, renal and skeletal anomalies, recurrent otitis, hypotonia, retinopathy, hip dysplasia
4	DS181038	Female	Seq (hg19) 19p13.2 (10140165_ 13379514) × 3 dn	CNV: 3,239,350	CNV1: 141	***CNV:*** *DNMT1, S1PR2, ICAM1, ICAM4, FDX2, TYK2, DNM2, SMARCA4, LDLR, QTL2, KANK2, DOCK6, EPOR, CCDC151, PRKCSH, ACP5, MAN2B1, DHPS, RNASEH2A, MAST1, KLF1, GCDH, CALR, NFIX, LYL1, TRMT1, NACC1, CACNA1A*	P	Delayed language development, delayed social development, hyperactivity, behavioral issues
5	DS183730	Female	Seq (hg19) 5q35.2q35.3 (175515930_ 177464729) × 1	CNV: 1,948,800	CNV: 26	***CNV:*** *SNCB, FGFR4, NSD1, SLC34A1, F12, DDX41, B4GALT7, PROP1*	P	Microcephaly, dysmorphism, developmental delay, squint, hypospadiasis, caudal regression, bulbous tip of nose, prominent little finger pads, high arched palate
6	DS183970	Female	Seq (hg19) Xp22.31 (6450180_ 8134280) × 3	CNV: 1,684,101	CNV: 5	***CNV:*** *STS*	LP	Delayed motor development, delayed language development, developmental regression, intellectual disability, normal karyotype, short philtrum, clinodactyly of fifth finger, brachydactyly of hands and feet, short stature
7	18PA002367	Female	Seq (hg19) 2q32.1q33.1 (187199536_ 200573191) × 1	CNV: 13,373,656	CNV: 41	***CNV:*** *COL3A1, COL5A2, SLC40A1, MSTN, HIBCH, GLS, STAT1, STAT4, HECW2, PGAP1, SF3B1, HSPD1, MARS2, SATB2*	P	In incubator for 1 month, elevated bilirubin, cleft lip and palate, multifocal gliosis bilateral, sinusitis right, unspecific symptoms
8	19CT045381	Male	Seq (hg19) 2p13.2 (71706517_ 71710050) × 1	CNV: 3534	CNV: 1	***CNV:*** *DYSF*	VOUS; P	Limb girdle muscular dystrophy, suspicion of dysferlinopathy

(*table continues*)

Table 1 (*continued*)

No.	Sample ID	Sex	Nomenclature	Size, bp	OMIM genes, *n*	OMIM morbid genes	Classification	Clinical features
9	18PA004196	Female	Seq [hg19] 13q11q34 (19027040_ 115169878) × 3, Xp22.33q28 (1-155,270,560) × 1∼2	CNV1:96,142,839 CNV2:155,270,560	CNV1: 271 CNV2: 722	***CNV1:*** *GJA3, GJB2, GJB6, FGF9, SGCG, SACS, MIPEP, CENPJ, ATP8A2, RNF6, SCAR3, RPL21, POLR1D, PDX1, FLT3, POMP, ALOX5AP, B3GLCT, BRCA2, KL, MAB21L1, SPART, RFXAP, SMAD9, EXOSC8, UFM1, FREM2, COG6, COD2, FOXO1, SLC25A15, TNFSF11, HTR2A, SUCLA2, NUDT15, ITM2B, RB1, LPAR6, RCBTB1, RNASEH2B, ATP7B, ALG11, DIAPH3, ATXN8OS, PIBF1, TBC1D4, CLN5, FBXL3, EDNRB, SPRY2, SLITRK1, SLITRK6, MIR17HG, GPC6, TGDS, CLDN10, DNAJC3, ZIC2, PCCA, NALCN, FGF14, ERCC5, SLC10A2, DAOA, LIG4, IRS2, COL4A1, COL4A2, NAXD, CARS2, ING1, F7, F10, PROZ, GRK1, CHAMP1 ; CNV2- SHOX, CSF2RA, NLGN4X, STS, ANOS1, GPR143, CLCN4, MID1, HCCS, AMELX, FRMPD4, TRAPPC2, OFD1, FANCB, PIGA, AP1S2, NHS, CDKL5, RS1, PHKA2, ADGRG2, PDHA1, SH3KBP1, RPS6KA3, CNKSR2, SMPX, MBTPS2, SMS, PHEX, PTCHD1, KLHL15, EIF2S3, PDK3, POLA1, ARX, IL1RAPL1, NROB1, GK, DMD, XK, CYBB, RPGR, OTC, TSPAN7, BCOR, ATP6AP2, USP9X, DDX3X, NYX, CASK, MAOA, NDP, KDM6A, SLC9A7, RP2, NDUFB11, RBM10, UBA1, MRX50, SYN1, CFP, SSX1, FTSJ1, PORCN, EBP, WAS, GATA1, HDAC6, PQBP1, SLC35A2, TFE3, WDR45, SYP, CACNA1F, CCDC22, FOXP3, USP27X, CLCN5, SHROOM4, BMP15, SSX2, KDM5C, IQSEC2, SMC1A, HSD17B10, HUWE1, PHF8, TSR2, FGD1, MAGED2, ALAS2, UBQLN2, ARHGEF9, AMER1, ZC4H2, LAS1L, MSN, AR, OPHN1, EFNB1, EDA, IGBP1, ARR3, KIF4A, DLG3, TEX11, IL2RG, MED12, NLGN3, GJB1, NONO, TAF1, OGT, HDAC8, PHKA1, XIST, SLC16A2, RLIM, NEXMIF, ABCB7, FGF16, ATRX, MAGT1, COX7B, ATP7A, PGK1, TBX22, BRWD3, POU3F4, ZNF711, POF1B, CHM, DIAPH2, PCDH19, SRPX2, TIMM8A, BTK, GLA, HNRNPH2, GPRASP2, PLP1, SERPINA7, TBC1D8B, PIH1D3, PRPS1, MID2, COL4A6, COL4A5, ACSL4, AMMECR1, CHRDL1, PAK3, DCX, ALG13, PLS3, CXorf56, UBE2A, UPF3B, RNF113A, NDUFA1, LAMP2, CUL4B, C1GALT1C1, GLUD2, GRIA3, THOC2, XIAP, STAG2, SH2D1A, OCRL, XPNPEP2, ZDHHC9, BCORL1, AIFM1, IGSF1, FRMD7, HS6ST2, GPC4, GPC3, PHF6, HPRT1, SLC9A6, FHL1, CD40LG, RBMX, GPR101, ZIC3, F9, ATP11C, SOX3, FMR1, AFF2, IDS, MAMLD1, MTM1, HMGB3, VMA21, NSDHL, BGN, ATP2B3, CCNQ, SLC6A8, BCAP31, ABCD1, SSR4, L1CAM, AVPR2, NAA10, HCFC1, MECP2, OPN1LW, OPN1MW, FLNA, EMD, RPL10, TAZ, ATP6AP1, GDI1, LAGE3, G6PD, IKBKG, DKC1, F8, RAB39B, CLIC2, TMLHE*	P	Miscarriage/spontaneous abortion

(*table continues*)

Table 1 (*continued*)

No.	Sample ID	Sex	Nomenclature	Size, bp	OMIM genes, *n*	OMIM morbid genes	Classification	Clinical features
10	18PA004223	Female	Seq (hg19) 7p22.3q36.3 (1_159,138,663) × 3	CNV: 159,138,663	CNV: 711	***CNV:*** *FAM20C, DNAAF5, MAD1L1, MRM2, LFNG, BRAT1, IQCE, CARD11, AP5Z1, WIPI2, ACTB, RNF216, PMS2, AIMP2, RAC1, GLCCI1, TMEM106B, CRPPA, AHR, TWIST1, DNAH11, IL6, FAM126A, KLHL7, GPNMB, GSDME, CYCS, HNRNPA2B1, SNX10, HOXA1, HOXA2, HOXA11, HOXA13, FKBP14, AQP1, GHRHR, PPP1R17, PDE1C, NT5C3A, RP9, BBS9, BMPER, NPSR1, TBX20, ANLN, NME8, SFRP4, POU6F2, CDK13, MPLKIP, SUGCT, GLI3, BLVRA, PGAM2, AEBP1, GCK, CAMK2B, NPC1L1, OGDH, CCM2, ADCY1, PKD1L1, IKZF1, DDC, EGFR, PSPH, CHCHD2, GUSB, ASL, KCTD7, SBDS, AUTS2, ELN, NCF1, POR, MDH2, HSPB1, YWHAG, ZP3, PTPN12, MAGI2, CD36, HGF, PCLO, SEMA3E, SEMA3A, ABCB4, ABCB1, ADAM22, CFAP69, AKAP9, KRIT1, GATAD1, PEX1, CDK6, SAMD9, SAMD9L, CALCR, COL1A2, SGCE, PON1, PON2, SLC25A13, DLX5, ASNS, TRRAP, ARPC1B, CYP3A5, AP4M1, TAF6, MAP11, STAG3, TFR2, ACTL6B, EPO, EPHB4, ACHE, SERPINE1, AP1S1, PLOD3, CUX1, PMPCB, SLC26A5, RELN, KMT2E, PUS7, COG5, SLC26A4, SLC26A3, DLD, LAMB1, PNPLA8, PPP1R3A, FOXP2, CAV1, MET, CFTR, TSPAN12, AASS, FEZF1, TAS2R16, POT1, PAX4, LEP, RBM28, IMPDH1, OPN1SW, FLNC, IRF5, TNPO3, SMO, MIR96, CEP41, BPGM, NUP205, AKR1D1, ATP6V0A4, TBXAS1, THAS, BRAF, AGK, WEE2, TAS2R38, PRSS1, TRPV6, KEL, CLCN1, NOBOX, TPK1, CNTNAP2, EZH2, KCNH2, NOS3, CDK5, ASB10, PRKAG2, KMT2C, XRCC2, DPP6, SHH, LMBR1, MNX1, DNAJB6, NCAPG2, WDR60*	P	Prenatal BoBs analysis finding of chromosome 7q11.2 duplication
11	19PA009631	Female	Seq (hg19) 21q11.2q22.3 (14436747_ 48129895) × 3	CNV: 33,693,149	CNV: 164	***CNV:*** *NRIP1, TMPRSS15, APP, SOD1, MRAP, CFAP298, SYNJ1, IFNAR2, IL10RB, IFNGR2, SON, DONSON, KCNE2, KCNE1, RUNX1, CLDN14, HLCS, PIGP, DYRK1A, KCNJ6, RIPK4, TMPRSS3, RSPH1, WDR4, CBS, CRYAA, SIK1, PDXK, CSTB, AIRE, PFKL, CFAP410, TSPEAR, ITGB2, COL18A1, COL6A1, COL6A2, FTCD, LSS, MCM3AP, PCNT*	P	Low-set ears, redundant skin and increased neck folds, clinical suspicion of genetic, endocrine, metabolic, cardiovascular disorder
12	19CT035301	Female	Seq (hg19) 21q11.2q22.3 (14391147_ 48129895) × 3	CNV: 33,738,748	CNV: 164	***CNV:*** *NRIP1, TMPRSS15, APP, SOD1, MRAP, CFAP298, SYNJ1, IFNAR2, IL10RB, IFNGR2, SON, DONSON, KCNE2, KCNE1, RUNX1, CLDN14, HLCS, PIGP, DYRK1A, KCNJ6, RIPK4, TMPRSS3, RSPH1, WDR4, CBS, CRYAA, SIK1, PDXK, CSTB, AIRE, PFKL, CFAP410, TSPEAR, ITGB2, COL18A1, COL6A1, COL6A2, FTCD, LSS, MCM3AP, PCNT*	P	Down syndrome
13	18PA006351	Female	Seq (hg19) 20p12.3p12.2 (7673410_ 11778409) × 3	CNV: 4,105,000	CNV: 9	***CNV:*** *PLCB1, PLCB4, SNAP25, MKKS, JAG1*	P	Increased nuchal transparency on antenatal ultrasound

(*table continues*)

Table 1 (*continued*)

No.	Sample ID	Sex	Nomenclature	Size, bp	OMIM genes, *n*	OMIM morbid genes	Classification	Clinical features
14	19CT041779	Female	Seq (hg19) 22q11.21 (18887399_ 20319532) × 3, 22q11.21 (20703333_ 21460600) × 3, (1-22)c × hmz	CNV1:1,432,134 CNV2: 757,268	CNV1: 30 CNV2: 12	***CNV1:*** *PRODH, SLC25A1, CDC45, GP1BB, TBX1, TXNRD2, COMT, TANGO2, RTN4R;* ***CNV2:*** *SCARF2, SERPIND1, PI4KA, SNAP29, LZTR1*	P; AOH	Pierre Robin syndrome, cleft palate
15	19CT022715	Female	Seq (hg19) 2p25.3p25.2 (95636_ 4658635) × 3, 15q11.2 (22747246_ 23377745) × 1, 21q22.2q22.3 (40115047_ 48038846) × 1	CNV1:4,563,000 CNV2:630,500 CNV3:7,923,800	CNV1: 13 CNV2: 4 CNV3: 75	***CNV1:*** *TPO, PXDN, MYT1L, TRAPPC12, RNASEH1, RPS7, COLEC11;* ***CNV2:*** *NIPA1;* ***CNV3:*** *RIPK4, TMPRSS3, RSPH1, WDR4, CBS, CRYAA, SIK1, PDXK, CSTB, AIRE, PFKL, CFAP410, TSPEAR, ITGB2, COL18A1, COL6A1, COL6A2, FTCD, LSS, MCM3AP, PCNT*	P; LP; P	Delayed motor and language development, intellectual disability, developmental regression
16	19CT022819	Female	Seq (hg19) 22q11.22q11.23 (22989833_ 25024532) × 3	CNV: 2,034,700	CNV: 27	***CNV:*** *BCR, IGLL1, CHCHD10, ST3, SMARCB1, MIF, SPECC1L, UPB1, GGT1*	P	Early puberty
17	19CT027331_ 19IN000429	Female	Seq (hg19) 7p22.3(13019_ 327230) × 3, 9q34.3 (137855270_ 141106614) × 1, Xp22.11 (24469876_ 24650929) × 3	CNV1:197,040 CNV2: 3,251,345 CNV3: 181,054	CNV1: 1 CNV2: 66 CNV3: 2	***CNV1:*** *FAM20C ;* ***CNV2:*** *MRPS2, SOHLH1, KCNT1, LHX3, CARD9, PMPCA, INPP5E, NOTCH1, AGPAT2, MAN1B1, GRIN1, TPRN, SLC34A3, TUBB4B, NSMF, EHMT1, CACNA1B ;* ***CNV3:*** *PDK3*	VOUS; P; VOUS	IUGR, ventriculomegaly, subaortic VSD, ostium secundum ASD, developmental delay, hypotonia
18	19CT031426	Female	Seq (hg19) 7q36.1 (147933931_ 152099456) × 1, 7q36.1q36.3 (152114731_ 159138663) × 1	CNV1:4,165,526 CNV2: 7,023,933	CNV1: 47 CNV2: 22	***CNV1:*** *CNTNAP2, EZH2, KCNH2, NOS3, CDK5, ASB10, PRKAG2, KMT2C;* ***CNV2:*** *KMT2C, XRCC2, DPP6, SHH, LMBR1, MNX1, DNAJB6, NCAPG2, WDR60*	P	Developmental delay, seizures, bilateral cleft palate, bilateral hearing loss
19	19CT034899	Female	4q13.3q22.1 (71347038_ 88071237) × 1	CNV: 16,724,200	CNV: 88	***CNV:*** *MUC7, AMTN, AMBN, ENAM, SLC4A4, ADAMTS3, ALB, AFP, ODAPH, SCARB2, FRAS1, ANTXR2, PRDM8, FGF5, HNRNPDL, COQ2, WDFY3*	P	Multiple congenital anomalies, brachydactyly of the hands and feet, frontal bossing, low-set ears, anteriorly displaced anus, hiatal hernia
20	19CT036094	Female	Seq (hg19) 22q11.21 (18887399_ 21709132) × 3, 5q31.2q33.1 (139409320_ 150628812) × 2 hmz, 9q21.32q22.32 (86701754_ 97846813) × 2 hmz	CNV:2,821,734 AOH1: 11,219,493 AOH2: 11,145,060	CNV: 45 AOH1: 142 AOH2: 45	***CNV:*** *PRODH, SLC25A1, CDC45, GP1BB, TBX1, TXNRD2, COMT, TANGO2, RTN4R, SCARF2, SERPIND1, PI4KA, SNAP29, LZTR1, GGT2*	P; AOH	Preterm newborn, cleft palate, facial dysmorphism, micrognathia, retrognathia
21	19CT039177	Female	Seq (hg19) 15q11.1q13.1 (20147946_ 28908545) × 4	CNV1: 8,760,600	CNV1: 25	***CNV:*** *NIPA1, MKRN3, MAGEL2, NDN, SNRPN, UBE3A, GABRB3, GABRA5, OCA2, HERC2*	P	Downslanting eyes, telecanthus, global developmental delay, feeding difficulty
22	19CT038325	Female	Seq (hg19) 9q22.1q22.33 (90810381_ 101604762) × 1, 22q11.21 (21040333_ 21469532) × 3	CNV1:10,794,382 CNV2: 429,200	CNV1: 60 CNV2: 8	***CNV1:*** *SECISBP2, AUH, ROR2, SPTLC1, ASPN, BICD2, FBP1, FANCC, PTCH1, ERCC6L2, HSD17B3, TDRD7, XPA, FOXE1, NANS, GABBR2, ANKS6, GALNT12;* ***CNV2:*** *SERPIND1, PI4KA, SNAP29, LZTR1*	P; LP	Clinical features of Gorlin syndrome, dysgenesis of corpus callosum, plantar dimple, macrocephaly, dysmorphic facial features

(*table continues*)

Table 1 (*continued*)

No.	Sample ID	Sex	Nomenclature	Size, bp	OMIM genes, *n*	OMIM morbid genes	Classification	Clinical features
23	19CT040411	Female	Seq (hg19) 9q21.11q22.2 (70726065_ 93177361) × 3, 9q22.2q22.31 (93179601_ 96128714) × 1∼2, 9q22.31q31.1 (96128715_ 102865014) × 1	CNV1: 22,451,297 CNV2: 2,949,114 CNV3:6,736,300	CNV1: 66 CNV2: 18 CNV3: 42	***CNV1:*** *PRKACG, FXN, TJP2, MIR204, TMC1, RORB, TRPM6, VPS13A, GNAQ, CEP78, PSAT1, HNRNPK, NTRK2, AGTPBP1, ISCA1, SECISBP2;* ***CNV2:*** *AUH, ROR2, SPTLC1, ASPN, BICD2;* ***CNV3:*** *FBP1, FANCC, PTCH1, ERCC6L2, HSD17B3, TDRD7, XPA, FOXE1, NANS, GABBR2, ANKS6, GALNT12, TGFBR1, ALG2, NR4A3, INVS*	P	Suspicion of chromosome abnormality
24	19CT041685	Female	Seq (hg19) 1p21.2p13.2 (100755510_ 114150909) × 1	CNV: 13,395,400	CNV: 86	***CNV:*** *CDC14A, GPR88, COL11A1, RNPC3, SLC25A24, GPSM2, TAF13, SORT1, GNAI3, GNAT2, AMPD2, ALX3, SLC6A17, RBM15, KCNA2, DRAM2, KCND3, WNT2B, SLC16A1, LRIG2*	P	Seizures, facial dysmorphism
25	18PA002136	Female	Seq (hg19) 2q32.2 (189857141_ 189870643) × 4, 19q13.2 (38931154_ 39038752) × 3, seq (hg19) (1-22)c × hmz	CNV1:13,503 CNV2:107,599	CNV1: 1 CNV2: 1	***CNV1:*** *COL3A1;* ***CNV2:*** *RYR1*	P; VOUS; AOH	Gastroschisis, intestinal alteration
26	19CT040686	Female	Seq (hg19) 2q22.3 (144542750_ 145228056) × 1, 16q23.1 (78388926_ 78423225) × 1	CNV1:685,307 CNV2: 34,300	CNV1: 2 CNV2: 1	***CNV1:*** *GTDC1, ZEB2;* ***CNV2:*** *WWOX*	P; LP	Developmental delay, dysmorphism, congenital heart disease
27	19CT037119	Female	Seq (hg19) Xp21.1 (31592844_ 32048417) × 1	CNV: 455,574	CNV: 1	***CNV:*** *DMD*	P	Autism
28	19CT038323	Female	Seq (hg19) 17p13.1 (7093128_ 7415897) × 1	CNV: 322,770	CNV: 25	***CNV:*** *ACADVL, CHRNB1*	LP	Central hypotonia, mild delayed milestones, soft dysmorphism
29	19CT018903	Female	Seq (hg19) 12q21.31 (80633447_ 80732246) × 1	CNV: 98,800	CNV: 1	***CNV:*** *OTOGL*	LP	Partial epilepsy with impairment of consciousness, active autistic disorder, delayed motor and language development, attention deficit disorder
30	19CT022820	Female	Seq (hg19) 17q21.31 (41521405_ 41735375) × 1	CNV:213,971	CNV: 3	***CNV:*** *MEOX1*	P	Developmental delay, speech delay, hitch hiker thumb, upslanting palpebral fissures, thin lips
31	19CT032039	Female	Seq (hg19) 5q23.2 (126605730_ 126669180) × 1	CNV: 63,451	CNV: 1	***CNV:*** *MEGF10*	P	Not provided
32	19CT011977	Male	Seq (hg19) 2q21.1 (131337236_ 131361335) × 1	CNV: 24,100	CNV: 1	***CNV:*** *CFC1*	P	Delayed motor and language development, developmental regression, intellectual disability
33	19CT014644	Male	Seq (hg19) 21q22.3 (43538247_ 48129895) × 1	CNV: 4,591,649	CNV: 55	***CNV:*** *TMPRSS3, RSPH1, WDR4, CBS, CRYAA, SIK1, PDXK, CSTB, AIRE, PFKL, CFAP410, TSPEAR, ITGB2, COL18A1, COL6A1, COL6A2, FTCD, LSS, MCM3AP, PCNT*	P	Multiple congenital malformations

(*table continues*)

Table 1 (*continued*)

No.	Sample ID	Sex	Nomenclature	Size, bp	OMIM genes, *n*	OMIM morbid genes	Classification	Clinical features
34	DS183979	Male	Seq (hg19) 2q37.1q37.3 (233643757_ 243199373) × 1	CNV: 9,555,617	CNV: 71	***CNV:*** *GIGYF2, ATG16L1, SAG, UGT1A1, COL6A3, MLPH, PER2, TRAF3IP1, TWIST2, NDUFA10, CAPN10, KIF1A, AGXT, D2HGDH, PDCD1*	P	Attention deficit hyperactive disorder, delayed language development, developmental regression, intellectual disability, short stature, no dysmorphism, first words at 5 years of age, hypopituitarism, neonatal hyperbilirubinemia, no consanguinity reported
35	DS180649	Male	Seq (hg19) 15q11.2q13.1 (23681146_ 28567945) × 3	CNV: 4,886,800	CNV: 20	***CNV:*** *MKRN3, MAGEL2, NDN, SNRPN, UBE3A, GABRB3, GABRA5, OCA2, HERC2*	P	Autism spectrum disorders, learning disability, fine motor delay, speech delay
36	DS185913	Male	Seq (hg19) 1q24.1 (165671310_ 165722209) × 0	CNV:50,900	CNV: 1	***CNV:*** *TMCO1*	P	Delayed motor development, delayed language development, developmental regression, intellectual disability, possible vascular malformation?, correction of palate pending, cleft palate, cerebral palsy
37	18PA005596	Male	Seq (hg19) 14q11.2q32.33 (20423820_ 107021901) × 2 hmz	CNV:86,598,082	CNV: 496	***CNV:*** *OSGEP, PNP, ANG, RPGRIP1, CHD8, SALL2, SLC7A7, MMP14, CEBPE, PABPN1, MYH6, MYH7, ZFHX2, NRL, PCK2, TINF2, TGM1, FOXG1, PRKD1, COCH, AP4S1, ATD, NUBPL, CFL2, PPP2R3C, PSMA6, NFKBIA, NKX2-1, PAX9, SEC23A, TRAPPC6B, FANCM, RPS29, MGAT2, DNAAF2, SOS2, L2HGDH, ATL1, NIN, PYGL, PTGDR, PTGER2, DDHD1, BMP4, GCH1, TMEM260, OTX2, KIAA0586, DACT1, SIX6, SIX1, TRMT5, PRKCH, SYNE2, ESR2, MTHFD1, SPTB, MAX, FUT8, GPHN, PIGH, RDH11, RDH12, ZFYVE26, ACTN1, SMOC1, PSEN1, DNAL1, COQ6, ALDH6A1, VSX2, ABCD4, NPC2, ISCA2, LTBP2, DLST, EIF2B2, MLH3, NEK9, FLVCR2, TTLL5, TGFB3, IFT43, ESRRB, IRF2BPL, POMT2, GSTZ1, VIPAS39, SPTLC2, TSHR, GALC, SPATA7, ZC3H14, TTC8, TDP1, CALM1, GPR68, CCDC88C, FBLN5, TRIP11, ATXN3, SLC24A4, SERPINA6, SERPINA1, SERPINA3, GSC, DICER1, GLRX5, TCL1B, TCL1A, AK7, VRK1, BCL11B, CCNK, EML1, YY1, DYNC1H1, TECPR2, TRAF3, AMN, MARK3, COA8, XRCC3, TDRD9, INF2, ADSS1, AKT1, ZBTB42, BRF1, PACS2*	P	Bell-shaped thoracic cage, congenital anomaly of spine, congenital hypotonia, micrognathia, scoliosis, congenital heart defects, dysmorphic facial features, polyhydramnios, rib anomalies, thoracic scoliosis, skin edema, umbilical cord abnormalities, ventricular septum abnormalities
38	DS182258	Male	Seq (hg19) Xp21.1 (32688830_ 32808729) × 0, 9q34.3 (140198815_ 140598814) × 1	CNV1: 119,900 CNV2: 400,000	CNV1: 1 CNV2: 8	***CNV1:*** *DMD;* ***CNV2:*** *NSMF, EHMT1*	P; LP	Growth hormone deficiency, Duchenne muscular dystrophy, 9q deletion

(*table continues*)

Table 1 (*continued*)

No.	Sample ID	Sex	Nomenclature	Size, bp	OMIM genes, *n*	OMIM morbid genes	Classification	Clinical features
39	19CT031828	Male	Seq (hg19) 8q23.3 (116650473_ 116795610) × 1	CNV:145,138	CNV: 1	***CNV:*** *TRPS1*	P	Clinical suspicion of trichorhinophalangeal syndrome
40	19CT036291	Male	Seq (hg19) 2q37.1 (232900013_ 233272231) × 3, 17q12 (34808905_ 36284204) × 1	CNV1:372,219 CNV2: 1,475,300	CNV1: 3 CNV2: 21	***CNV1:*** *DIS3L2;* ***CNV2:*** *ZNHIT3, PIGW, ACACA, HNF1B*	P; VOUS	Autism, diplegic cerebral palsy, partial idiopathic epilepsy with seizures of localized onset
41	19CT035796	Male	Seq (hg19) 2p16.3 (48013836_ 48076735) × 1	CNV:62,900	CNV: 2	***CNV:*** *MSH6, FBXO11*	P	Hypotonia, delayed motor and language development, developmental regression, intellectual disability
42	19CT035800	Male	Seq (hg19) 21q22.13q22.2 (38558547_ 40076346) × 1, 21q22.3 (43845295_ 44088388) × 3, 21q22.3 (46064045_ 47385246) × 1	CNV1:1,517,800 CNV2:243,094 CNV3:1,321,202	CNV1: 8 CNV2: 4 CNV3: 12	***CNV1:*** *DYRK1A, KCNJ6;* ***CNV2:*** *RSPH1;* ***CNV3:*** *TSPEAR, ITGB2, COL18A1*	P; VOUS; P	Microcephaly
43	19CT035812	Male	Seq (hg19) 3q29 (197354615_ 197686192) × 3, 7p14.1 (40150784_ 40321018) × 3, 7q11.23 (72648431_ 74149730) × 1	CNV1: 331,578 CNV2:170,235 CNV3:1,501,300	CNV1: 4 CNV2: 2 CNV3: 26	***CNV1:*** *RUBCN, RPL35A;* ***CNV2:*** *MPLKIP, SUGCT;* ***CNV3:*** *ELN*	VOUS; VOUS; P	Congenital malformations, cardiac abnormalities, clinical suspicion of Williams syndrome
44	19CT038105	Male	Seq (hg19) (1-22) × 2, (X) × 1∼2, (Y) × 0∼1	Mosaic X (1-2 copies); mosaic Y (0∼1)	Entire X and Y chromosomes	Entire X and Y chromosomes	P	Recurrent miscarriages
45	18PA007554	Male	Seq (hg19) 8q24.3 (142647831_ 146364022) × 3, 22q13.31q13.33 (46709933_ 51304566) × 1	CNV1:3,716,192 CNV2: 4,594,634	CNV1: 59 CNV2: 32	***CNV1:*** *SLURP1, CYP11B1, CYP11B2, GPIHBP1, MAFA, FAM83H, PUF60, PLEC, OPLAH, GPAA1, CYC1, DGAT1, SLC52A2, SLC39A4, TONSL, RECQL;* ***CNV2:*** *TRMU, ALG12, MLC1, TUBGCP6, SBF1, SCO2, TYMP, CHKB, ARSA, SHANK3, ACR*	P	Developmental delay, seizures, dysmorphism
46	18PA008791	Male	Seq (hg19) Xp22.12 (20001830_ 20496429) × 2	CNV:494,600	CNV: 2	***CNV:*** *RPS6KA3*	P	Ventriculomegaly, suspected neuronal migration defect, microcephaly
47	18PA008616	Male	Seq (hg19) 9q34.11 (130395515_ 130422214) × 1	CNV:26,700	CNV: 1	***CNV:*** *STXBP1*	P	Generalized convulsive epilepsy, autism
48	DS180675	Male	Seq (hg19) 5q35.3 (178,930,830_ 179,633,929) × 1 dn, 13q31.1q31.3 (86,587,765_ 92,648,539) × 2 hmz	CNV:703,100 AOH:6,060,775	CNV: 9 AOH: 9	***CNV:*** *LTC4S, SQSTM1;* ***AOH:*** *MIR17HG*	P; AOH	Brain atrophy, delayed motor development, global developmental delay, leukocyte abnormalities, epilepsy, severe language development delay, slight hepatosplenomegaly, microcephaly, long face.

(*table continues*)

Table 1 (*continued*)

No.	Sample ID	Sex	Nomenclature	Size, bp	OMIM genes, *n*	OMIM morbid genes	Classification	Clinical features
49	18PA002170	Male	Seq (hg19) 22q11.21 (18884533_ 20347332) × 1, 22q11.21 (20717933_ 21520532) × 1	CNV1: 1,462,800 CNV2: 802,600	CNV1: 30 CNV2: 12	***CNV1:*** *PRODH, SLC25A1, CDC45, GP1BB, TBX1, TXNRD2, COMT, TANGO2, RTN4R;* ***CNV2:*** *SCARF2, SERPIND1, PI4KA, SNAP29, LZTR1*	P	Malformation of heart and great vessels, congenital cardiomyopathy, large interventricular perimembrane, stenosis of the left pulmonary artery, compression of the esophagus
50	19CT040646	Male	Seq (hg19) 22q11.21 (18884633_ 20351032) × 1, 22q11.21 (20703833_ 21520632) × 1	CNV1: 1,466,400 CNV2: 816,800	CNV1: 30 CNV2: 12	***CNV1:****PRODH, SLC25A1, CDC45, GP1BB, TBX1, TXNRD2, COMT, TANGO2, RTN4R;* ***CNV2:*** *SCARF2, SERPIND1, PI4KA, SNAP29, LZTR*	P	Mother with history of tetralogy of Fallot and clinical diagnosis of DiGeorge syndrome
51	19CT041737	Male	Seq (hg19) 1q21.1q21.3 (145365236_ 151481581) × 3	CNV: 6,116,346	CNV: 81	***CNV:*** *HJV, RBM8A, PEX11B, GJA5, GJA8, FCGR1A, SF3B4, VPS45, PRPF3, TARS2, ECM1, ADAMTSL4, CTSK, PRUNE1, ZNF687, RFX5, SELENBP1, PSMB4, POGZ*	P	Mixed receptive-expressive language disorder, unspecified congenital malformation of face and neck, unspecified childhood disorder of social functioning
52	DS181740	Male	Seq (hg19) 7q35q36.1 (145448731_ 148600230) × 1, 7q36.1 (148843231_ 151338630) × 1, 7q36.1q36.3 (152440831_ 158744830) × 1	CNV1: 3,151,500 CNV2: 2,495,400 CNV3: 6,304,000	CNV1: 3 CNV2: 37 CNV3: 19	***CNV1:*** *CNTNAP2, EZH2;* ***CNV2:*** *KCNH2, NOS3, CDK5, ASB10, PRKAG2;* ***CNV3:*** *DPP6, SHH, LMBR1, MNX1, DNAJB6, NCAPG2, WDR60*	P	Microcephaly, dysmorphism, developmental delay, squint, hypospadiasis, caudal regression, bulbous tip of nose, prominent little finger pads, high arched palate
53	19CT045173	Male	Seq (hg19) 16p11.2 (28824426_ 29052425) × 1, 22q11.21 (18890033_ 20325632) × 1	CNV1:228,000 CNV2:1,435,600	CNV1: 9 CNV2: 30	***CNV1:*** *TUFM, ATP2A1, CD19, LAT;* ***CNV2:*** *PRODH, SLC25A1, CDC45, GP1BB, TBX1, TXNRD2, COMT, TANGO2, RTN4R*	P	Behavioral and developmental disorders
54	19CT038102	Male	Seq (hg19) 15q26.3 (100809220_ 100843345) × 1	CNV: 34,126	CNV: 1	***CNV:*** *ADAMTS17*	LP	Autism spectrum disorder
55	19CT026287	Male	Seq (hg19) 3p26.1 (4398615_ 4478037) × 1, 19p13.2 (7199291_ 7300940) × 3	CNV1:79,423 CNV2:101,650	CNV1: 1 CNV2: 1	***CNV1:*** *SUMF1;* ***CNV2:*** *INSR*	LP; VOUS	Abnormal internal genitalia, cleft palate with bilateral cleft lip
56	19CT035296	Male	Seq (hg19) 2p16.3 (50629310_ 50730635) × 1	CNV: 101,326	CNV: 1	***CNV:*** *NRXN1*	P	Global developmental delay, hypotonia, family history of spinal muscular atrophy type 1
57	19CT012365	Male	Seq (hg19) 10p12.1 (27359279_ 27391022) × 1	CNV:31,744	CNV: 1	***CNV:*** *ANKRD26*	P	Microcephaly, irregular fingers, toes, flat foot, bat-like ears, dysmorphic facial features, speech and motor delay, intellectual disability, developmental delay

In cases where there is more than one classification, please follow the classification based on the International System for Human Cytogenetic Nomenclature for multiple variants [CNV1, CNV2, CNV3, etc (in bold)] in the same order.

ADHD, attention-deficit/hyperactivity disorder; AOH, absence of heterozygosity; ASD, atrial septal defect; BoBs, bacterial artificial chromosomes (BACs) on beads; CNV, copy number variant; ID, identifier; IUGR, intrauterine growth retardation; LP, likely pathogenic; LP-GS, low-pass genome sequencing; OMIM, Online Mendelian Inheritance in Man; P, pathogenic; VOUS, variant of uncertain significance; VSD, ventricular septal defect.

Table 2 Summary of 5X LP-GS Testing in 331 Clinical Cases

P/LP				VOUS	AOH	Neg
57 (17.2)				57 (17.2)	27 (8.2)	190 (57.4)
Type of CNV	Diagnostic findings related to phenotype	Carrier findings	Diagnostic findings unrelated to phenotype			
	49 (86.0)	7 (12.3)	1 (1.8)			
Microdeletion/ microduplication syndromes	33 (67.3)	1				
Intragenic	10 (20.4)	6	1			
Trisomy	4 (8.2)					
Triploidy	1 (2.0)					
UPD	1 (2.0)					

Data are given as *n* (%). $N = 331$ total cases; $n = 180$ males; $n = 151$ females.

AOH, absence of heterozygosity; CNV, copy number variant; LP, likely pathogenic; LP-GS, low-pass genome sequencing; Neg, negative; P, pathogenic; UPD, uniparental isodisomy; VOUS, variant of uncertain significance.

M.R.H.). A standard analysis pipeline was established for the analysis and interpretation of benign CNVs published in population databases, such as Database of Genomic Variants and other databases to assess the pathogenicity of a CNV [Online Mendelian Inheritance in Man (OMIM), Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER), Human Gene Mutation Database (HGMD), Clinical Genome Resource dosage sensitivity map, and PubMed].

Representative Clinical Cases

Detection of Single-Exon CNV (Exon 1 of the *TRPS1* Gene)

A blood sample from a 13-year−old male was referred for LP-GS testing with a clinical suspicion of trichorhinophalangeal syndrome. An approximately 14,138-bp heterozygous copy number loss of chromosome 8q23.3 was detected (Figure 2A) [International System for Human Cytogenetic Nomenclature (ISCN): seq(hg19) 8q23.3(116650473_116795610) × 1]. This region encompassed the 5′ untranslated region and exon 1 of the *TRPS1* gene associated with autosomal dominant trichorhinophalangeal syndrome types I and III (OMIM number 604386). *TRPS1* encodes a transcription factor involved in the regulation of the genes that control bone and cartilage growth in skeletal development. Multiple studies have reported disease phenotypes associated with intragenic and whole gene deletions of *TRPS1*, which was consistent with the clinical phenotype submitted for clinical testing in this individual.[14−16]

Detection of Trisomy 13 and Mosaic Monosomy X

A dual diagnosis of a double aneuploidy was detected in a products of conception specimen from a spontaneous loss of pregnancy. This specimen was submitted for the LP-GS test, which demonstrated both trisomy 13 and mosaic monosomy X in Figure 2B [ISCN: seq (hg19) 13q11q34(19027040_115169878) × 3, Xp22.33q28(1 to 155,270,560) × 1 approximately 2]. The prevalence and frequency of 45,X in literature on pregnancy losses and live births demonstrates that approximately 99% nonmosaic Turner syndrome fetuses do not survive gestation.[17] A retrospective cohort study showed live births among 50% of individuals with a prenatal diagnosis of trisomy 13 syndrome.[18] Trisomy 13 conceptuses have life-threatening medical complications, and if live-born, the median survival time is 7 to 10 days.[19]

Detection of Uniparental Isodisomy 14

A dried blood spot sample from a 1-month−old male with bell-shaped thoracic cage, congenital anomaly of spine, congenital hypotonia, micrognathia, scoliosis, congenital heart defects, dysmorphic facial features, polyhydramnios, rib anomalies, thoracic scoliosis, skin edema, umbilical cord abnormalities, and ventricular septum abnormalities was submitted for the LP-GS test. AOH for the entire chromosome 14 (14q11.2q32.3) was identified in this assay [ISCN: seq (hg19) 14q11.2q32.33(20423820_107021901) × 2 hmz] in Figure 2C. This finding is consistent with uniparental isodisomy of chromosome 14 (OMIM number 608149). The clinical features of paternal UPD 14 were consistent with the clinical findings of this individual.[20]

Dual Diagnosis of Kleefstra Syndrome + DMD

An 8-year−old male presented with clinical features suggestive of Kleefstra syndrome 1, growth hormone deficiency, and muscular dystrophy. Previous genetic testing by chromosomal microarray identified a 9q34.3 microdeletion involving the *EHMT1* gene, consistent with a diagnosis of Kleefstra syndrome. However, because muscular dystrophy is not a clinical feature reported in individuals with Kleefstra syndrome, 5X LP-GS testing was ordered. In addition to the previously identified 9q34.3 deletion involving the *EHMT1* gene (Figure 3A), the 5X LP-GS test identified a pathogenic

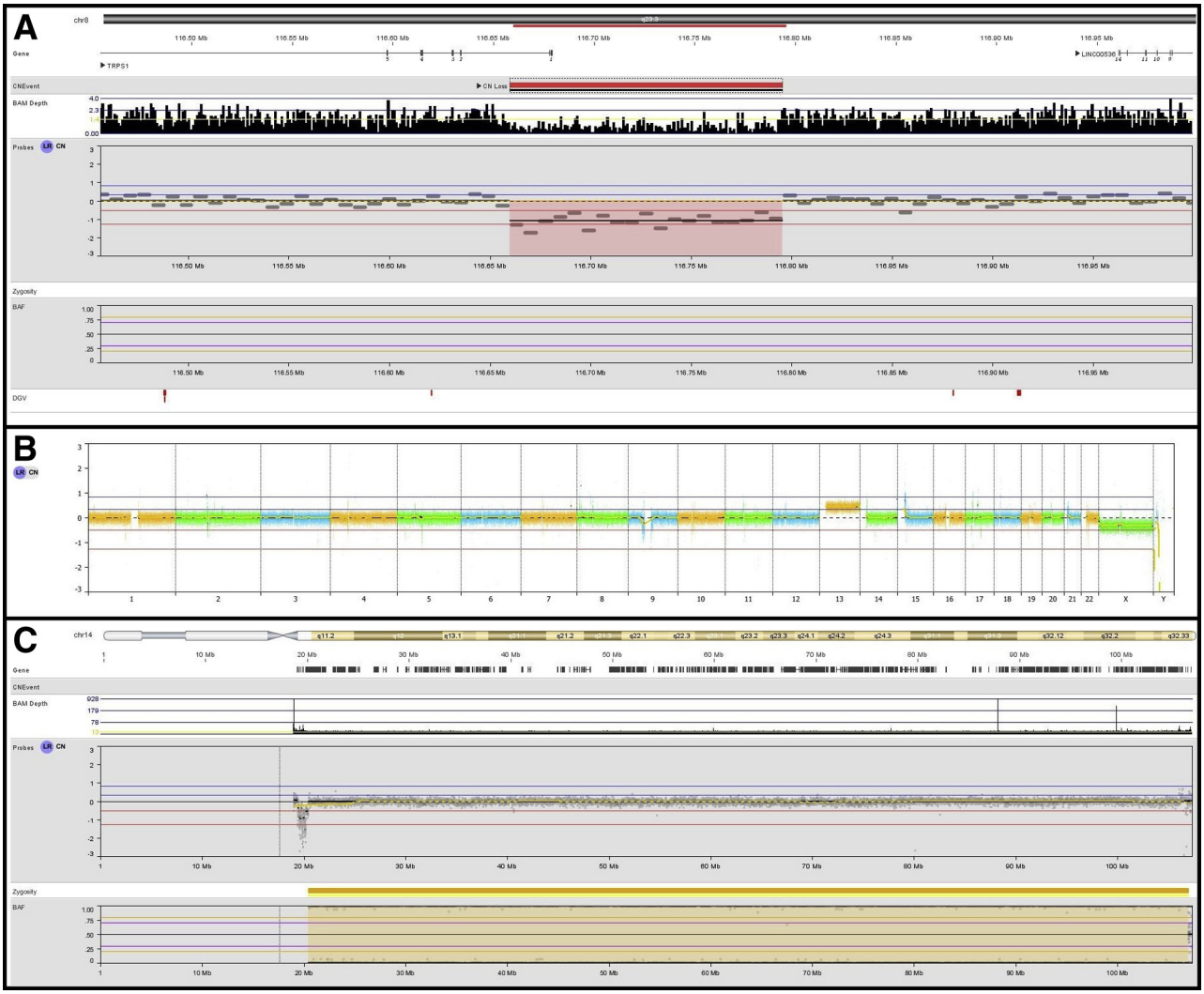

Figure 2 **A:** Intragenic copy number (CN) loss of *TRPS1* gene on chromosome (chr) 8q23.3. CN variant analysis was performed using Biodiscovery's NxClinical 5.0 software. The BAM depth track depicts the sequencing reads in this sample. The probes track depicts the virtual probes calculated using the Multi Scale Reference (MSR) algorithm, and the CN track within the probes shows the copy number state of 1 encompassing the 5′ untranslated region and exon 1 of the *TRPS1* gene. **B:** Dual diagnosis of a double aneuploidy in a products of conception specimen. Whole genome view track from NxClinical 5.0 software shows both trisomy 13 and mosaic monosomy X, both of which are common aneuploidies in pregnancy losses. Virtual probes (generated by the MSR algorithm) as the log ratio (LR)/CN state tracks are shown across the genome with different alternating colors per chromosome. A moving average of the copy number of the probes. Chr 1-22, X, and Y are shown from left to right. Chr 13 shows a CN state of 3, whereas chr X shows a CN state of approximately 1.5 (mosaic monosomy X). **C:** Detection of absence of heterozygosity of chromosome 14 (yellow highlight of entire chr 14) demonstrating uniparental isodisomy 14 by NxClinical 5.0 software. The BAM depth and probes depict a CN state of 2, whereas the B-allele frequency (BAF) track shows loss of heterozygous probes (BB = 1.00; AA = 0). DGV, Database of Genomic Variants.

intragenic deletion of the *DMD* gene (Figure 3B). This copy number loss (heterozygous deletion) was approximately 119 kb in size and included exons 8 to 9 of the *DMD* gene [ISCN: seq (hg19) Xp21.1(32688830_32808729) × 0, 9q34.3(140198815_140598814) × 1], which results in an out-of-frame deletion (*http://www.dmd.nl*, last accessed December 1, 2019). The results of the 5X LP-GS test were consistent with the patient's additional clinical feature of muscular dystrophy (an additional diagnosis of Duchenne/Becker muscular dystrophy).

Blending of the Phenotypes due to Two Pathogenic CNVs

An 11-year−old male presented with aggressive tantrums along with language delay (starting at 5 years of age) and motor delay. Karyotyping studies performed on this individual were negative. The detection of two pathogenic deletions, 16p11.2 distal deletion syndrome (Figure 4A) and the 22q11.2 deletion in Figure 4B (DiGeorge/velocardiofacial syndrome deletion) [ISCN: seq (hg19) 16p11.2(28824426_29052425) × 1, 22q11.21(18890033_20325632) × 1] is shown. Looking at the clinical features reported with these two microdeletions, this

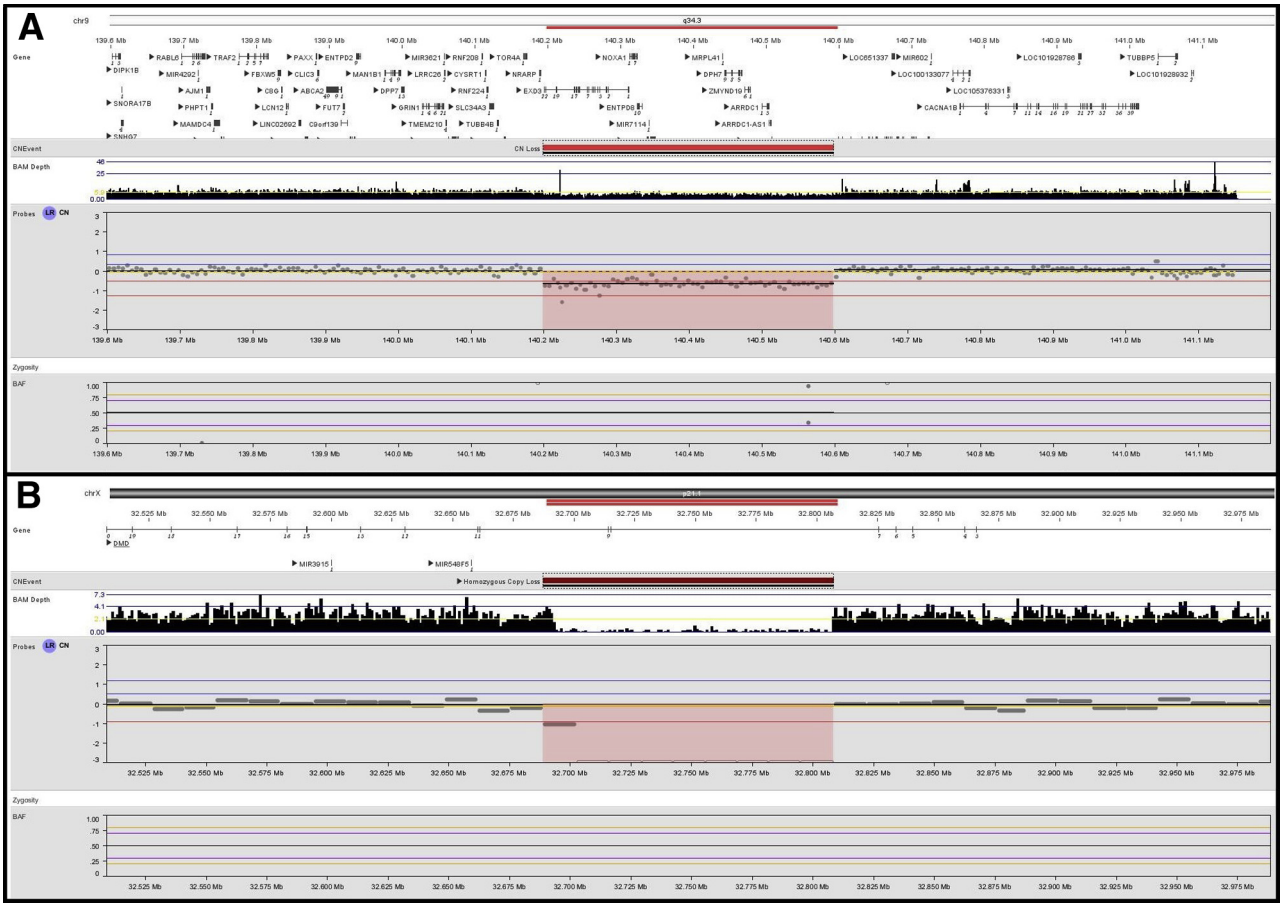

Figure 3 A case of dual diagnosis of Kleefstra syndrome + Duchenne muscular dystrophy (DMD). Although the chromosomal microarray (CMA) platform detected the *EHMT1* deletion (**A**), the intragenic *DMD* deletion (exons 8 to 9; **B**) was picked up by the low-pass genome sequencing test. Copy number (CN) variant analysis performed using Biodiscovery's NxClinical 5.0 software shows the BAM depth track depicting the sequencing reads in this sample. The probes track depicts the virtual probes (CN state = 0) calculated using the Multi Scale Reference algorithm and demonstrates the lack of sequencing reads (sharp dip in the BAM depth track) in a male with only one copy of the X chromosome (chr). BAF, B-allele frequency; CN, copy number; LR, log ratio.

patient has a blended phenotype with significant overlap, including intellectual disability and behavioral problems, between the 16p11.2 deletion and 22q11.21 deletion. Independently, the 16p11.2 deletion contributes to the following clinical features, such as developmental delay, obesity, behavioral problems, schizophrenia, and craniofacial dysmorphism (OMIM number 613444), whereas the 22q11.2 deletion is characterized by congenital heart defect, cleft palate, immunodeficiency, inner ear anomalies, dysmorphism, intellectual disability, hypocalcemia, short stature, neuropsychiatric and behavioral disorders, renal and urinary tract anomalies, gastrointestinal anomalies, and seizures (OMIM number 188400).

Discussion

CMA has been the first-tier test for individuals with developmental delay, ID, autism, congenital anomalies, and dysmorphic features.[4] For over a decade, diagnostic laboratories have been using the CMA test for routine clinical use. With the reducing cost of sequencing technologies and a focus on generating a comprehensive platform for assessing clinically significant SNVs and CNVs from a single assay, several studies have demonstrated the clinical utility or diagnostic yield of genome sequencing technologies (GS at $\geq$30X depth).[21–25] A position statement of the American College of Medical Genetics and Genomics on the clinical utility of genetic and genomic services paves the way for GS becoming a standard of care.[26] Similarly, several studies have documented the use of low-pass or low-resolution GS ($\leq$5X depth) as a tool to validate clinically relevant CNVs in a prenatal setting at a significantly reduced cost than CMA.[12] One of the major considerations for validating an LP-GS based test is the average coverage or depth needed across the genome to be equivocal to the gold standard CMA test. Studies by Dong et al[27] (2017) have shown the reliable performance of low-pass GS performed at 0.25X coverage for CNV detection. Our assessment of the performance of LP-GS at <2X depth demonstrates that this depth does not allow for accurate genotyping of the single-nucleotide polymorphisms to be used for B-allele frequency determination, which allows for the

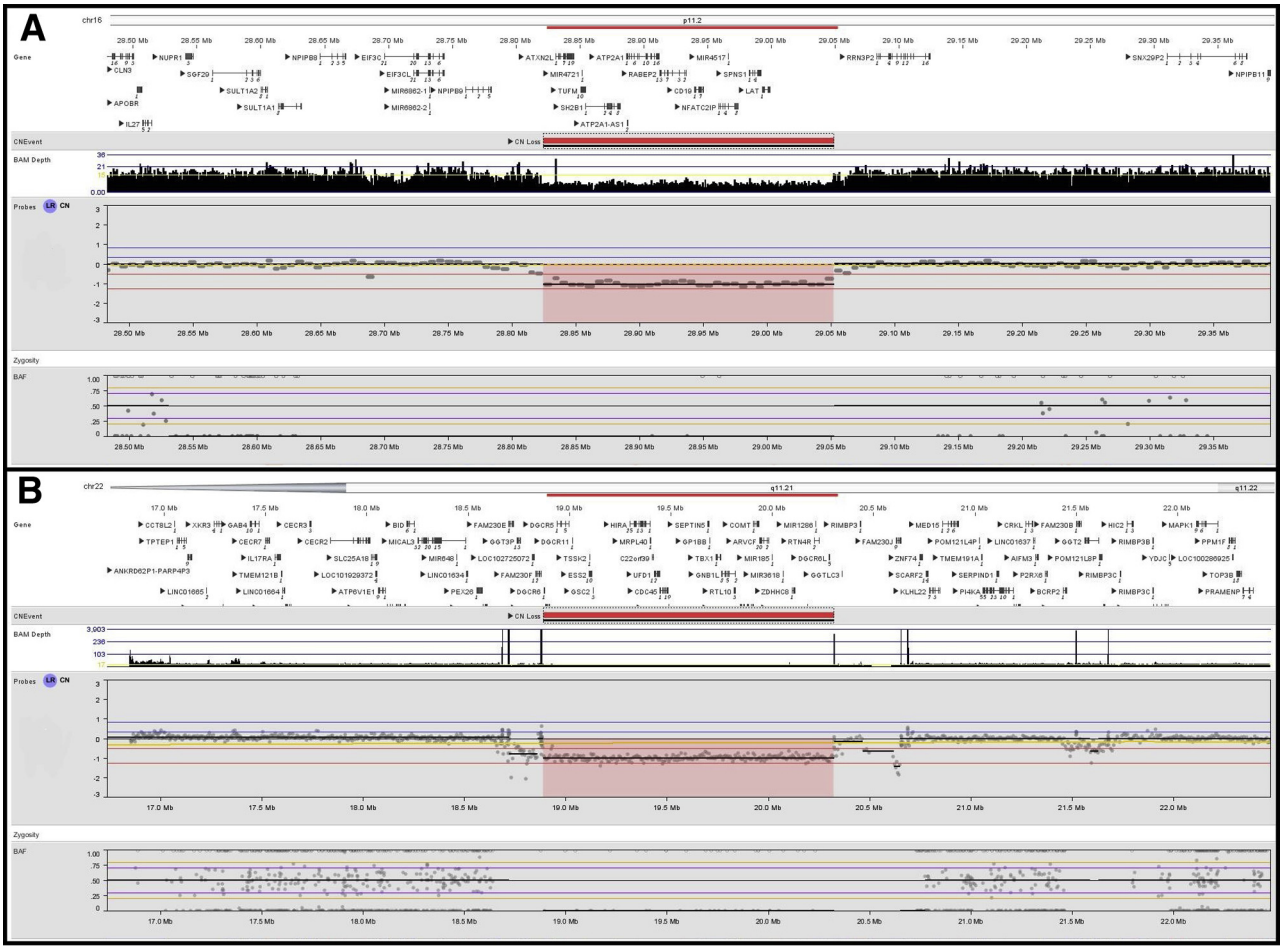

Figure 4 A case of blended diagnosis of 16p11.2 distal deletion syndrome (**A**) and 22q11.21 deletion syndrome (**B**). Copy number variant (CNV) analysis was performed using Biodiscovery's NxClinical 5.0 software and shows the BAM depth track depicting the sequencing reads in this sample. The probes track depicts the virtual probes (CN state = 1) calculated using the Multi Scale Reference algorithm for both pathogenic CNVs. BAF, B-allele frequency; chr, chromosome; CN, copy number; LR, log ratio.

detection of regions of homozygosity/AOH or uniparental isodisomy specimens (A.C., S.S., M.R.H., Z.M., E.S., A.M., L.S., R.L., and B.R.R.N., unpublished data). This is consistent with the 1000 Genome data, where approximately 8X sequencing depth is needed for reliable assessment of the single-nucleotide polymorphisms. Hence, we assessed that approximately 5X depth of the LP-GS test is required to reliably and accurately detect microdeletions/microduplications, intragenic deletions/duplications, and AOH regions. The validation study on 78 samples at approximately 5X LP-GS analysis demonstrates the ease of use and diagnostic yield (or clinical utility) of using an LP-GS platform as a replacement to CMA. Experiments were performed to compare approximately 30X and approximately 5X sequencing data from six samples, which demonstrated the sequencing variant concordance across the genome. Three haplotype map samples run in three independent runs also demonstrated significant CNV concordance between the published CNV data.[13] The LP-GS test performed on 33 samples with previously characterized CNVs by karyotyping, fluorescence *in situ* hybridization, and/or CMA demonstrated 100% specificity, sensitivity, accuracy, and precision. Data analysis and interpretation and reporting of CNV data from GS assays have been a challenge for the past decade for clinical/diagnostic laboratories. An evaluation of an Edico home brew tool, Variantyx, Golden helix, and BioDiscovery's NxClinical 5.0 software on the 5X LP-GS data set revealed NxClinical software to have superiority of CNV visualization, analysis, and classification of CNVs for reporting (A.C. and M.R.H., unpublished data). In addition, challenging samples, such as a mosaic interstitial deletion, mosaic trisomy, and mosaic marker chromosome delineation, were only identified by NxClinical software (Table 1). Assessing AOH regions can be difficult by various low-pass GS methods, and the 1000 Genome data have established that approximately 8X coverage and depth is needed for single-nucleotide polymorphism accuracy from GS data. Our data set with AOH samples also demonstrated that approximately 5X LP-GS has better accuracy at detecting multiple AOH samples (from established cases of consanguinity) and uniparental isodisomy (Supplemental Table S2).

In addition, the diagnostic yield of the 5X LP-GS test was assessed on 331 samples received in a global laboratory

network. The diagnostic yield of the 5X LP-GS test (17.2%) for pathogenic/likely pathogenic CNVs was comparable to the published CMA yield (approximately 5% to 18%), depending on the array platform used in clinical practice. In addition, variants of uncertain significance and AOH regions were identified that were recommended for additional testing to resolve the variants of uncertain significance and identify diagnostic homozygous pathogenic variants by higher-depth sequencing (approximately 30X). Additional testing of the CNVs detected by LP-GS was not needed on the basis of the CNV concordance (by karyotyping, fluorescence *in situ* hybridization, and/or array comparative genomic hybridization) detected from the exhaustive validation performed in the laboratory.

Several of the clinically used CMA platforms worldwide have different capabilities and limits of CNV and AOH detection (Supplemental Tables S5 and S6). The detection of CNVs by these platforms relies heavily on the coverage and placement of probes across the genome and hence clinically significant CNVs can be missed for CNVs that occur in off-target regions. In the dual diagnosis case of Kleefstra syndrome + DMD, the array platform picked up the *EHMT1* intragenic deletion but missed the two-exon *DMD* deletion simply because of lack of probe placement over those two exons within the *DMD* gene (Figure 3A). Notably, both deletions including the *EHMT1* and *DMD* genes were detected by the 5X LP-GS. This confirmation of the diagnosis of the DMD disorder by the 5X LP-GS test will allow for improved medical management of the index patient while also having significant clinical counseling implications for the patient's mother and other family members. This case demonstrates the bias in probe location and resolution of the microarray platform used and the superiority of the 5X LP-GS test, which entails a uniform, nonbiased GS-based method for CNV detection.

Not only have we detected cases of dual diagnosis, but also cases where two CNVs are contributing to a blended phenotype (Figure 4). This highlights the power and potential of the 5X LP-GS test over all the current methods used for clinical testing and patient care.

A cost comparison of multiplexing low-pass genome samples was performed, and the sequencing capabilities of a low-pass genome were assessed. As per capacity described by Illumina, NovaSeq can run 48 and 144 5X LP-GS on an S2 and S4 flow cell, respectively, which is 50% reduction in cost compared with microarray (exact amount not stated as manufacturer discounts vary). This demonstrates the tremendous reduction in per-sample sequencing cost and is more cost-effective than any CMA platform being used for clinical testing worldwide.

The ability of LP-GS at approximately 5X depth to accurately detect CNVs and AOH from the entire human genome at a significantly lower cost has also been established by multiple assays performed in this study.

The detection of pathogenic CNVs by CMA has been widely used as a gold standard. The performance metrics of 5X LP-GS were established to be better than standard CMA used previously for characterization of samples used in this study. Specifically, the 5X LP-GS approach was an effective method for the diagnosis of chromosomal disorders or microdeletion/microduplication syndromes. Our clinical testing experience has allowed us to identify intragenic deletions and duplications, cases with dual diagnosis, and blended phenotypes and has the ability to use different types of clinical samples without any bias in compromising the 5X LP-GS results. Clinical specificity and sensitivity on the validation data set demonstrate 100% accuracy when compared with samples previously run by conventional methods, such as karyotyping, fluorescence *in situ* hybridization, and CMA. The clinical samples received from Malaysia, Columbia, and India demonstrate the usefulness of a high-performing, affordable GS test for individuals where self-pay for genetic testing is a norm. Unlike reported publications of approximately 0.25X GS, the 5X LP-GS test detected clinically relevant CNVs and AOH, which was the determining factor to maintain a depth of coverage at approximately 5X for validating this low-pass GS as a clinical test. Currently, our analysis pipeline is unable to detect inversions and balanced translocations, and future work will be on optimizing our software tools for their detection. Because of the lower cost, higher resolution, and sensitivity, LP-GS is predicted to eventually replace CMA. Compared with CMA, NGS methods for GS are an alternative state-of-the-art technology promising improved detection of genetic abnormalities with unprecedented resolution.

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Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2020.03.008*.

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