Review

The Spectrum of Cytogenomic Abnormalities in Patients with Developmental Delay and Intellectual Disabilities

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Current clinical cytogenomics laboratory uses array comparative genomic hybridization (aCGH) or single nucleotide polymorphism (SNP) chip as first-tier test supplemented with routine karyotyping and fluorescent in situ hybridization (FISH) for patients with developmental delay (DD), intellectual disability (ID), multiple congenital anomalies (MCA) and autistic spectrum disorders (ASD). A spectrum of cytogenomic abnormalities including numerical chromosomal abnormalities, unbalanced and balanced structural and cryptic rearrangements, and recurrent genomic disorders have been detected 10~20% of patients with DD/ID/MCA/ASD and collectively present in approximately 0.8% of a general population. The characterization of genomic coordinates and gene contents for these abnormalities has enabled accurate mapping of candidate genes and correlating genotypes with phenotypes and thus more informative genetic counseling. Future application of WGS will expand this spectrum of cytogenomic abnormalities by including complex and cryptic structural variants. Further delineation of molecular mechanisms of these cytogenomic abnormalities and development of novel therapeutic approaches will ultimately lead to disease-specific personalized management and precision treatment.
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INTRODUCTION

In the field of medical genetics, technologic innovations have been the driving force in improving the efficacy of genetic diagnosis and in expanding the spectrum of disease-causing mutations.[1] Clinical cytogenetics, as an integral part of medical genetics, has been routinely applied to the study of human chromosomal abnormalities and their correlated disease manifestations from patients with developmental delay (DD), intellectual disability (ID), multiple congenital anomalies (MCA) and autistic spectrum disorders (ASD). A conventional cytogenetics laboratory detects numerical and structural chromosomal abnormalities using cell-based Giemsa-stained banding pattern (G-band) on metaphase chromosomes and fluorescent in situ hybridization (FISH) analysis on metaphase chromosomes or interphase chromatin. In the past decade, the first-tier application of DNA-based oligonucleotide array comparative genomic hybridization (aCGH) or single nucleotide polymorphism (SNP) chip analysis has enabled the detection of pathogenic copy number variants (CNVs) and thus evolved clinical cytogenetics to cytogenomics.[2,3] Curated clinical resources for evidence-based interpretation of cytogenomic findings have been established.[4] This review outlines the analytical validities of key cytogenomic technologies, highlights the diagnostic efficacy for the spectrum of cytogenomic abnormalities, and discusses the potential applications of next generation whole genome sequencing (WGS). The continuous progress from clinical cytogenomic service has resulted in rational disease classification and better genetic counseling and concurrently promoted further basic research to dissect the molecular mechanisms and to develop therapeutic interventions for patients affected by cytogenomic abnormalities.

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KEY TECHNOLOGIES IN A CLINICAL CYTO-GENOMIC LABORATORY

In 1956, Tjio and Levan correctly described that a normal human metaphase contains 46 chromosomes.[5] This observation allowed the identification of numerical chromosomal abnormalities like trisomy 21 for Down syndrome, 45,X for Turner syndrome, 47,XXY for Klinefelter syndrome, trisomy 13 for Patau syndrome, and trisomy 18 for Edwards syndrome.[6-10] In 1968, Caspersson et al. reported differentiate Quinacrine staining of chromosomes and prompted the development of various chromosome banding techniques.[11] Giemsa staining on trypsin-treated chromosome spreads presents unique G-band 'barcodes' for

each pair of chromosomes under a microscope. A normal human G-band ideogram was created as a standard for accurate grouping, numbering and pairing of human chromosomes based on their size, centromere position, defined regions and bands; this organized chromosomal profile of an individual is referred to as a karyotype.[12] Despite an effective tool to detect numerical and structural chromosomal abnormalities, the banding method has two obvious technical limitations: the requirement of viable tissues for setting up cell culture to capture metaphases and the low analytical resolution of chromosomal G-bands. The size of a human genome is 3,000 Mb (megabases) and estimated total number of protein-coding genes is about 20,000. So the average size of a chromosome G-band in a medium 500-band level is about 6 Mb and contains 40 coding genes. The lack of genomic mapping for involved gene content of many detected chromosomal abnormalities had been the major obstacles for accurate karyotype-phenotype correlation and candidate gene identification.

In 1982, FISH technology using labeled DNA probes hybridized onto metaphase chromosomes was developed to map genes onto specific chromosomal G-band regions.[13] This gene mapping tool was immediately applies to cytogenetic diagnosis. FISH on metaphase chromosomes, using labeled DNA probes in the size of 100-800 kilobase (Kb), has enhanced the analytical resolution and allowed accurate diagnosis of submicroscopic genomic disorders (also termed contiguous gene syndromes or microdeletion syndromes), such as DiGeorge syndrome (OMIM#188400) by a deletion at 22q11.2, Prader-Willi syndrome (OMIM#176270) and Angelman syndrome (OMIM#105830) by a deletion at 15q11.2. FISH can also be performed directly on interphase nuclei, which overcame the limitation of cell culture and extended its diagnostic application toward rapid screening of chromosomal and genomic abnormalities. Multiplex FISH panels with differentially labeled probes have been developed for prenatal screening of common aneuploidies involving chromosomes X, Y, 13, 18 and 21 and for postnatal detection of cryptic subtelomeric rearrangements.[14,15] The abnormality detection rate is 3.7% by conventional karyotyping for large numerical and structural chromosomal abnormalities and up to 6.8% when combined with FISH analysis for targeted genomic disorders and subtelomeric rearrangements.[16]

In 1992, to overcome frequent cell culture failure and poor metaphase quality in karyotyping solid tumor samples, Kallioniemi et al. developed comparative genomic hybridization (CGH) using differently labeled test and control DNAs co-hybridized onto normal metaphase chromosomes to measure copy number changes.[17] In 1995, Schena et al. developed a microarray-based technology to quantitatively monitor multiple gene expression.[18] A hybrid of these CGH and microarray technologies formed the novel aCGH technology for a high resolution analysis of copy number changes through the genome. A decade later, high density oligonucleotide microarrays or SNP chips following industrial standards along with user-friendly analytical software packages were developed by several companies. These genomic technologies filled the gap between the Mb-range chromosome G-bands and Kb-level gene structure and were quickly validated for diagnosis of CNVs.[19] CNVs are defined as gains or losses of genomic materials larger than 1 Kb in size and they present as benign polymorphic in approximately 12% of the genome from normal human populations.[20] Given the analytical resolution of 50~300 Kb for a routine aCGH, the clinical sensitivity is close to 100% and the clinical specificity is estimated to be 99.4% for detecting a reference set of recurrent microdeletions and microduplications ranging from 500 Kb-3.0 Mb.[21] A multi-center comparison of 1,499 patients using the same oligonucleotide platform (Agilent 44K) showed a 12% abnormality detection rate, and about 53% of the abnormal findings are less than 5 Mb and thus beyond the analytical resolution of routine karyotyping.[22] Although cytogenetic testing has gradually become a supplemental or confirmatory procedure, karyotyping is still the gold standard to detect numerical chromosomal abnormalities and structural rearrangements and FISH is also the 'cell-based' method of choice to determine mosaic patterns. Approximately 45% of genomic imbalances are larger than 3-5 Mb and could be confirmed by high resolution G-banding; most recurrent genomic disorders, subtelomeric rearrangements and mosaic patterns can be readily confirmed by clinically-validated commercial FISH probes.

An international system for human cytogenetic nomenclature (ISCN) was first introduced in 1978 and has been continuously updated to the current 2013 version for a systematic documentation of chromosomal and genomic abnormalities.[12] The analytical validities and technical capacity of chromosome, FISH and aCGH are summarized in **Table 1**. Recently, WGS has been used to study balanced chromosomal rearrangements and hidden genomic rearrangements.[23] Nomenclature for describing chromosomal and genomic rearrangements in a nucleotide level has been suggested and a systematic approach to the reporting of medically relevant WGS findings has been proposed.[24,25]

THE SPECTRUM OF CYTOGENOMIC ABNORMALITIES

The prevalence of ID/DD and ASD are reported to be 1~3% and 0.67%, respectively.[26] Other common neurodevelopment disorders including speech and language delay, schizophrenia and epilepsy are also subjected for cytogenomic testing. The diagnostic yield by an integrated cytogenomic analysis of aCGH, FISH and karyotype is 10-20%.[2,22] The diagnostic yield could be varied by the criteria of patient referrals and the resolution of genomic analysis. For example, of the 1,354 consecutive pediatric patients analyzed by 44K and 180K Agilent oligonucleotide aCGH, pathogenic abnormalities were detected in 176 patients (a 13% diagnostic yield). These abnormalities were classified into chromosomal and cryptic structural abnormalities seen in 95 patients (54%), recurrent genomic disorders in 66 patients (37.5%), and common aneuploidies in 15 patients (8.5%).[27]

Table 1. Analytical Validities and Diagnostic Capacity of Cytogenomic Analyses.

	Analytical Validity*				Types of abnormalities detected**							Culumative Diagnostic Yield	Human Cytogenetic Nomenclature
	Spatial Resolution	Analytical Resolution	Sensitivity	Specificity	Chr Num Abn	Bal Struc Abn	Unbal Struc Abn	CNV	UPD ROH	Exonic	Mosaic		
Cell Based G-banding												**3.70%**	**ISCN1978/1981**
Routine (400-550 bands)		5 ~ 7 Mb			+	+	+	-	-	-	**>6%**		**ISCN1985/1991**
High Resolution (550-850 bands)		3 ~ 5 Mb			+	+	+	-	-	-	**>6%**		
Cell Based FISH												**6.80%**	**ISCN1995**
Gene/locus-specific		100-800 Kb	~98%	~98%	+	+	+	+	-	-	**>3~5%**		
Regional specific (cen/subtel)		>100 Kb	~98%	~98%	+	+	+	+	-	-	**>3~5%**		
DNA-based aCGH (Agilent)												**10-20%**	**ISCN2005**
CGH 44K	68 Kb	400~500 Kb	>99%	>99%	+	-	+	+	-	-	**>20%**		**ISCN2009/2013**
CGH 180K	17 Kb	100~120 Kb	>99%	>99%	+	-	+	+	-	-	**>20%**		
CGH+SNP 180K	17 Kb	100~120 Kb	>99%	>99%	+	-	+	+	+	-	**>20%**		
CGH+SNP 400K	7.5 Kb	40~50 Kb	>99%	>99%	+	-	+	+	+	+	**>20%**		

* Sensitivity and specificity of FISH based on laboratory validation and of aCGH based on ref. #19
** Chr = chromosome, num = numerical, bal = balanced, unbal = unbalanced, Abn = abnormalities; CNV = copy number variant, UPD = uniparent disomy, ROH = Region of homoygosity
+ detectable, - undetectable; percentage of mosaic detection based on chromosome analysis of 50 metaphases, FISH assay of 200 cell and ref#32 for aCGH.

Table 2. The Spectrum of Cytogenomic Abnormalities and Their incidences.*

Type of Abnormality	Commonly Seen Abnormalities	Estimated Incidence
Numerical Abnormality		
Sex Chromosome Aneuploidy		
--Male	47,XXY; 47,XYY; other X/Y aneuploidy	1/360 male births
--Female	45,X; 47,XXX; other X aneuploidy	1/580 female births
Autosomal Aneuploidy	47,+21; 47,+18; 47,+13; other aneuploidy	1/700
Total		1/260
Structural Abnormality		
Unbalanced Rearrangements	Deletions, duplications, ring, marker chromosomes, etc.	1/1,600
Balanced rearrangements	Robertsonian, reciprocal translocation, inversion, etc.	1/490
Total		1/375
Genomic Disorders	microdeletion/duplication at 22q11.2, 16p11.2, 1q21.1, 15q13, 7q11.23, 15q11-q13, 17q21.31, 16p13.11, etc.	1/550
All Cytogenomic Abnormalities		1/120

*The incidences for numerical and structural abnormalities from ref#28, and genomic disorders from ref#1

The spectrum of cytogenomic abnormalities ranges from numerical chromosomal abnormalities, unbalanced and balanced structural chromosomal abnormalities, submicroscopic recurrent genomic disorders, to cryptic or intragenic copy number changes. From a large newborn survey, the overall incidence of male sex chromosomal abnormalities (47,XXY for Klinefelter syndrome, 47,XYY, and other X or Y aneuploidy) is 1/360 male births and of female sex chromosomal abnormalities (45,X for Turner syndrome, 47,XXX, and other X aneuploidy) is 1/580 female

births. The most commonly seen numerical chromosomal abnormality is Down syndrome (trisomy 21) with an incidence of 1/800. The overall incidence for autosomal numerical abnormalities (trisomy 21, 18, 13 and others) is 1/700. The most commonly seen balanced rearrangement is Robertsonian translocation with an incidence of 1/1100. There are many types of unbalanced rearrangements including deletions, duplications, ring chromosomes and supernumerary marker chromosomes. The overall incidence of unbalanced chromosomal rearrangements is 1/1600 and of balanced rearrangements is 1/490.[28] The most commonly seen genomic disorder is DiGeorge syndrome (22q11.2 deletion) with an incidence of 1/8000. The overall incidence of frequently detected 14 genomic disorders is estimated to be 1/550.[1] **Table 2** lists the spectrum of cytogenomic abnormalities and their incidences. The overall incidence for all cytogenomic abnormalities is about 1/120 in a general population.

Chromosomal and Cryptic Structural Abnormalities

With its much higher analytical resolution than chromosome G-banding, aCGH analysis can delineate the genomic coordinates and gene contents for almost all chromosomally visible numerical and structural imbalances. This genomic information facilitates fine mapping of critical regions or intervals containing candidate dosage-sensitive genes through subtractive comparison of overlapped deletions and duplications. For example, cytogenomic mapping defined an 11q14.1-q23.2 interstitial deletion from a complex chromosomal rearrangement and reveal FZD4 haploinsufficiency as cause for exudative vitroretinopathy.[29] Similar approach has been applied to other interstitial deletions, duplication and subtelomeric rearrangements in autosomes and sex chromosomes.[30-33] This integrated cytogenomic analysis has also been used in prenatal diagnosis to define genomic imbalances from structural chromosomal abnormalities and thus provide accurate gene content, phenotype predication and risk estimation for prenatal genetic counseling.[34,35]

Cytogenomic analysis can also resolve the genomic structures, mutagenesis mechanisms and mitotic or meiotic behaviors from puzzling chromosomal structural abnormalities like ring chromosomes or supernumerary marker chromosomes. For example, ring chromosome 20 syndrome is a rare chromosomal disorder characterized by refractory epilepsy with seizures in wakefulness and sleep, behavior problems, and mild to severe cognitive impairment. The aCGH analysis revealed two distinct groups of patients: 75% were mosaic for the r(20) and a normal cell line with no detectable deletions or duplications of chromosome 20 in either cell line, and 25% had non-mosaic ring chromosomes with a deletion at one or both ends of the chromosome. The age of onset of seizures inversely correlated with the percentage of cells containing the ring chromosome.[36] Complex interstitial duplication and distal deletion was detected in a ring chromosome 13.[37] Unique structural rearrangement and distinct mitotic behavior were observed in two case of ring chromosome 21.[38] Clinical classification by different type of ring chromosome was proposed.[36,38] For supernumerary marker chromosomes, aCGH analysis has proved very effective in defining the breakpoints, unexpected structural complexity, copy number changes, and gene content.[39,40] Another interesting observation from aCGH applications on two large series is the detection of low-level mosaicism of numerical and structural abnormalities in approximately 0.5% of patients referred for DD/ID/MCA.[41,42] It was suggested that the DNA extracted from the white blood cells can reflect mosaic pattern more accurately than culture stimulated lymphocytes. A cytogenomic approach combining cell-based methods of FISH on direct prepared interphase cells and extensive karyotyping on metaphase cells with DNA-based estimation from aCGH log2 ratio or SNP pattern was proposed for dissecting mosaic patterns.[38]

Hidden genomic aberrations in complex chromosomal rearrangements or apparently balanced translocations were also detected by aCGH.[37,43] Of patients presenting abnormal phenotypes and an apparently balanced translocation, approximately 29-40% has cryptic breakpoint-associated or unrelated imbalances of paternal origin.[44,45] Several disease-causing mechanisms induced by a balanced translocation including loss of function by gene disruption, gain of function by gene fusion and aberrant expression by positional effect, have been demonstrated. For example, Cacciagli et al. detected a de novo balanced translocation t(10;13)(p12;q12) in a patient with severe speech delay and major hypotonia.[46] This translocation disrupted the ATP8A2 gene. This gene is highly expressed in the brain, suggesting the patient's mental disability is likely due to the halpoinsufficiency of the ATPA2 gene. Brownstein et al. reported a case with over-expression of the α-Klotho gene induced by a balanced translocation t(9;13)(q21.13;q13.1) and established the association α-Klotho over-expression with hypophosphatemic rickets and hyperparathyroidism.[47] Application of paired-end genomic sequencing or breakpoint-targeted capture sequencing on five ASD/DD patients carrying a balanced rearrangement revealed unexpected sequence complexity as an underlying feature of karyotyping balanced alterations.[48] Cost-effective diagnostic sequencing analysis for balanced rearrangements detected in patients with ID/DD/ASD should be implemented in the near future.

Recurrent Genomic Disorders

Genomic disorders refer to microdeletions and microduplications mediated by non-allelic homologous recombination (NAHR) within regional low copy repeats (LCRs). A dozen of recurrent genomic disorders such as DiGeorge syndrome caused by a deletion at 22q11.2, Williams-Beuren syndrome (OMIM#194050) by a deletion of 7q11.23, Prader-Willi syndrome and Angelman syndrome by a deletion at 15q11.2 have been recognized clinically and routinely diagnosed by FISH testing. The application of genomic analysis enables not only more accurate diagnosis of these previously recognized genomic disorders but also the detection of many novel recurrent genomic disorders. In 2006, the first genomic disorder identified by aCGH is a 500 Kb microdeletion at 17q21.31 containing the MAPT gene

(microtubular associated protein tau) from patients with a clearly recognizable ID, hypotonia and a characteristic face.[49,50] This later termed Koolen syndrome (OMIM#610443) is caused either by heterozygous mutation in the KANSL1 gene or a 17q21.31 deletion. The KANSL1 gene encodes a nuclear protein that plays a role in chromatin modification. It is a member of histone acetyltransferase (HAT) complex. The reciprocal 17q21.31 microduplication syndrome (OMIM#613533) manifests some degree of psychomotor retardation, poor social interaction, and communication difficulties reminiscent of ASD.[51] Since then, many genomic disorders have been reported. The aCGH analysis on 15,767 pediatric patients with ID/DD revealed that about 14.2% of them are caused by pathogenic CNVs over 400Kb, and approximately 60% of these pathogenic CNVs are within 45 known genomic disorder regions.[52] A study of human populations for the polymorphic inversions at 17q21.31 observed that the H2 haplotype occurred at the highest frequencies in South Asian and Southern Europe; this H2 haplotype is susceptible to de novo deletions that lead to developmental delay and learning difficulties.[53] Population genetic studies for genomic disorders of other loci could define predisposing genomic structures and recurrence risk for different ethnic groups at different geographic regions.

The microdeletion and microduplication of the same genomic locus offer an opportunity to study dosage-sensitive genes, especially for the opposite phenotypes from haploinsufficient and triple-sensitive genes. Comparison of clinical features of 7q11.23 microdeletion for Williams syndrome and reciprocal microduplication syndrome (OMIM#609757) noted different neurologic and behavior problems. The 7q11.23 microdeletion shows relative strength in expressive language and excessive sociability. To the contrary, the 7q11.23 microduplication has speech and language delay, deficit of social interaction and aggressive behavior. The FZD9, LIMK1, CLIP2 and GTF21RD1 genes have been suggested to be the candidate genes for neurologic and behavior phenotypes.[54] Microdeletion syndrome at 16p11.2 (OMIM#611913) and microduplication syndrome at 16p11.2 (OMIM#614671) were initially associated with ASD but a subsequent study revealed mirror body mass index phenotypes. Microdeletion at 16p11.2 is often associated with obesity, macrocephaly and ASD, while reciprocal microduplication is associated with underweight, microcephaly and schizophrenia.[55,56] It has been estimated that the most frequently seen genomic disorders account for a 4.5% diagnostic yield in pediatric patients and an estimated 0.18% prevalence in a general population.[1]

Uniparental Disomy (UPD), Regions of Homozygosity (ROH), and Variants of Unknown Significance (VOUS)

UPD is defined as the inheritance of both homologs of a chromosome pair from a single parent. When both homologs are from that parent, it is denoted as heterodisomy or heteroUPD. If both copies are from one parental homolog, it is termed as isodisomy or isoUPD. UPD of chromosomes 6, 7, 11, 14 and 15 have been known to cause diseases. Paternal UPD of chromosome 15, patUPD15, causes Prader-Willi syndrome while maternal UPD, matUPD15 causes Angelman syndrome. Segmental duplication of maternal 11p15 or paternal deletion of 11p15 causes decreased expression of IGF2, manifesting with impaired growth or Silver–Russell syndrome. Segmental duplication of paternal 11p15, paternal UPD, or maternal imprinting mutations of 11p15 lead to increased expression of IGF2, manifesting with overgrowth and Beckwith–Wiedemann syndrome.[57] Current validated CGH-SNP aCGH and SNP chip can detect chromosomal and segmental isoUPD but the detection of heteroUPD requires concurrent parental study.[58] The clinical significance of ROH segments is not clear. One possible disease-causing mechanism could be the presence of autosomal recessive phenotype by the doubling of a single mutation within the ROH segment. Other findings such as VOUS detected in approximately 9.3% of pediatric cases will require follow up parental study to determine the parental origin of VOUS and even further functional analysis to understand their clinical significance.[59]

FUTURE DIRECTIONS AND CONCLUDING REMARKS

The aCGH or SNP chip analysis has brought pediatric and prenatal genetic evaluation into the genomic era. This progress has contributed greatly to our understanding of genetic etiology in 12%-20% of pediatric patients with DD/ID/MCA/ASD. In additional to the technical progress in cytogenomic diagnosis, the implementations of knowledge-based genetic counseling, rational clinical action and follow up familial studies could be of direct benefit for a substantial proportion of patients.[60,61] For example, the aggressive behavior from patients with a 15q13.3 deletion involving the CHRNA7 gene could benefit from treatment with the NChR allosteric modulator and acetylcholinesterase (AChE) inhibitor, galantamine.[62] Research application of WGS defined paired-duplication marked cryptic inversion and breakpoints of translocation and inversion.[63,64] Future clinical use of WGS could further expand the spectrum of cytogenomic abnormalities by delineating VOUS, ROH, and more cryptic structure variants to single nucleotide level. As we gain better understanding of molecular mechanisms of these cytogenomic abnormalities through functional analysis and develop novel target therapeutic approaches, disease-specific management and treatment could be introduced in the near future.

CONFLICT OF INTEREST
None.

REFERENCES

1. Wei Y, Xu F, Li P. Technology-driven and evidence-based genomic analysis for integrated pediatric and prenatal genetic evaluation. J Genet Genomics. 2013;40(1):1-14.
2. Miller DT, Adam MP, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet. 2010;86(5):749-764.
3. Manning M, Hudgins L, Professional Practice and Guidelines Committee. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. Genet Med. 2010;12(11):742-745.

4. Xiang B, Xu F, Zeng W, Zi D, Ma D. Navigating web-based resources for genetic testing of chromosome abnormalities, CNVs and gene mutations. N A J Med Sci. 2014;7(4):163-170.
5. Tjio JH, Levan A. The chromosome number of Man. Hereditas 1956;42(1-2):1-6.
6. Lejeune J, Gautier M, Turpin R. Study of somatic chromosomes from 9 mongoloid children. C R Hebd Seances Acad Sci. 1959;248(11):1721-1722.
7. Ford CE, Jones KW, Polani PE, de Almeida JC, Briggs JH. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). Lancet. 1959;273(7075):711-713.
8. Jacobs PA, Strong JA. A case of human intersexuality having a possible XXY sex-determining mechanism. Nature. 1959;183(4657):302–303.
9. Patau K, Smith DW, Therman E, Inhorn SL, Wagner HP. Multiple congenital anomaly caused by an extra autosome. Lancet. 1960;275(7128):790–793.
10. Edwards JH, Harnden DG, Cameron AH, Crosse VM, Wolff OH. A new trisomic syndrome. Lancet. 1960;275(7128):787-790.
11. Caspersson T, Farber S, Foley GE, et al. Chemical differentiation along metaphase chromosomes. Exp Cell Res. 1968;49(1):219–222.
12. Shaffer LG, McGowan-Jordan J, Schmid M (eds). ISCN (2013): An international system for human cytogenetic nomenclature, S.Karger, Basel 2013.
13. Langer-Safer PR, Levine M, Ward DC. Immunological method for mapping genes on Drosophila polytene chromosomes. Proc Natl Acad Sci USA. 1982;79(14):4381-4385.
14. Ried T, Landes G, Dackowski W, Klinger K, Ward DC. Multicolor fluorescence in situ hybridization for the simultaneous detection of probe sets for chromosomes 13, 18, 21, X and Y in uncultured amniotic fluid cells. Hum Mol Genet. 1992;1(5):307-313.
15. Ning Y, Roschke A, Smith AC, et al. A complete set of human telomeric probes and their clinical application. Nat Genet. 1996;14(1):86-89.
16. Shaffer LG, American College of Medical Genetics Professional Practice and Guidelines Committee. American College of Medical Genetics guideline on the cytogenetic evaluation of the individual with developmental delay or mental retardation. Genet Med. 2005;7(9):650-654.
17. Kallioniemi A, Kallioniemi OP, Sudar D, et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science. 1992;25895083):818-821.
18. Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science. 1995;270(5235):467–470.
19. Xiang B, Li A, Valentin D, Novak N, Zhao H-Y, Li P. Analytical and clinical validity of whole genome oligonucleotide array comparative genomic hybridization for pediatric patients with mental retardation and developmental delay. Am J Med Genet. 2008;146A(15):1942-1954.
20. Redon R, Ishikawa S, Fitch KR, et al. Global variation in copy number in the human genome. Nature. 2006;444(7118):444-454.
21. Xu F, Li P. Chapter 1: Cytogenomic abnormalities and dosage-sensitive mechanisms for intellectual and developmental disabilities. In Ahmad Salehi (eds) Developmental Disabilities--Molecules Involved, Diagnosis and Clinical Care. InTech, 2013:pp1-30.
22. Xiang B, Zhu H, Shen Y, et al. Genome-wide oligonucleotide array CGH for etiological diagnosis of mental retardation: A multi-center experience of 1,499 clinical cases. J Mol Diagn. 2010;12(2):204-212.
23. Talkowski ME, Rosenfeld JA, Blumenthal I, et al. Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. Cell. 2012;149(3):525-537.
24. Ordulu Z, Wong KE, Currall BB, et al. Describing sequencing results of structural chromosome rearrangements with a suggested next-generation cytogenetic nomenclature. Am J Hum Genet. 2014;94(5):695-709.
25. McLaughlin HM, Ceyhan-Birsoy O, Christensen KD, et al.; MedSeq Project. A systematic approach to the reporting of medically relevant findings from whole genome sequencing. BMC Med Genet. 2014;15:134.
26. Shevell M, Ashwal S, Donley D, et al; Quality Standards Subcommittee of the American Academy of Neurology; Practice Committee of the Child Neurology Society. Parctice parameter: evaluation of the child with global developmental delay: report of the quality standards subcommittee of the child neurology society. Neurology. 2003;60(3):367-380.
27. Xu F, Li L, Schulz VP, et al. Cytogenomic mapping and bioinformatic mining reveal interacting brain expressed genes for intellectural disabilities. Mol Cytogenet. 2014;7(1):4.
28. Nussbaum RL, McInnes RR, Willard HF, Hamosh A. Thompson & Thompson Genetics in Medicine, 7th edition. p76.
29. Li P, Zhang HZ, Huff S, et al. Karyotype-phenotype insights from 11q14.1-q23.2 interstitial deletions: FZD4 haploinsufficiency and exudative vitroretinopathy in a patient with a complex chromosome rearrangement. Am J Med Genet. 2006;140A(24):2721-2729.
30. Khattab M, Xu F, Li P, Bhandari V. A de novo 3.54 Mb deletion of 17q22-q23.1 associated with hydrocephalus: A case report and review of literature. Am J Med Genet. 2011;155A(12):3082-3086.
31. Rossi MR, DiMaio M, Xiang B, et al. Clinical and genomic characterization of distal duplications and deletions of chromosome 4q: Study of two cases and review of the literature. Am J Med Genet. 2009;149A(12):2788-2794.
32. Cook S, Wilcox K, Grommisch B, Li P, Xu F**.** Prenatal diagnosis of Xq26.1-q26.3 duplication in two fetuses of a woman with gonadal mosaicism. N A J Med Sci. 2014;7(4):176-179.
33. Wei Y, Gao X, Yan L, Xu F, Li P, Zhao Y. Prenatal diagnosis and postnatal follow up of partial trisomy 13q and partial monosomy 10p: A case report and review of the literature. Case Rep Genet. 2012;821347.
34. Li P, Pomianowski P, DiMaio SM, et al. Genomic characterization of prenatally detected chromosomal structural abnormalities using oligonucleotide array comparative genomic hybridization. Am J Med Genet. 2011;155A(7):1605-1615.
35. Xu ZY, Geng Q, Luo FW, Xu F, Li P, Xie JS. Multiplex ligation-dependent probe amplification and array comparative genomic hybridization analyses for prenatal diagnosis of cytogenomic abnormalities. Mol Cytogenet. 2014;7(1):84.
36. Conlin LK, Kramer W, Hutchinson AL, et al. Molecular analysis of ring chromosome 20 syndrome reveals two distinct groups of patients. J Med Genet. 2011;48(1):1-9.
37. Zhang HZ, Xu F, Seashore M, Li P. Unique genomic structure and distinct mitotic behavior of ring chromosome 21 in two unrelated cases. Cytogenet Genome Res. 2012;136(3):180-187.
38. Xu F, DiAdamo AJ, Grommisch B, Li P. Interstitial duplication and distal deletion in a ring chromosome 13 with pulmonary atresia and ventricular septal defect: A case report and review of the literature. N A J Med Sci. 2013;6(4):208-212.
39. Tsuchiya KD, Opheim KE, Hannibal MC, et al. Unexpected structural complexity of supernumerary marker chromosomes characterized by microarray comparative genomic hybridization. Mol Cytogenet. 2008;1:7.
40. Kleefstra T, de Leeuw N, Wolf R, et al. Phenotypic spectrum of 20 novel patients with molecularly defined supernumerary marker chromosomes 15 and a review of the literature. Am J Med Genet. 2010;152A(9):2221-2229.
41. Ballif BC, Rorem EA, Sundin K, et al. Detection of low-level mosaicism by array CGH in routine diagnostic specimens. Am J Med Genet. 2006;140A(24):2757-67.
42. Cheung SW, Shaw CA, Scott DA, et al. Microarray-based CGH detects chromosomal mosaicism not revealed by conventional cytogenetics. Am J Med Genet. 2007;143A(15):1679-1686.
43. Higgins AW, Alkuraya FS, Bosco AF, et al. Characterization of apparently balanced chromosomal rearrangements from the developmental genome anatomy project. Am J Hum Genet. 2008;82(3):712-722.
44. De Gregori M, Ciccone R, Magini P, et al. Cryptic deletions are a common finding in ''balanced'' reciprocal and complex chromosome rearrangements: A study of 59 patients. J Med Genet. 2007;44(12):750–762.
45. Baptista J, Mercer C, Prigmore E, et al. Breakpoint mapping and array CGH in translocations: comparison of a phenotypically normal and an abnormal cohort. Am J Hum Genet. 2008;82(4):927–936.
46. Cacciagli P, Haddad MR, Mignon-Ravix C, et al. Disruption of the ATP8A2 gene in a patient with a t(10;13) de novo balanced translocation and a severe neurological phenotype. Eu J Hum Genet. 2010;18(12):1360-1363.
47. Brownstein CA, Adler F, Nelson-Williams C, et al. A translocation causing increased α-Klotho level results in hypophosphatemic rickets

and hyperparathyroidism. Proc Natl Acad Sci USA. 2008;105(9):3455-3460.
48. Talkowski ME, Ernst C, Heilbut A, et al. Next-generation sequencing strategies enable routine detection of balanced chromosome rearrangements for clinical diagnostics and genetic research. Am J Hum Genet. 2011;88(4):469-481.
49. Koolen DA, Vissers LE, Pfundt R, et al. A new chromosome 17q21.31 microdeletion syndrome associated with a common inversion polymorphism. Nat Genet. 2006;38(9):999-1001.
50. Shaw-Smith C, Pittman AM, Willatt L, et al. Microdeletion encompassing MAPT at chromosome 17q21.3 is associated with developmental delay and learning disability. Nat Genet. 2006;38(9):1032-1037.
51. Kirchhoff M. Bisgaard A-M, Duno M, Hansen FJ, Schwartz MA. 17q21.31 microduplication, reciprocal to the newly described 17q21.31 microdeletion, in a girl with severe psychomotor developmental delay and dysmorphic craniofacial features. Eu J Med Genet. 2007;50(4):256-63.
52. Cooper GM, Coe BP, Girirajan S, et al. A copy number variation morbidity map of developmental delay. Nat Genet. 2011;43(9):838-846.
53. Donnelly MP, Paschou P, Grigorenko E, et al. The distribution and most recent common ancestor of the 17q21 inversion in humans. Am J Hum Genet. 2010;86(2):161-171.
54. Merla G, Brunetti-Pierri N, Micale, Fusco C. Copy number variants at Williams-Beuren syndrome 7q11.23 regions. Hum Genet. 2010;128(1):3-26.
55. Weiss LA, Shen Y, Korn JM, et al; Autism Consortium. Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med. 2008;358(7):667-675.
56. Jacquemont S, Reymond A, Zufferey F, et al. Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. Nature. 2011;478(7367):97-102.
57. Weksberg R, Shuman C, Beckwith JB. Beckwith-Wiedemann syndrome. Eu J Hum Genet. 2010;18(1):8–14.
58. Papenhausen P, Schwartz S, Risheg H, et al. UPD detection using homozygosity profiling with a SNP genotyping microarray. Am J Med Genet. 2011;155A(4):757-768.
59. Kaminsky EB, Kaul V, Paschall J, et al. An evidence-based approach to establish the functional and clinical significance of copy number variants in intellectual and developmental disabilities. Genet Med. 2011;13(9):777-784.
60. Darilek S, Ward P, Pursley A, et al. Pre- and postnatal genetic testing by array-comparative genomic hybridization: genetic counseling perspectives. Genet Med. 2008;10(1):13-18.
61. Coulter ME, Miller DT, Harris DJ, et al. Chromosomal microarray testing influences medical management. Genet Med. 2011;13(9):770-776.
62. Cubells JF, Deoreo EH, Harvey PD, et al. Pharmaco-genetically guided treatment of recurrent rage outbursts in an adult male with 15q13.3 deletion syndrome. Am J Med Genet. 2011;155A(4):805-810.
63. Brand H, Collins RL, Hanscom C, et al. Paired-duplication signatures mark cryptic inversions and other complex structural variation. Am J Hum Genet. 2015;97(1):170-176.
64. Suzuki T, Tsurusaki Y, Nakashima M, et al. Precise detection of chromosomal translocation or inversion breakpoints by whole-genome sequencing. J Hum Genet. 2014;59(12):649-654