SEAMLESS CGH DIAGNOSTIC TESTING

GENETISURE DX POSTNATAL ASSAY — Informed decisions start with a complete microarray platform for postnatal analysis

For In Vitro Diagnostic Use

INTENDED USE:

GenetiSure Dx Postnatal Assay is a qualitative assay intended for the postnatal detection of copy-number variations (CNV) and copy-neutral loss of heterozygosity (cnLOH) in genomic DNA obtained from peripheral whole blood in patients referred for chromosomal testing based on clinical presentation. GenetiSure Dx Postnatal Assay is intended for the detection of CNVs and cnLOH associated with developmental delay, intellectual disability, congenital anomalies or dysmorphic features. Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation and counseling, as appropriate. Interpretation of assay results is intended to be performed only by healthcare professionals who are board-certified in clinical cytogenetics or molecular genetics.

The assay is intended to be used on the SureScan Dx Microarray Scanner System and analyzed by CytoDx Software.

WARNING:

This device is not intended to be used for standalone or diagnostic purposes, pre-implantation or prenatal testing or screening, population screening or the detection of, or screening for, acquired or somatic genetic aberrations.

Trusted Answers. Together.

PR7000-0592

Printed in USA, February 15, 2017
5991-7736ENE

CE IVD

LEARN MORE AT:
www.agilent.com/genomics/genetisuredx

Europe
info_agilent@agilent.com

Agilent custom and catalog arrays and CytoGenomics software are for Research Use Only. Not for use in diagnostic procedures.

Cartagenia Bench Lab is marketed in the USA as exempt Class I Medical Device and in Europe and Canada as a Class I Medical Device.

This information is subject to change without notice.

Agilent Technologies

Agilent Technologies

OUR FOCUS: IMPROVING QUALITY OF LIFE

The Genetic Disease Landscape

Genetic defects can be responsible for a wide range of disorders, from intellectual disability to congenital dysmorphisms, neuromuscular disorders, epilepsy and autism. Either a single gene or chromosomal anomalies are the cause of about 25% to 30% of all major congenital dysmorphisms, such as cleft lip and heart defects[1].

At least 10% of all neonatal intensive-care unit admissions involve the presence of congenital dysmorphisms[2].

In addition, genetic defects can be responsible for developmental delay (DD) and intellectual disability (ID), including autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD). Many of these are copy-number variants, deleted or duplicated regions of the genome that can range in size from very small to entire chromosomes. Although the average age of diagnosis for pervasive developmental disorder can be as late as four years, research has shown that early treatment therapies for ASD, for example, can optimize long-term prognosis, and treatment is less effective as children get older[3].

Median global prevalence of ASD alone was an estimated 62/10,000 in 2012[4], and the 17% increase in DD and ID disorders in the U.S. between 1996 and 2008 was driven largely by increases in ASD[5].

An early genetic diagnosis can therefore be critical to the prognosis of the child, as it can enable interventions that may prevent, anticipate or more successfully treat complications[6]. Diagnosis can also facilitate financial support, educational assistance and membership in support groups.

Genetic anomalies account for 25% to 50% of ID cases, and this number increases with the severity of the disability[7].

A definitive genetic diagnosis could provide the answers and rapidly change the focus of a medical investigation from finding the cause to appropriate medical care.

CHROMOSOMAL MICROARRAY TECHNOLOGY

Enabling Clinical Diagnosis of Genetic Disorders: From Research to Clinic

Chromosomal microarrays use a modified *in situ* hybridization technology that allows detection and mapping of gDNA sequence copy differences between two genomes in a single experiment. Analysis of fluorescence intensity of probes with respect to their genomic location enables detection of regions where copy-number variations (CNVs) and copy-neutral loss of heterozygosity (cnLOH) may occur.

aCGH is recognized by the American College of Medical Genetics and Genomics (ACMG), the Child Neurology Society (CNS) and the American Academy of Neurology (AAN) as the first-tier test for diagnosis of genetic anomalies associated with developmental disabilities[8,9,10].

The European Society of Human Genetics (ESHG) has established guidelines for array-based, whole-genome "molecular karyotyping" in constitutional genetic diagnosis for the detection of submicroscopic imbalances[11].

Guidelines[11] for quality assurance of aCGH also have been established by the European Cytogeneticists Association (ECA), the Canadian College of Medical Geneticists (CCMG) and the Human Genetics Society of Australasia (HGSA).

Microarrays are a proven technology used in hundreds of laboratories around the world. Agilent aCGH arrays have established a standard of excellence for the characterization of genetic diseases with more than 10 years of research use and 10,000 published papers.

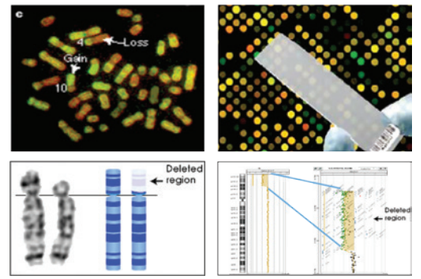

Figure 1. A visual representation of the type of information that is obtained using array CGH, aCGH (shown in top panels) versus FISH (shown in bottom panels). aCGH involves hybridization to multiple independent probes which improves the statistical confidence in the results obtained. The results obtained with FISH offer less resolution and quantitative comparison between test and reference samples.

Higher diagnostic yield, increased resolution and greater sensitivity make aCGH a superior method compared with karyotyping or FISH. Medical geneticists now consider aCGH to be the standard test for detecting CNVs linked to a unique patient or to one of the many known genetic disorders that cannot be detected by karyotype alone.[8,12,13]

THE GENETISURE DX POSTNATAL ASSAY

The GenetiSure Dx Postnatal Assay uses Agilent's proprietary aCGH for copy-number and LOH analysis, enabling cytogeneticists to accurately detect genetic anomalies associated with developmental delay, intellectual disability, congenital anomalies and dysmorphic features in children and adults. The GenetiSure Dx Postnatal solution includes all components required to process your microarray samples and perform data analysis.

Designed for what matters

The GenetiSure Dx Postnatal Assay is designed to enable identification of copy-number and copy-neutral change across the genome. Each microarray contains approximately 107,000 probes optimized for copy-number (CN) analysis as well as 59,000 biallelic SNP probes. The CN probes are distributed across the entire genome with a higher density in regions designated to be of clinical interest.

High assay resolution

Agilent probes, high-quality 60-mers, enable confident calls with as few as five consecutive probes. The probes target 94% of the genome with at least five CN probes per 400 Kb and resulting in median resolution of approximately 150 Kb. Regions identified to be clinically relevant are targeted with increased probe density, resulting in median resolution of approximately 25 Kb.

The presence of dedicated SNP probes enables detection of copy-neutral changes, such as uniparental disomy (UPD) and LOH.

The SNP probes target 91% of the genome, with at least 100 SNP probes per 10 Mb. The median resolution for LOH is approximately 8 Mb

The assay is able to detect mosaic amplifications and deletions spanning 100 or more probes.

Built-in quality controls

The GenetiSure Postnatal Dx Assay includes a series of in-process QC checks, external controls and array QC metrics that let users easily monitor and assess the quality of results.

Easy and streamlined assay workflow

The assay is comprehensive and easy to implement in your laboratory.

- Only 500 ng of genomic DNA, extracted from 200 µl of whole blood, are required for testing.
- The accuracy of the GenetiSure Dx Postnatal Assay results is not affected by increased levels of hemoglobin, conjugated bilirubin, unconjugated bilirubin or triglycerides in the patient's whole blood in EDTA, or by storage of the blood for up to seven days.
- The workflow is fully optimized and easy to set up. As there is no PCR amplification step, segregation of lab space is not required.
- The whole workflow, including interpretation, can be run in three days.

Assay Validation – Results You Can Trust

The GenetiSure Dx Postnatal Assay has been validated and tested extensively. Below is a summary of the different studies performed on the assay.

Study	Description
Accuracy	The analytical-accuracy study demonstrated the accuracy of the GenetiSure Dx Postnatal Assay using a panel of approximately 600 samples from different sources that exhibited aberrations across the entire genome. The average confirmation rates were 93.5% for larger CNVs (>20 probes) and 92.5% for small CNVs (5 to 20 probes). For cnLOH intervals, the average confirmation rate was 90.1%. The confirmation rate (%) is the number of assay-identified aberrations confirmed using a reference method divided by the total number of the assay-identified aberrations.
Clinical Validity	The GenetiSure Dx Postnatal Assay, which included 900 samples, was validated in a clinical study involving several partner laboratories. The study demonstrated that the assay has clinical validity and diagnostic utility, as compared to standard-of-care methods. The diagnostic yield for the assay, when considering only CNVs, was 15%, which was comparable to the diagnostic yield obtained for the same samples at the collection sites using non-Agilent microarray methods. This yield increased to 20% when cnLOH aberrations were also considered, exceeding the diagnostic yield at the collection sites.
Limit of Detection	To determine the analytical sensitivity, or Limit of Detection (LOD), of the GenetiSure Dx Postnatal Assay, a study was conducted to evaluate the minimum and maximum amounts of DNA acceptable as the assay input. Results support the use of 500 ng as the recommended input amount. The data demonstrated that performance did not decline down to 375 ng. For CNVs only, the LOD could be further reduced to 250 ng.
Reproducibility	The results of this study demonstrated that the GenetiSure Dx Postnatal Assay was reproducible when performed at multiple laboratory sites by different operators over multiple days and is suitable for implementation in a clinical laboratory environment.
Precision	The results of the precision study demonstrated that assay results are not affected by multiple extractions of the same sample, multiple reagent lots or scanners.
Cross Contamination	The presence of contamination could result in corrupt and inaccurate patient data. This study was designed to determine whether cross contamination occurs during the routine assay workflow, and if so, what the impact on data would be. No suspected cross contamination was detected.
Whole Blood Stability	Whole blood specimens from the collection site may be stored prior to processing, or they may need to be transferred to a remote laboratory for processing; therefore, a whole blood stability study was performed. Whole blood specimens stored for up to 10 days at 2°C to 8°C prior to gDNA isolation, and then processed with the GenetiSure Dx Postnatal Assay, produced acceptable results.
Interfering Substances	The GenetiSure Dx Postnatal Assay uses gDNA isolated from patient whole blood. Specimens may be obtained from patients with endogenous conditions resulting in hemolysis, bilirubinemia or lipemia. An interfering study was performed to determine the effects of these conditions on the results of the test. The study demonstrated that the assay results are not altered by the presence of excessive hemoglobin, triglycerides (triolein) or bilirubin (conjugated or unconjugated) in the patient whole blood specimen.

Analysis and Reporting of Results Tailored to Cytogeneticists

Resolving the analysis bottleneck

Agilent CytoDx software has been designed specifically for use with the GenetiSure Dx Postnatal Assay. CytoDx addresses the needs of cytogeneticists for analysis and triage of their data using a streamlined, validated workflow. It also contains optimized algorithms for accurate detection of copy-number changes and copy-neutral variations, including LOH and UPD. The validated analysis workflow enables suppression, classification, editing, linking to external databases, annotations of aberrations and report generation.

Variant analysis

Agilent Bench Lab software enables labs to automate variant-analysis pipelines and drives more efficient triage and reporting of clinically relevant structural variants. Labs can quickly test multiple hypotheses, build robust variant-assessment pipelines and draft clinical-grade reports with ease, all while building an in-house database of clinical findings. The result is faster report turnaround times, patient-centric analysis, robustness through standardization and a standard of care based on an internal database of previous findings.

CLINICAL PERFORMANCE

DATA YOU CAN COUNT ON

The GenetiSure Dx Postnatal Assay is a qualitative assay intended for postnatal detection of CNVs and cnLOH in genomic DNA obtained from peripheral whole blood in patients referred for chromosomal testing based on clinical presentation. The GenetiSure Dx Postnatal Assay is intended for the detection of CNVs and cnLOH associated with developmental delay, intellectual disability, congenital anomalies or dysmorphic features. Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation and counseling, as appropriate.

Gains and Losses

Reported below are some examples of samples run with the GenetiSure Dx Postnatal Assay, reporting gains and losses of various sizes that are suggestive of different clinical conditions.

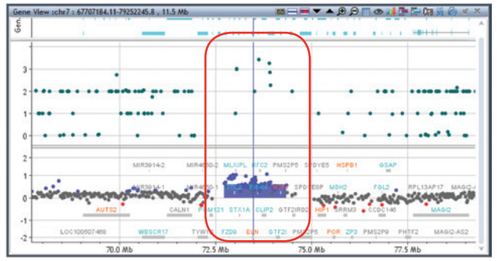

Figure 1. A 1.6 Mb gain, in a region containing 88 probes, was detected at Chr. 7q11.23. The patient underwent genetic testing because of presentation with developmental delays and autism symptoms. The reported aberration suggests a microduplication syndrome.

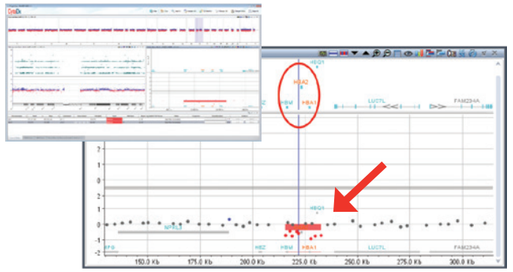

Figure 2. A 17 Kb deletion at Chr. 16p13.3, which includes 12 CGH probes, was detected. The deleted region spanned two HBA genes and was easily identified by the Gene track in CytoDx. The findings were consistent with patient clinical indication of an alloimmunization history and Hirschsprung's-like symptoms.

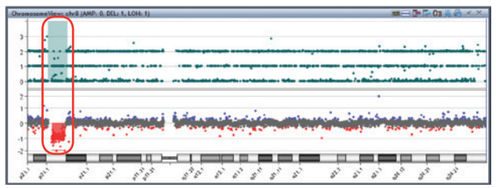

Figure 3. A deletion event was confirmed by both CGH and SNP probes. Figure 3 illustrates how the Agilent ADM-2 algorithm called a large, 4.3 Mb hemizygous deletion on the p arm of chromosome 8, spanning 186 CGH probes. The LOH-calling algorithm determined that in the same region there were only single A or B SNP alleles (0 or 1 uncut) instead of the combination of AA, AB and BB SNP alleles (0, 1 and 2 uncut alleles) present in a normal diploid genome. The patient underwent genetic testing because of positive results on a previous test on different technology and for a family history of autism. Deletions in 8p23.1 are linked to a microdeletion/CDH syndrome.

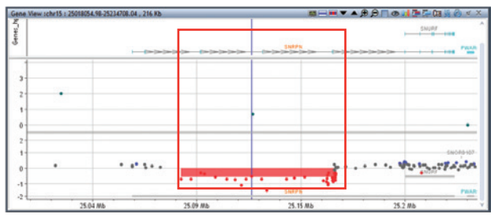

Figure 4. A 74.5 Kb hemizygous deletion encompassed by 41CGH probes at Chr. 15q11.2 was detected. This deletion partially overlaps the SNRPN gene, which is present in the OMIM database. Results confirmed the reason for referral, which was an indication of Angelman Syndrome by methylation studies.

Copy-Neutral Changes

Copy-neutral loss of heterozygosity is also detectable using the Agilent aCGH assay. For each SNP probe, gDNA that has been cut at the restriction site results in a different fluorescent signal than that produced by uncut gDNA. Genotyping of SNPs enables subsequent detection of cnLOH intervals, identified in the software by locating genomic regions with a statistically significant scarcity of heterozygous calls.

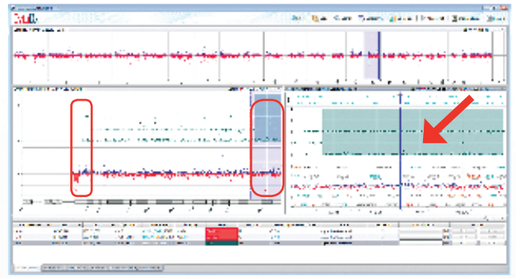

Figure 5. Shown is an example of a 10.5 Mb cnLOH at Chr. 15q26.1- q26.3. Also visible is an additional small LOH region close to centromere. AOH at centromere and telomere is consistent with UPD related to MII nondisjunction. Findings were consistent with clinical phenotype of Prader-Willi syndrome, but additional methylation tests would be required to confirm the diagnosis.

Whole Chromosome Aberration

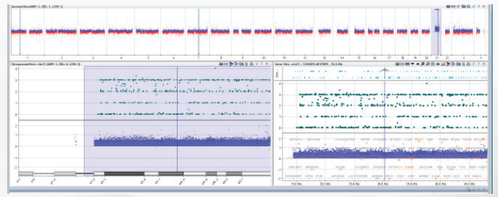

Figure 6. Down syndrome (DS) is one of the most frequent congenital birth defects and the most common genetic cause of mental retardation. DS presents with a complex clinical spectrum of variable features affecting most organ systems. The reason for referral of this patient was Tetralogy of Fallot with Absent Pulmonary Valve. In most cases, DS results from the presence of an extra copy of chromosome 21, as reported in the above sample. Trisomy of Chr. 21 is highlighted both from copy-number and SNP probes[14].

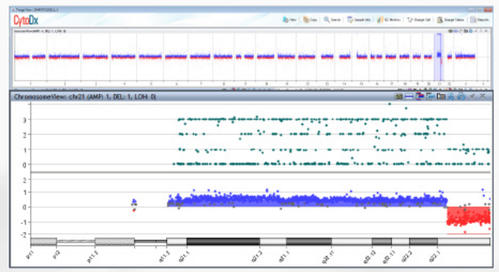

Figure 7. This sample reported a 29 Mb amplification at 21q11.2-q22.3 and a 4.5 Mb deletion at 21q22.3. This aberration is suggestive of a ring chromosome resulting in a partial for Chr. 21 and was confirmed by karyotype.

A COMPLETE WORKFLOW, FROM DNA TO RESULT

Assay Selection

Sample Processing

Data Generation & Analysis

Data Interpretation

CE-IVD Testing

GenetiSure Dx Postnatal Array

- CGH+SNP microarray designed and validated for postnatal samples
- Increased resolution in targeted genomic regions of known dose-sensitivity and significant clinical interest
- Whole-genome CN and LOH in a single assay
- High-fidelity 60-mer oligonucleotides based on the Agilent OLS technology

Dx Reagents

- Labeling, hybridization and washing reagents are validated for diagnostic use and optimized for use with the GenetiSure Dx Postnatal Array
- GenetiSure Dx DNA labeling kit for direct labeling of DNA
- All reagents have been designed to streamline sample processing

SureScan Dx

- Provides confidence in your results with excellent sensitivity and wide dynamic range
- Easy to use and flexible due to continuous slide loading and random scanning capability
- Fully integrated with data analysis software for a seamless workflow

CytoDx

- Streamlined workflow for data analysis
- Validated algorithms for analysis of the Agilent GenetiSure Dx Postnatal Array
- Pre-loaded tracks and links to external databases for data interpretation and evidence support

Cartagenia Bench Lab

- Easy variant assessment and reporting: ACMG variant classification is integrated
- Streamline data interpretation to shorten turnaround times
- Minimal impact on your IT infrastructure
- Create and manage your local knowledge database

Research Use

Catalog & Custom CGH/CGH+SNP Arrays

Agilent can provide a variety of CGH array designs for clinical research and, with our customization capabilities, support for the development of customer-defined content arrays and informatics by laboratories interested in developing and validating their own tests using instrumentation for multiplex systems.

www.genomics.agilent.com/cgh_snp

CytoGenomics

- Workflow-based, easy-to-use analysis tool for CGH and CGH+SNP analysis
- Contains powerful algorithms for accurate copy-number and LOH calls
- Designed specifically for cytogenetic research to put data into biological context

COMPREHENSIVE ANSWERS FOR YOUR LABORATORY NEEDS

Ordering Information

Components	Agilent Part Number	Contents
Agilent GenetiSure Dx Postnatal Assay	K1201A	6 slides 4x and 6 coverslips, 24 samples
Agilent GenetiSure Dx DNA Labeling Kit	K1201-64100	25 sample and 25 reference reactions
Agilent GenetiSure Dx Hybridization Kit	K1201-64200	25 hybridization slides reagents
Agilent GenetiSure Dx Wash Buffer Set	K1201-64300	8 L wash buffer 1; 4 L wash buffer 2 reagents
Agilent GenetiSure Dx Cot-1 Human DNA	K1201-64400	625 µL, 1 µg/µL reagent

Equipment	Agilent Part Number
SureScan Dx Microarray Scanner Bundle	G5761AA
Hybridization Chamber Kit, SureHyb enabled, Stainless	G2534A
Microarray Hybridization Oven	G2545A
Hybridization Oven Rotator Rack	G2530-60029

Details & Specifications

GenetiSure Dx Postnatal Array – Agilent Part Number K1201A

Feature	Specifications
Format	4x180K
Arrays/Slide	4
Biological Features	~107,000 (CGH) + ~59,000 (SNP)
Internal Quality Control Features	8,121
Probe Spacing	Median for CGH probes: ~25 Kb across whole genome; 3.5 Kb in regions of clinical interest
Resolution	Median resolution for CNV: ~150 Kb overall; ~25 Kb in targeted regions LOH median resolution of 8 Mb
Regions of clinical interest	Target genomic regions of known dose-sensitivity and significant clinical interest, designated by international cytogenetics communities and recommended for coverage in chromosomal microarray (CMA) tests
Design based on	UCSC hg19 (NCBI Build 37, February 2009)
Manufacturing	Agilent 60-mer SurePrint technology

SureScan Dx Microarray Scanner – Agilent Part Number G5761A

Feature	Specifications
Dynamic Range	>10^4 (16-bit data format), >10^5 (20-bit data format), >10^6 with XDR
Dynamic AutoFocus	Continually adjusts scanner's focus, keeping features in focus at all times
Resolution	2, 3, 5, or 10 microns
Autoloader	24-slide cassette allows for hands-off operation
Integrated Barcode Reader	Reads code 128 (A,B,C), Code 39, Code 93, and CODABAR
Compatible Dyes	Cyanine 3 and Cyanine 5, and Alexa 647, 555, and 660
PMT Adjustment	Automatic PMT gain calibration before each run, Allows adjustment of levels from 100% (default) to 1%
Detection Limit	0.01 chromophores per square micron
Pixel Placement Error	1 pixel @ 5 micron resolution
Uniformity	5% CV global non-uniformity; average local non-uniformity is typically 1% based on 100 micron features
Scan time	Two-color simultaneous data acquisition in 16 minutes per for 3-micron scans and 24 minutes for 2-micron scans (scan region of 61 mm x 21.6 mm)

References

1. Queißer-Luft, A., and Spranger, J. Congenital Malformations. *Dtsch Arztebl*, 103(38), 2464-2471 (2006).
2. Jones, K.L., and Adam, M.P. Evaluation and Diagnosis of the Dysmorphic Infant. *Clin Perinatol*, 42(2), 243 (2015).
3. Mandell, D.S., *et al.* Factors associated with age of diagnosis among children with autism spectrum disorders. *Pediatrics*, 116(6), 1480-1486 (2005).
4. Elsabbagh, M., *et al.* Global prevalence of autism and other pervasive developmental disorders. *Autism Res*, 5(3), 160-179 (2012).
5. Boyle, C.A., *et al.* Trends in the prevalence of developmental disabilities in US children. *Pediatrics*, 127(6), 1034-1042 (2011).
6. Hunter, A.G.W. Medical genetics: 2. The diagnostic approach to the child with dysmorphic signs. *CMAJ*, 167(4), 367-372 (2002).
7. Kaufman, L., *et al.* The genetic basis of non-syndromic intellectual disability: A review. *J Neurodevelop Disord*, 2, 182-209 (2010).
8. Satya-Murti, S., *et al.* Chromosomal Microarray Analysis for Intellectual Disabilities. American Academy of Neurology (2013). https://www.aan.com/uploadedFiles/Website_Library_Assets/Documents/3.Practice_Management/1.Reimbursement/1.Billing_and_Coding/5.Coverage_Policies/13%20ChromoMicroIntelDisabil.pdf
9. Michelson, D.J., *et al.* Evidence report: Genetic and metabolic testing on children with global developmental delay, Report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology*, 77(17), 1629–1635 (2011).
10. South, S.T., *et al.* ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: Revision 2013. *Genet Med Genet Med*, 15(11), 901-909 (2013).
11. Vermeesch, J.R., *et al.* Guidelines for molecular karyotyping in constitutional genetic diagnosis. *Eur J Hum Genet*, 15, 1105-1114 (2007).
12. Miller, D.T., *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*, 86(5), 749-764 (2010).
13. Kiang, J.U., and Koo, S.H. Evolving applications of microarray technology in postnatal diagnosis (Review). *Int J Mol Med*, 30, 223-228 (2012).
14. Lyle, R., *et al.* Genotype – Phenotype correlations in Down syndrome identified by array CGH in 30 cases of partial trisomy and partial monosomy chromosome 21. *Eur J Hum*, 17(4), 454-466 (2009).