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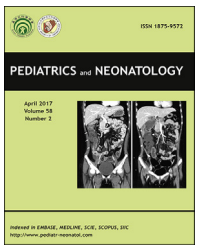

REVIEW ARTICLE

Current Genetic Testing Tools in Neonatal Medicine

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With the growing understanding of the magnitude of genetic diseases in newborns and equally rapid advancement of tools used for genetic diagnoses, healthcare providers must have a sufficient knowledge base to both recognize and evaluate genetic diseases in the neonatal period. Genetic assessment has become an essential aspect of medicine, and professionals need to know when genetic evaluation is indispensable. Much progress has been made in recent years in utilizing massively parallel sequencing for rapid diagnosis of genetic conditions in neonates. Next-generation sequencing is increasingly being used for noninvasive prenatal diagnosis, and it may become an essential component of newborn screening. This review will define some basic genetic terms and concepts, explain the gamut of genetic testing available for early diagnosis of genetic diseases, and describe some common chromosomal abnormalities, genomic disorders, and single-gene diseases relevant to neonatal medicine.

1. Introduction

The exponential growth of the diagnostic technologies in genetics in the past decade has enabled state-of-the-art evaluation of newborns and infants. Now, we have the technology to provide genetic testing on a single cell in an embryo before implantation,[1,2] and to extract fetal DNA from maternal plasma to test for significant genomic imbalances.[3,4] With the growing awareness of the role of genetics in human diseases, susceptibility to complex diseases, and innate responsiveness to environmental triggers such as drugs, toxins, and infectious agents, it is crucial for physicians to implement individualized medicine in clinical practice, and apply the best diagnostic and therapeutic tools for pediatric care.

Early detection and management of inborn errors of metabolism through clinical/biochemical evaluation and deciphering of newborn screening are essential in this population for improved clinical outcomes. Abnormal biochemical results such as hyperammonemia, hypoglycemia, and severe lactic acidosis are important indicators of

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possibly life-threatening genetic diagnoses, and should be evaluated promptly. While craniofacial dysmorphism and/or multiple congenital anomalies frequently herald a genetic evaluation, neonatal hypotonia, feeding difficulties, seizures, and altered mental status often portend an underlying genetic etiology. In large tertiary centers, genetic consultation is frequently obtained for newborns with specific congenital cardiovascular malformations, cardiomyopathy, neonatal liver disease with direct hyperbilirubinemia, cystic kidney disease, structural brain defects, congenital diaphragmatic hernia, disorders of sexual differentiation, and skeletal dysplasias/limb malformations, etc. The spectrum of malformations that can affect a fetus or neonate is wide, with several hundred genetic conditions being linked to a genetic causation. Growth anomalies in newborns including overgrowth, asymmetric growth, significant growth retardation, and micro- or macrocephaly are other important considerations for genetic evaluation. Over 4000 Mendelian disorders are known to have a genetic etiology at present, and a significant fraction of these present in the perinatal period with one or more of these clinical presentations. Perhaps the largest data come from the study in British Columbia,[5] where the incidence of genetic disorders was estimated to be 5.32%, when only significant congenital anomalies were taken into consideration (individuals followed up for 25 years). The incidence of genetic disorder was higher in newborns if all congenital anomalies were included (7.94%). While it may be difficult to clearly estimate the burden of genetic diseases presenting in the 1st month of life, it is remarkable that over 800 genetic disorders have been catalogued in the Online Mendelian Inheritance in Man presenting in the newborn period (www.omim.org). In these instances, a molecular diagnosis has a significant impact not only on the recurrence risk for families, but also on providing appropriate medical care and health intervention for the newborn. Therefore, it is critical that healthcare providers understand the basics of the multitude of genetic tests that are currently in place, and that they be familiar with the fundamental genetic concepts to assess children suspected to have inherited disorders.

2. Basics of clinical genetics

With the exception of the germ cells and a few highly specialized cell types that lack DNA (e.g., red blood cells), each human cell contains 23 pairs of chromosomes ($n = 46$). The chromosome pairs are called homologous chromosomes, except for the sex chromosome in males, where a Y chromosome is present with the X chromosome. The genetic information is contained in the DNA, packaged tightly in the 23 pairs of chromosomes within the nucleus (Figure 1A). Genes are segments of DNA that contain exons (coding sequences), introns (noncoding regions), and regulatory sequences that are important for proper gene expression. The genome collectively refers to the total genetic information in a cell. It is estimated that humans have ~22,000 genes, providing the blueprint in the form of RNA, to direct protein synthesis necessary for maintaining structure, function, and regulation of body. While most mutations occur in the coding region, promoters and other regulatory elements can also have sequence alterations, affecting protein functions, thus interfering with normal cellular processes. There are now thousands of single-gene disorders that are well characterized, with several being recognized in the neonatal period, such as cystic fibrosis, phenylketonuria, spinal muscular atrophy, congenital adrenal hyperplasia, Stickler syndrome, CHARGE syndrome, osteogenesis imperfecta, Rubinstein—Taybi syndrome, and Noonan syndrome (Table 1). These single-gene disorders, also called monogenic disorders, occur as autosomal dominant, autosomal recessive, or X-linked disease. These disorders are routinely diagnosed by analytes, biochemical assays and/or DNA-based studies.

Chromosomal abnormalities constitute a major category of genetic diseases, which result in most cases from errors in the two cell divisions of meiosis causing an abnormal gamete. The ensuing imbalance can involve the entire chromosome, causing either trisomy or monosomy (aneuploidy) as a consequence of nondisjunction, when the homologous chromosomes fail to separate during cell division. These can also be seen as structural rearrangements, observed as insertions, translocations, inversions, deletions, duplications, and complex rearrangements. The effects of chromosomal imbalance are largely due to altered dosage of genes that are important for various cellular functions. The brain, being most vulnerable to such genomic alterations, is often involved, ultimately influencing intellectual function. The burden of constitutional cytogenetic abnormalities is considerably higher in spontaneous miscarriages, detected in about 50% of all first-trimester abortuses.[13] The frequency of karyotype abnormalities in stillbirths is also significant, reaching around 6%.[14] At birth, chromosomal abnormalities (autosomes and sex chromosomes) are ascertained in one in 160 live born infants.[15] Of these, the most common cytogenetic abnormality encountered is Down syndrome (trisomy 21), seen in approximately one in 800 live births, arising mainly from meiosis I error. Cardiac screening is recommended in all infants born with Down syndrome since 45—50% are known to have heart defects.[8] Atrioventricular septal defect is the most common congenital cardiovascular malformation, followed by ventricular septal defect, atrial septal defect, and tetralogy of Fallot.[16] Malformations such as duodenal atresia and Hirschsprung disease are more common in Down syndrome than in other cytogenetic disorders. G-banded karyotype analysis continues to be the mainstay of the evaluation of aneuploidies in present day, including trisomy 21, trisomy 18, trisomy 13, and Turner syndrome (45,X) (Table 1). Congenital cardiovascular malformations are observed in about 30% of females with Turner syndrome,[17—19] with left-sided lesions such as bicuspid aortic valve and coarctation of the aorta (CoA) being the most common defects.[20,21] Hypoplastic left heart syndrome is observed in some females with Turner syndrome.[22] Trisomy 18 and 13, both associated with significantly high early mortality, are recognizable at birth by distinct craniofacial features and congenital anomalies. Intrauterine growth retardation, polyvalvular heart disease, and clenched fists with over-riding fingers should promptly alert healthcare providers to the possibility of trisomy 18.[23] A newborn with cleft lip and palate, eye anomalies (microphthalmia and anophthalmia), scalp defects, holoprosencephaly, and

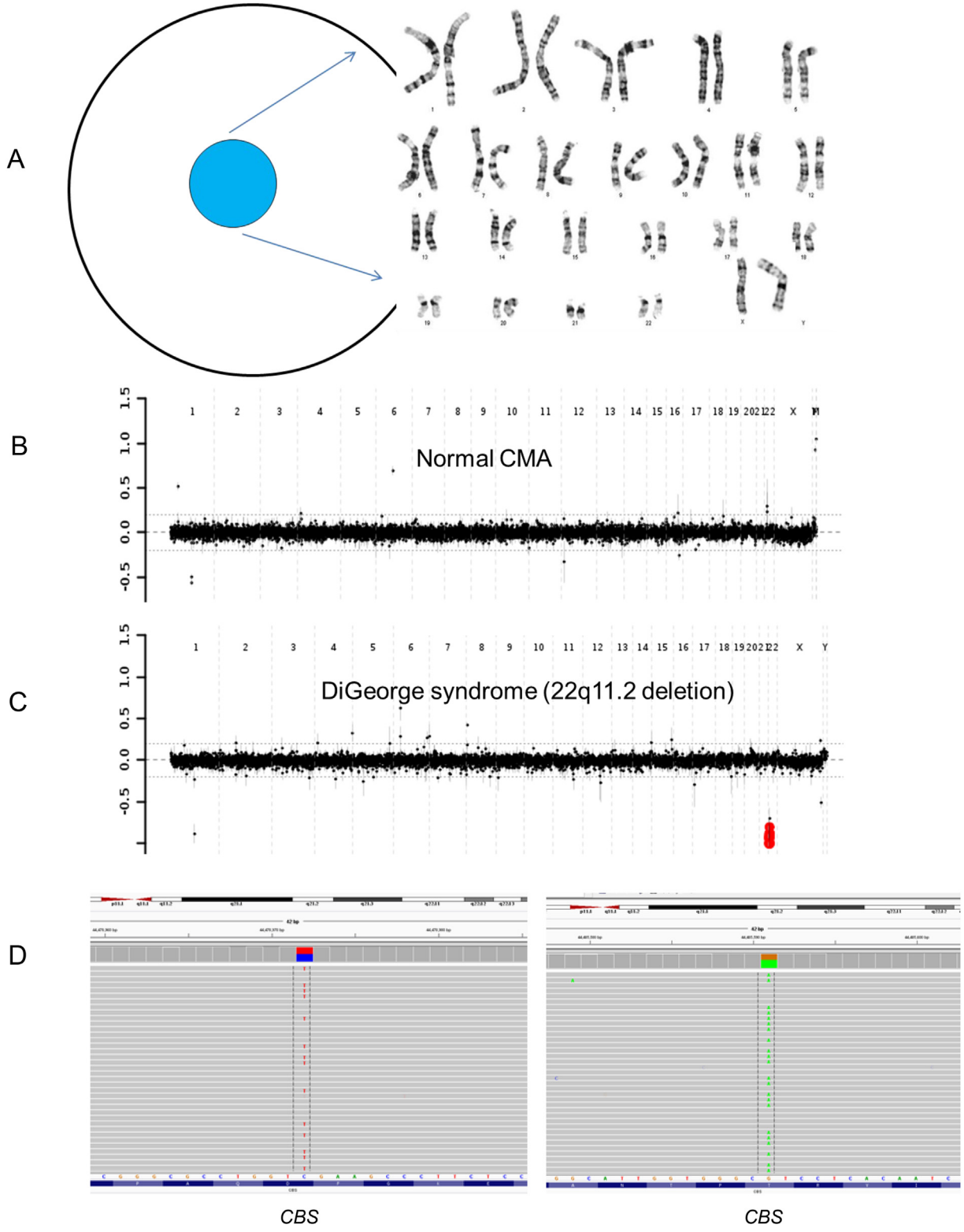

Figure 1 Diagnostic tests available for neonatal testing. (A) A normal karyotype in a female infant (resolution of 5—7 Mb). Chromosomal study evaluating 23 pairs of chromosomes is the standard test for confirming aneuploidies such as Down syndrome (trisomy 21) and Turner syndrome (45,X) in the newborns. (B) A normal chromosomal microarray analysis (significantly higher resolution, about 30 kb) in a newborn infant. A comparison is made with (C) abnormal CMA (chromosomal microarray analysis) results in an infant with DiGeorge syndrome, caused by submicroscopic deletion of 22q11.2. (D) Diagnostic results from whole exome sequencing, aligned to hg19 with a previously described analysis pipeline.[6] The figure shows aligned reads in the Integrative Genomics Viewer[7] and demonstrates two heterozygous variants in *CBS* (c.G1330A, p.D444N; c.C572T,p.T191M) associated with homocystinuria. Subsequently, Sanger sequencing demonstrated that the variants were inherited in trans, with each parent being heterozygous for one of the variants.

Table 1 Examples of genetic testing of some of the disorders observed in the newborn period.

Type of genetic disorders	Genetic disorder	Notable clinical features in the neonatal period	Gene(s) implicated in the syndrome	Recommended diagnostic testing in the neonatal period				
				Karyotype	Chromosomal microarray analysis	Analyte/gene/panel DNA testing/other	Whole exome sequencing	Newborn screening
Chromosomal abnormalities	Down syndrome[8]	Dysmorphic features, AVSD, hypotonia	–	+	–	–	–	–
	Turner syndrome[6]	Cystic hygroma, webbed neck, CoA, HLHS (rare), renal abnormalities	–	+	–	–	–	–
	*Trisomy 18[17]	Dysmorphic features, growth retardation, polyvalvular heart disease, clenched fists	–	+	–	–	–	–
	*Trisomy 13[18]	Growth retardation, cleft lip/palate, microphthalmia, scalp defects, holoprosencephaly, postaxial polydactyly	–	+	–	–	–	–
	Cri-du-chat syndrome (5p minus)[7]	Cat-like cry, round face, microcephaly, CVM	–	+	+	–	–	–
	Cat-eye syndrome[9]	TAPVR, TOF, imperforate anus, iris coloboma, biliary atresia, malrotation of the gut, preauricular tags or pits, renal malformation	–	+	+		–	–
Genomic disorders	†DiGeorge syndrome/ velcardiofacial syndrome	Conotruncal heart defects, hypocalcemia, absent or hypoplastic thymus, cleft palate, dysmorphic features, long digits	*TBX1*	–	+	–	–	–
	Prader–Willi syndrome	Neonatal hypotonia, feeding difficulties	*SNRPN*	–	+	Methylation study for Prader–Willi/Angelman syndrome	–	–
	1p36 deletion	Dysmorphic features, sensorineural hearing loss, seizures, brain abnormalities, CVM, LVNC	*RERE*	–	+	–	–	–
	Miller–Dieker syndrome	Cerebral agyria/pachygyria or type I lissencephaly, corpus callosum dysgenesis/agenesis, microcephaly, seizures, distinctive facies	*PAFAH1B1 (LIS1)*	–	+	–	–	–

Single gene disorders	Spinal muscular atrophy	Hypotonia, muscle weakness, absence of deep tendon reflexes	*SMN1*	−	−	*SMN1* deletion analysis	−	−
	Smith—Lemli—Optiz syndrome	Growth retardation, microcephaly, congenital anomalies, 2—3 syndactyly of the toes	*DHCR7*	−	−	Sterol panel (7-dehydocholesterol elevation); *DHCR7* sequence analysis	−	−
	Cornelia de Lange syndrome	Growth retardation, hirsutism, synophrys, highly arched eyebrows, upper limb reduction defects including oligodactyly, CVM	*NIPBL*, *RAD21*, *SMC3*, *HDAC8*, *SMC1A*	−	+ (rare)	Single gene testing; NGS panel	+**	−
	Rubinstein—Taybi syndrome	Dysmorphic features, broad thumbs and great toes, cryptorchidism, renal anomalies	*CREBBP*, *EP300*	−	+	*EP300*, *CREBBP* sequence analysis	+**	−
	CHARGE syndrome	Choanal atresia, coloboma, external, middle, and inner ear abnormalities, hearing loss, cranial nerve dysfunction, feeding difficulties, CVM, urogenital malformations, EA, TEF, cleft lip/palate, postnatal growth retardation	*CHD7*	−	+ (rare deletion/duplication)	*CHD7* sequence analysis	+**	−
	Holt—Oram syndrome	Preaxial radial ray anomaly, ASD, cardiac conduction defect	*TBX5*	−	+ (rare deletion/duplication)	*TBX5* sequence analysis	−	−
	Kabuki syndrome	Elongated palpebral fissures with eversion of the lateral part of the lower eyelid; arched and broad eyebrows; cupped ears, CVM, other congenital anomalies	*KMT2D* (*MLL2*), *KDM6A*	−	+ (rare deletion/duplication)	*KMT2D* and *KDM6A* sequence analysis	+**	−
	Stickler syndrome	Pierre Robin sequence (micrognathia, cleft palate, glossoptosis), myopia, hearing loss	*COL2A1*, *COL11A1*, *COL11A2*, *COL9A1*, *COL9A2*, *COL9A3*	−	−	NGS panel	+**	−
	Osteogenesis Imperfecta	Fractures in the newborn period, blue sclera	*COL1A1/2*	−	+ (rare)	*COL1A1*, *COL1A2* sequence analysis	−	−
	Zellweger syndrome spectrum disorder	Hypotonia, large anterior fontanel, liver dysfunction, seizures, bony stippling of the patella	*PEX1* (most common); at least 12 *PEX* genes implicated	−	−	VLCFA (elevation of C26:0 and C26:1, and the ratios C24/C22 and C26/C22); skin fibroblasts for confirmation; *PEX1* sequence analysis, NGS panel	−	−

(*continued on next page*)

Table 1 (*continued*)

Type of genetic disorders	Genetic disorder	Notable clinical features in the neonatal period	Gene(s) implicated in the syndrome	Recommended diagnostic testing in the neonatal period				
				Karyotype	Chromosomal microarray analysis	Analyte/gene/panel DNA testing/other	Whole exome sequencing	Newborn screening
	Noonan syndrome/ RASopathies	Hydrops, dysmorphic features, pulmonary valve stenosis, HCM, cryptorchidism	*PTPN11*, *RAF1*, *SOS1*, *NRAS*, *BRAF*, *MAP2K1*	−	−	NGS panel	+	−
	Alagille syndrome	Distinctive facies, cholestatic jaundice, bile duct paucity on liver biopsy, butterfly vertebrae, posterior embryotoxon, TOF	*JAG1*, *NOTCH2*	−	+	*JAG1*, *NOTCH2* sequence analysis	−	−
	Tuberous sclerosis	Cardiac rhabdomyomas, infantile spasms, subependymal nodules, cortical dysplasia, hypomelanotic macules	*TSC1*, *TSC2*	−	+	*TSC1*, *TSC2* sequence analysis	−	−
	Congenital adrenal hyperplasia due to 21-Hydroxylase-deficiency	Poor feeding, hypotension, hyponatremia, hyperkalemia, metabolic acidosis, adrenal crisis, virilization in females	*CYP21A2*	−	−	Serum 17-OHP (markedly elevated), androgens (elevated); *CYP21A2* sequence analysis	−	+
	Phenylketonuria	No physical signs of hyperphenylalaninemia at birth—Impaired brain development in untreated children resulting in ID, microcephaly	*PAH*	−	+ (rare)	Plasma phenylalanine above 120 μmol/L and altered ratio of phenylalanine to tyrosine, *PAH* sequence analysis	−	+
	Galactosemia	Sepsis, feeding difficulties, liver dysfunction, coagulation problems, jaundice	*GALT*	−	−	Erythrocyte galactose-1-phosphate (elevated), erythrocyte GALT enzyme activity (absent); *GALT* sequence analysis	−	+
	Cystic fibrosis	Meconium ileus, pancreatic insufficiency	*CFTR*	−	−	Sweat chloride test; *CFTR* sequence analysis	−	+
	Congenital disorder of glycosylation[10]	Hypotonia, seizures, cerebellar hypoplasia, liver dysfunction, coagulation defects, protein-losing enteropathy	Over 80 congenital disorders of glycosylation	−	−	Carbohydrate-deficient transferrin	+	−
	PURA-related disorder[11]	Hypotonia, abnormal EEG, feeding and respiratory difficulties, myopathic facies	*PURA*	−	−	NGS (panel of genes linked with ID)	+	−
	Neonatal	Intrauterine growth retardation,	*ABCC8*,	−	+	NGS panel	+	−

	diabetes mellitus	hyperglycemia, glycosuria	*EIF2AK3*, *FOXP3*, *GATA6*, *GCK*, *INS*, *KCNJ11*, *PDX1*, *ZFP57*				
	Familial hyperinsulinism (neonatal presentation)	Severe hypoglycemia, inappropriately elevated serum concentration of insulin, seizures, hypotonia, poor feeding, apnea	*ABCC8*, *KCNJ11*, *GCK*, *GLUD1*, *HNF4A*, *HADH*, *UCP2*	−	+ (rare)	NGS panel	+	−
	Epileptic encephalopathy[12]	Neonatal seizures	*KCNQ2*, *KCNQ3*, *KCNT1*, *SCN1A*, *SCN1B*, *SCN2A*, *SCN8A*, *SIK1*, *STXBP1*, *ALDH7A1*, *ARX*, *SLC25A22*, *PLCB1*, *SLC13A5*	−	+	NGS panel	+	−
	Congenital myotonic dystrophy	Severe weakness, hypotonia, respiratory insufficiency	*DMPK*	−	−	CTG repeat expansion in the noncoding region of *DMPK*	−	−
	Congenital central hypoventilation syndrome	Hypoventilation when awake and/or sleeping, Hirschsprung disease, autonomic dysfunction	*PHOX2B*	−	+	*PHOX2B* polyalanine expansion; *PHOX2B* sequence analysis	−	−
Methylation defects	Beckwith–Wiedemann syndrome	Overgrowth, macroglossia, omphalocele, neonatal hypoglycemia, ear creases	Abnormal methylation on the maternal chromosome at imprinting center; paternal UPD 11p15; *CDKN1C* mutation	−	+	Methylation analysis; *CDKN1C* sequence analysis	−	−

ASD = atrial septal defect; AVSD = atrioventricular septal defect; CHARGE = mnemonic for *c*oloboma, *h*eart defects, choanal *a*tresia, *r*etarded growth and development, *g*enital abnormalities, and *e*ar anomalies; CoA = coarctation of the aorta; CVM = cardiovascular malformation; EA = esophageal atresia; FISH = fluorescence *in situ* hybridization; GALT = galactose-1-phosphate uridyltransferase; HCM = hypertrophic cardiomyopathy; HLHS = hypoplastic left heart syndrome; ID = intellectual disability; LVNC = left ventricular noncompaction; NGS = next-generation sequencing; TAPVR = total anomalous pulmonary venous return; TEF = tracheoesophageal fistula; TOF = tetralogy of Fallot; UPD = uniparental disomy; VLCFA=very long chain fatty acids.

+** = If initial studies are non-diagnostic.

* FISH is available for rapid diagnosis and should be considered in the evaluation.

† FISH is available for 22q11.2 deletion.

postaxial polydactyly would likewise need to be evaluated for trisomy 13.[24] Fluorescence *in situ* hybridization is available for rapid diagnosis of these critical aneuploidy syndromes. Concomitant presence of imperforate anus and total anomalous pulmonary venous return in a newborn, especially in the presence of preauricular pits and tags, iris coloboma, gut malrotation, or biliary atresia, should alert the physician to the probability of cat eye syndrome. This syndrome is typically caused by a marker chromosome resulting in partial tetrasomy (i.e., 4 copies) of the region spanning the p arm and part of 22q11 arm (Table 1).[9] Many other cytogenetic abnormalities present in the neonatal period, which require prompt detection for optimal clinical management and counseling.

3. Chromosomal microarray analysis

For the past five decades, G-banded karyotype analysis has been used for the evaluation of birth defects in children, offering resolution of genetic imbalance of over 5–10 Mb in size. The introduction of the fluorescence *in situ* hybridization technique in clinical genetics in 1990 brought in the era of molecular cytogenetics. For the first time, fluorescence *in situ* hybridization allowed detection of disease-causing submicroscopic events that were often below the detection threshold of G-banded karyotype analysis. The characterization of these submicroscopic events in human diseases, also called structural variations or DNA copy number variations (CNVs), has been revolutionary in the field of human genetics. CNVs involving all chromosomes have been identified in the past decade due to the growing use of array-comparative genomic hybridization [chromosomal microarray analysis (CMA)]. The higher sensitivity of array-comparative genomic hybridization, often within 10–30 kb resolution, offers advantages over routine G-banded karyotype analysis when identifying genomic imbalances that are submicroscopic (Figures 1B and 1C). The microarray technology is based on hybridization of patient's labeled DNA against a healthy reference control. The measurement of signal intensity ratio of patient's DNA to reference DNA allows identification of gains or losses of chromosomal material. These CNVs involve deletion or duplication of genomic segments responsible for genomic disorders.[25] Genomic disorders, usually affecting dosage-sensitive gene(s), are increasingly being recognized as important players in human birth defects, as shown by the increasing use of CMA in clinical practice. Offering an increasing detection rate of about 15–20%, CMA is now considered as a first-tier test in the evaluation of children with multiple congenital anomalies.[26] The 22q11.2 deletion syndrome, also known as DiGeorge syndrome or velocardiofacial syndrome, is a prototype genomic disorder, which is frequently observed with conotruncal heart defects.[27] Neonates born with tetralogy of Fallot, truncus arteriosus, or interrupted aortic arch type B are routinely evaluated for the typical ~3 Mb or nested 1.5 Mb deletion of 22q11.2 (Figure 1C). Occurrence of cleft palate absent or hypoplastic thymus, and/or hypocalcemia with conotruncal heart defects warrants evaluation of DiGeorge syndrome/velocardiofacial syndrome in newborns. Another relevant example of a genomic disorder presenting in the newborn period is Prader–Willi syndrome. Significant neonatal hypotonia with feeding difficulties should alert the physician to evaluate the baby for loss of the paternally expressed genes on 15q11.2 related to this diagnosis (Table 1).[28] This can occur as a result of either deletion of 15q11.2 on the paternal allele or maternal uniparental disomy of chromosome 15. While several other microdeletion syndromes are clinically recognizable in the neonatal period, such as 1p36 microdeletion syndrome (dysmorphic features, hypotonia, feeding difficulties, total or partial absence of the corpus callosum, seizures, septal defects, left ventricular noncompaction),[29,30] Miller–Dieker syndrome (lissencephaly, seizures, atrial septal defect), and Alagille syndrome (*JAG1* deletion on 20p12; characteristic facies, cholestasis, peripheral pulmonic stenosis), many genomic disorders are ascertained after the 1st month of life, particularly those associated with neurocognitive delays, such as Williams–Beuren syndrome (7q11.23 deletion; supravalvular aortic stenosis, failure to thrive, hypercalcemia) and Smith–Magenis syndrome (17p11.2 deletion; behavior disorder, sleep disturbance, craniofacial, septal defects). Undoubtedly, CMA undertaken for evaluation of dysmorphic features and congenital anomalies in the newborn period often uncovers these relevant genetic disorders that require prolonged multidisciplinary care. In many tertiary care centers, CMA is now routinely performed for congenital cardiovascular malformations,[31] with diagnostic yield as high as 20% in children with syndromic cardiac defects.[32–34]

While CMA is a powerful tool in identifying the genetic cause of multiple congenital anomalies, it is important to note that not all structural variations observed on CMA are pathogenic. Some are benign and largely characterized as copy number polymorphisms.[35] In other instances, CNVs may be involved in complex disease traits, with incomplete penetrance and variable expressivity.[36] Since CNVs are common in the genomes of healthy individuals,[37,38] it can often be challenging to attribute pathogenicity to loci that are frequently involved in structural rearrangement in the unaffected population. These variations in apparently healthy individuals are also catalogued in detail and deposited in large databases that are publically available, such as Database of Genomic Variants (http://projects.tcag.ca/variation). The CNVs established in disease population are also systematically chronicled in large publically available databases such as DECIPHER (https://www.sanger.ac.uk/research/areas/humangenetics/ddd) and The International Standards for Cytogenomic Arrays (ISCA) (www.iscaconsortium.org).

It is important to note that there are different platforms for CMA studies. Oligonucleotide-based arrays provide an excellent substrate for detecting DNA copy number changes, including single exon deletions.[36] Single-nucleotide polymorphism arrays can detect CNVs, as well as long contiguous stretches of copy number neutral regions of absence of heterozygosity. These can be associated with uniparental disomy or parental consanguinity, increasing the risk for autosomal recessive conditions. Single-nucleotide polymorphism arrays can also detect low-level mosaicism (presence of 2 populations of cells with different genotypes in 1 individual) and triploidy (3 copies of every chromosome). Combined oligonucleotide/single-nucleotide polymorphism arrays are also available at

some laboratories, offering the advantages of each method. As the methodology is designed to detect copy number imbalances, CMA is unable to detect balanced rearrangements such as reciprocal translocations and chromosomal inversions.

4. Newborn screening

The most successful application of genetic testing has undoubtedly been in the field of newborn screening, aimed at identifying treatable conditions. Every year, millions of presymptomatic newborns are tested for critical genetic, endocrine, metabolic, and hemoglobin disorders using a single 3-mm dried blood spot sample. Routine screening of blood using electrospray tandem mass spectrometry methods was introduced into state newborn screening programs in the 1990s. Early detection of amino acidemia (phenylketonuria, tyrosinemia, maple syrup urine disease, etc.), galactosemia, organic acidemias (propionic acidemia, methylmalonic acidemias, biotinidase deficiency, etc.), urea cycle defects (citrullinemia, argininosuccinic aciduria, argininemia), and fatty acid oxidation defects (medium-chain acyl-CoA dehydrogenase-*MCAD* deficiency, very long-chain acyl-CoA dehydrogenase-*VLCAD* deficiency, other carnitine uptake and transporter defects) has assisted in reducing mortality, morbidity, and disabilities in at-risk newborns. Plasma amino acids, urine organic acids, acylcarnitine profile, and ammonia are important diagnostic studies to validate the metabolic abnormalities detected on newborn screening. In addition, screening at birth for congenital adrenal hyperplasia, congenital hypothyroidism, cystic fibrosis, severe combined immunodeficiency, and other conditions associated with T-cell lymphopenia has been crucial in judicious management of these disorders. Most states in the USA have mandatory newborn screening for at least 29 primary conditions and 25 recommended secondary targets (with opt-out policies for parents), following the recommendation by a newborn screening expert group convened by the American College of Medical Genetics.[39] Screening for some of the lysosomal storage disorders has been introduced in some states with established benefits of early treatment pertaining to enzyme replacement or hematopoietic stem cell transplantation.[40]

5. Whole exome sequencing

While the application of cytogenetics and molecular cytogenetics has been pivotal in the evaluation of newborns with suspected genetic diseases, DNA-based sequencing studies have been equally important with groundbreaking and innovative utility in newborn evaluation. Mendelian disorders, such as spinal muscular atrophy, congenital muscular dystrophy, congenital sensorineural hearing loss, and polycystic kidney disease in newborns, have traditionally been evaluated by single-gene DNA sequencing studies or gene-panel evaluation. Infants experiencing unprovoked seizures within the first 28 days of life without a history of asphyxia, ischemia, or infection are now increasingly being evaluated for a genetic etiology using next-generation sequencing. Several single genes are associated with early infantile epileptic encephalopathy, with onset in the first months of life, including *ARX*, *SLC25A22*, *KCNQ2*, *STXBP1*, etc.[41] Massive parallel sequencing or next-generation sequencing is now increasingly being utilized in neonatal intensive care setting for rapid genetic diagnosis.[42,43] Collectively, the 180,000 exons (termed exome) only account for about 1.5% of the human genome, but they contribute to 80—85% of all the known disease-causative variants. Several studies have demonstrated the utility of whole exome sequencing (WES) in critically ill neonates with genetic disorders, providing prompt diagnoses for personalized care (Table 1).[11,12] Some published examples of rare disorders unearthed by WES or whole genome sequencing in seriously ill neonates include congenital disorder of glycosylation[10] and *KCNQ2* mutation[12] (Table 1). While the prohibitive cost of whole genome sequencing limits its use at present, WES has been proved to be successful in clinically diagnosing about 25—30% of children suspected to have genetic diseases.[44,45] Although it is extremely powerful, there is a concern of uncovering incidental findings (unrelated to the reason for testing of the newborn) that can be relevant for child's future health, or can impact the well-being of parents and other family members. For this consideration, it is highly recommended that WES be performed with detailed pretest counseling, generally offered by genetic counselors or clinical geneticists skilled in addressing these concerns related to genomic testing.[46]

6. Mitochondrial disease in newborns

The increasing awareness of the presentation of mitochondrial disease in neonatal period has resulted in systematic evaluation of this disorder in the newborn critical care units. Mitochondria are energy-generating power plants of the cell, having their own small genome containing 37 genes that encode 13 proteins, 22 tRNAs, and two rRNAs. Inherited as both an autosomal recessive and an autosomal dominant disease due to nuclear DNA mutation, mitochondrial disease is also caused by sporadic or maternally inherited mitochondrial DNA changes, leading to dysfunction of the mitochondrial respiratory chain.[47—49] The mitochondrial electron transport chain is the essential final pathway for aerobic metabolism producing the majority of energy driving cellular reactions for vital tissues. A disruption of this function can virtually involve any organ system in the body, seen clinically as lactic acidosis, encephalopathy, skeletal myopathy, cardiomyopathy, liver disease, respiratory difficulties, swallowing dysfunction, sensorineural deafness, and/or ocular disease. Electron transport chain studies have traditionally been the mainstay in the diagnosis of mitochondrial diseases, although with the emergence of high-throughput technologies, next-generation sequencing is increasingly being used for the diagnosis of such diseases.[50,51]

7. Summary

In summary, the phenomenal advancement of molecular technologies in the diagnosis of genetic disease in recent years has dramatically changed the landscape of neonatal

medicine. While the utility of whole genome sequencing is being deliberated currently for its inclusion in the newborn screening program,[52] the implementation of this technique in the clinical evaluation of critically ill neonates is imminent. It is vital for pediatric healthcare providers to be cognizant of the available diagnostic tools for providing the best clinical care to their patients with genetic disorders.

Conflicts of interest

The author has no conflicts of interest relevant to this article.

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