CHAPTER NINETEEN

Clinical and Genomic Approaches for the Diagnosis of Craniofacial Disorders

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Abstract

With the rapid development of readily accessible molecular diagnostic tools, a growing number of patients and families with craniofacial anomalies will have access to a confirmed molecular diagnosis. This chapter provides an overview to current clinical and molecular resources and approaches used by diagnostician today.

Clarifying the underlying cause of a congenital defect is necessary to provide proper counseling, identify carrier/risk status of family members, inform prognosis and direct appropriate management, treatments, and surveillance recommendations. The use of molecular testing has evolved to confirm a suspected clinical diagnosis, establish a diagnosis in an unclear condition and end a diagnostic odyssey for many children with underlying syndromes, but the use of these techniques to understand common nonsyndromic malformations like clefts and craniosynostosis is still an active area of research that will contribute to clinical care in the future.

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1. INTRODUCTION

A diagnostic evaluation for a craniofacial disorder is based on the knowledge from embryology, dysmorphology, clinical genetics, and pediatrics. With the rapid development of readily accessible molecular diagnostic tools, patients, and families will have access to a confirmed molecular diagnosis, shortening their diagnostic odyssey, and facilitating their clinical care and genetic counseling. As a consequence, families and medical providers are turning to the clinical and basic science research communities for recommendations and targeted therapies to improve outcomes and prognoses.

Clarifying the underlying cause of a congenital defect is necessary to provide proper counseling, identify carrier/risk status of family members, inform prognosis and direct appropriate management, treatments, and surveillance recommendations. The use of molecular testing has evolved to confirm a suspected clinical diagnosis, establish a diagnosis in an unclear condition and end a diagnostic odyssey for many children with underlying syndromes, but the use of these techniques to understand common nonsyndromic malformations like clefts and craniosynostosis is still an active area of research that will contribute to clinical care in the future. It is not the intention of this chapter to encompass all elements of a diagnostic evaluation as one can refer to a growing number of texts, articles, and databases regarding the diagnostic approach to a dysmorphic child (Aase, 1990; Epstein, Erickson, & Wynshaw-Boris, 2008; Ferretti, 2006; Graham & Sanchez-Lara, 2014; Hall, Allanson, Gripp, & Slavotinek, 2007; Johns Hopkins University, n.d.; Jones, Jones, & Campo, 2013; London Medical Databases, n.d.). Rather, this chapter will provide an overview to some of the current molecular resources and approaches used today.

2. CLINICAL EVALUATION AND DYSMORPHOLOGY

The field of dysmorphology has played a major role in the prenatal and postnatal clinical diagnostic evaluation of patients with craniofacial malformations. A comprehensive diagnostic consultation includes a preevaluation review of photographs and prior records including data from prior molecular, cytogenetic and biochemical tests, and other procedures; a thorough pregnancy, birth, family, medical, surgical, and developmental history; and detailed review of systems. It is particularly important to not overlook the prenatal exposure history and timing for common recognized teratogens (Abbott, 2010), and to be able to recognize a teratogenic

syndrome and be able to reassure parents regarding common exposures to medications regarding medications, which are not teratogenic. The importance of common maternal conditions (e.g., obesity, diabetes, alcoholism), which increase the risk for certain malformations, can help to prevent future malformations. One of the most valuable tools in an evaluation is a thorough history and pedigree analysis, which can alert the clinician of potential Mendelian disorders with incomplete penetrance and variable expressivity. The physical examination should always include standard growth measurements plotted appropriately and adjusted for prematurity and sex. One should also follow a prescribed examination format whenever possible to avoid overlooking clues in the proband and other potentially affected relatives. A differential diagnosis can then be formulated and additional data may be collected to narrow this list of conditions or confirm a suspected diagnosis. Follow-up data can include imaging, additional subspecialty consultations, examining photographs at a younger age, imaging studies, or laboratory studies (molecular, cytogenetic, or biochemical testing). It is also often helpful to just reevaluate the patient after a short time if the child is very young or records are not complete.

An individual with "dysmorphic features" has a pattern of morphologic abnormalities that is outside the normal range of development either by descriptive observation or measurement (Hall et al., 2007). Clinicians may refer to such a collection of major and minor anomalies in an individual as a "syndromic" based solely on their experience. Most care providers for dysmorphic patients develop skills in recognizing the "gestault" of common patterns of malformation, but for less frequent malformation syndromes, molecular diagnosis can be quite helpful. Recognizing the pattern of major and minor anomalies through a thorough history and examination is critical for an accurate diagnosis.

A *syndrome* can be defined as a particular set of anomalies occurring together with some frequency. For example patients with Treacher Collins syndrome (Fig. 1B) often have malar hypoplasia, lower lid colobomas, micrognathia, and occasionally cleft palate or microtia, but there are many nuances in diagnosing different syndromes associated with mandibulofacial dysostosis, and the accuracy of a clinical diagnosis can hinge on a single feature such as the presence or absence of hand abnormalities or microcephaly. Multiple structural defects can also result from a *sequence* in which a single primary defect in morphogenesis produces multiple abnormalities through a cascading process of secondary and tertiary problems in morphogenesis.

Isolated structural defects can arise through any of four basic mechanistic categories: *deformations, malformations, disruptions,* and *dysplasias* (Fig. 1; Table 1).

Figure 1 Examples of a deformation, malformation, disruption, and dysplasia. (A) Deformation example: top view of a child's head with plagiocephaly and left-sided flattening of the occiput (Note the anterior position of the left ear as a secondary finding). (B) Malformation example: child with Treacher Collins syndrome (lower lid colobomas, microtia/anotia, micrognathia, wearing a BAHA for conductive hearing loss). (C) Disruption example: multiple facial defects secondary to amniotic band sequence. (D) Dysplasia example: child with craniometaphyseal dysplasia, a progressive bony dysplasia with thickening of the craniofacial bones.

Deformational abnormalities are produced by intrauterine mechanical forces that distort otherwise normal fetal structures. Deformational anomalies often occur late in gestation as the fetus outgrows the size of the uterus and fetal constraint becomes more apparent. It is thought that after an abnormal mechanical stress is removed, the defect may begin to correct spontaneously, or correct with gentle bracing to counter the initial deforming forces. Intrauterine constraint can be secondary to factors specific to the mother (small pelvic outlet, primigravida, bicornuate uterus, or uterine fibroids),

Table 1 Morphogenesis of Congenital Anomalies

Deformations	Response of normal tissue to a mechanical force
Malformations	Primary error in tissue morphogenesis
Disruptions	Breakdown of previously normal tissues
Dysplasias	Abnormal gross structure because of abnormal tissue architecture

to the individual pregnancy (multiple gestation or intra-abdominal pregnancy), or to the individual fetus (oligohydramnios, malformations such as bilateral renal agenesis or abnormal fetal presentations, e.g., breech presentation) (see Graham & Sanchez, 2016).

In contrast, *malformations* denote a primary problem in the morphogenesis of a tissue (Opitz, 2000). An isolated malformation can arise as a localized error in embryonic development with a multifactorial etiology and varied recurrence risk or higher birth prevalence among certain populations. A multiple malformation syndrome results in a primary developmental anomaly of two or more systems in which all of the structural defects are secondary to a single underlying etiology, which can be environmental (teratogenic), genetic (e.g., single-nucleotide variant) or chromosomal. *Disruptions* are structural defects caused by the active destruction and breakdown of normal tissues (e.g., amniotic band syndrome/amnion rupture sequence (Iqbal, Derderian, Cheng, Lee, & Hirose, 2015)). A fourth category of problems, termed *dysplasias*, results in abnormal structure because the tissues from which individual structures are formed are abnormal; for example, the defective cartilage and bone in various skeletal dysplasias (Lachman & Taybi, 2007).

It is often helpful to prepare a differential diagnosis for major malformations while tracking and comparing their associated *minor anomalies*. *Major malformations* are disturbances of embryogenesis and these defects affect medically important structures such as the palate, eyes, brain, heart, and kidneys. Most major malformations occur as isolated defects although they can often be essential to meeting the diagnostic criteria for a recognizable syndrome. *Minor anomalies* and *common variants* (prevalence >4%) are considered subtle developmental disturbances and are of no serious medical or cosmetic consequence but may be rather common in the general population. Minor anomalies and common variants often occur in structures that are embryologically complex such often as the face, ears, hands, and feet; the distinction between these categories is one of degree and prevalence (Aase, 1990; Merks, van Karnebeek, Caron, & Hennekam, 2003). Mendelian disorders are typically very rare and have highly penetrant phenotypes. Outside of

pharmacogenetics, the adoption and clinical utility of rare and more common genetic variants with low penetrance is yet to be determined (Fig. 2).

The use of standardized nomenclature and of phenotype descriptors is essential to both clinical and molecular diagnostics. Over the course of several years, an international group of dysmorphology experts set out to define this work by dividing into in six working groups, each focused on a particular area of the human body. In 2005 and 2006, this international working group met face-to-face developed a standardized set of definitions and terms now known as the Elements of Morphology which describe the physical variations now used in human phenotypic analyses. The Elements of Morphology, resulted in six articles proposing consensus definitions for almost 400 phenotypic variations of the head and face; periorbital region; ear, nose, and philtrum; mouth and lips; and hands and feet (Carey, Allanson, Hennekam, & Biesecker, 2012). Today, integration of clinical and phenotypic data is critical to filtering genomic results and ultimately clinical molecular reporting. The Human Phenotype Ontology (HPO) project (http://www.human-phenotype-ontology.org) has provided an expanded structure and consists of a comprehensive set of over 10,000 terms describing human phenotypic abnormalities and several thousand subclass relations between the various HPO classes (Kohler et al., 2014).

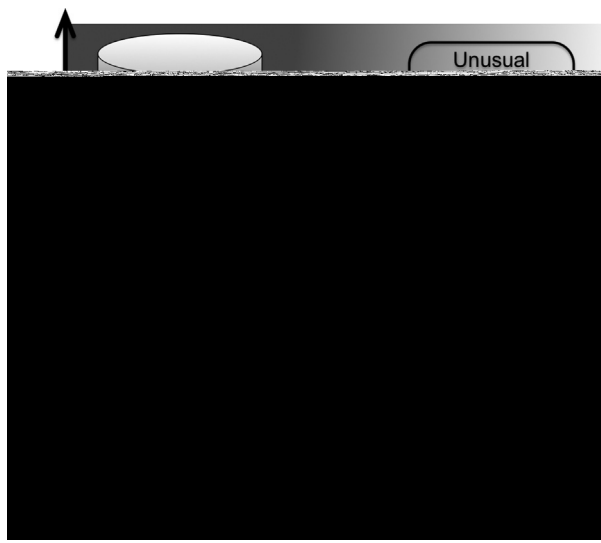

Figure 2 Illustration of categories/conditions with various allele frequencies (*x*-axis) and penetrance (*y*-axis). Currently only highly penetrant and rare conditions are assessed in a clinical setting. Rare and less penetrant variants are difficult to identify and characterize for clinical utility. *(Figure adapted from McCarthy et al., 2009)*

3. CHROMOSOMAL ABERRATIONS

In 1956, Tjio and Levan were the first to accurately describe that humans have 46 chromosomes. This discovery was technically the first very low-resolution whole-genome analysis and opened the field of clinical cytogenetics. As standard-staining techniques such as G-Banding and other visualization methods evolved, the technical ability to detect aneuploidy (e.g., Trisomy 13), large structural rearrangements and some defined microdeletion syndromes became common in clinical practice. Chromosomal aberrations can be associated with up to half of early spontaneous abortions, 6% of stillbirths, around 5% of couples with a history of two or more miscarriages, and approximately 0.5% of newborns (Patil, Rao, & Majumdar, 2014). G-banding chromosome analysis performed at the 350–500 band level does not typically detect segments smaller than 5–10 Mb (Gardner, Sutherland, & Shaffer, 2012). Florescent *in situ* hybridization (FISH) techniques and subtelomere FISH, which bind to specific regions of the chromosome by matching complementary sequences, have improved the ability to detect small cryptic deletions and rearrangements and has a resolution as low as 35 kb (Fig. 4C) (Bejjani & Shaffer, 2008). One primary limitation of G-Banding and FISH apart from the resolution is the need for fixed intact or cultured cells.

4. EVOLUTION OF COPY NUMBER DETECTION

After the first draft of the human genome was published, almost any genomic region could be fluorescently labeled and available commercially for diagnostic purposes. Subtelomeric FISH became one of the most helpful tools to screen chromosomes for cryptic terminal deletions or duplications related to unbalanced translocations (Durmaz et al., 2015). The next wave of technology came from multiplexing thousands of genomic regions on chromosome array chips to efficiently screen the whole genome for cryptic copy number anomalies. As array density and resolution evolved, there was a transition from lower resolution bacterial artificial chromosome arrays to comparative genomic hybridization analyses, oligo and single nucleotide polymorphism (SNP) arrays. The latter two are now often combined and these are the most common array types in use today. Arrays containing SNPs have an advantage over other types of arrays because they can detect copy neutral loss of heterozygosity flagging potential consanguinity and recessive

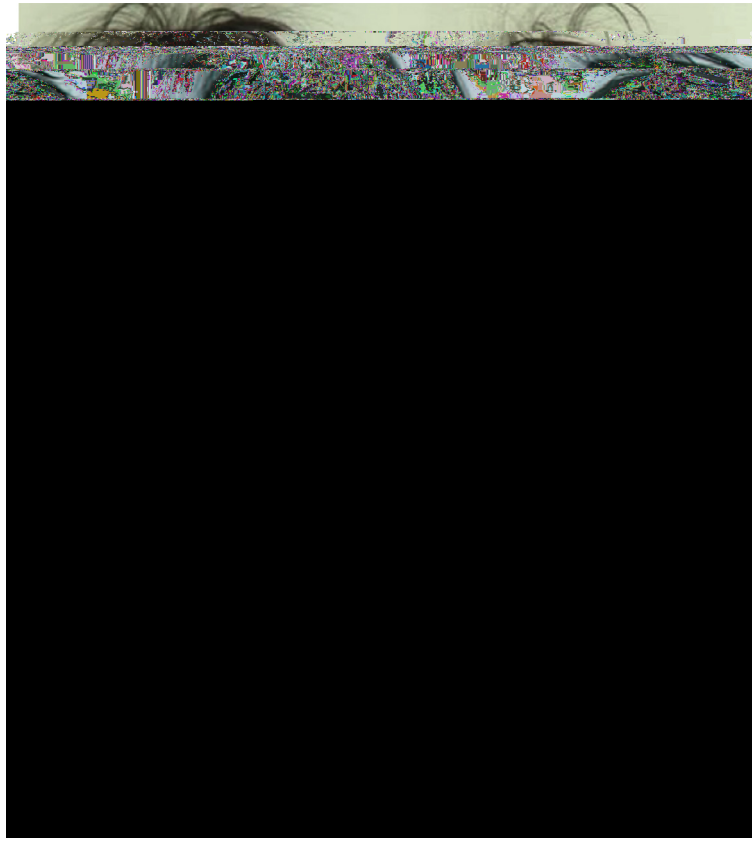

Figure 4 (A and B) Female patient with cleft lip and palate secondary to Cri Du Chat syndrome (5p minus microdeletion syndrome). (C) Florescence *in situ* hybridization (FISH) green probe for 5p confirmed presence of a single copy. (D) Chromosome microarray detected a 33.7 Mb deletion significantly larger than the typical 5p minus deletion.

candidate genes, and can flag individual chromosomes raising concern for uniparental disomy (Alkuraya, 2012). Arrays have significantly advanced our understanding of chromosomal disorders and facilitated the ability to compare an individual's genomic breakpoints with gene content (Fig. 4). A chromosomal microarray cannot detect balanced chromosomal rearrangements such as translocations and inversions, nor can it assess positional information for insertions. Other molecular techniques such as quantitative real-time polymerase chain reaction (qPCR) or multiplex ligation-dependent probe amplification are used to detect, quantify, and confirm chromosomal copy number changes but these cannot screen a large number

of regions across the whole genome. Exon-specific oligo arrays are a more cost-effective way to screen a large number of genes for copy number changes. There are now several copy number detecting bioinformatic algorithms using next-generation sequencing (NGS) (Abel & Duncavage, 2013; Legault, Girard, Lemieux Perreault, Rouleau, & Dube, 2015; Xie & Tammi, 2009). These NGS-based copy number techniques are beginning to transition into the clinical setting where they will likely replace current chromosomal microarray techniques. Extensive validation and optimization are still needed to increase the diagnostic sensitivity and reduce the number of false positives that require follow-up confirmatory testing.

The diagnostic yield of a karyotype or chromosomal microarray analysis used to screen craniofacial malformations varies by type and category. For example, in some studies, the diagnostic yield for isolated nonsyndromic single-suture craniosynostosis cases is very low or near zero. Yet in syndromic craniosynostosis, the yield ranges from 6.7% to 28%. The vast majority (85%) of craniosynostosis cases with an abnormal karyotype or array are typically midline (metopic and sagittal) sutures (Johnson & Wilkie, 2011; Lattanzi et al., 2012; Wilkie et al., 2010). Karyotype and chromosome microarray studies in individuals with oral clefts also have varying degrees of diagnostic yield depending on whether if the clefts were detected prenatally or postnatally. Maarse et al. summarized a comprehensive review of prenatal and postnatal chromosomal and microarray studies (Maarse et al., 2012). Of 407 fetuses with oral clefts, cleft lip and palate had the highest prevalence of associated anomalies (54%, range 39.1–66%). There were 23 cases of cleft lip without cleft palate, and three of these had associated anomalies while only one had a chromosomal defect. Studies that grouped both cleft lip and/or cleft palate had a lower prevalence of associated anomalies (29.9%, ranging 17.2–57.1%). The prevalence of chromosomal defects in cleft cases with associated anomalies was 50% (74/146) while it was 0.9% in cases with clefts that were formerly presumed to be isolated. Of 28,953 postnatally assessed infants, almost all chromosomal abnormalities were found in association with additional anomalies. Cleft palate was the category most frequently associated with other anomalies (45.9%, range 22.2–78.3%). The prevalence of associated anomalies in cleft lip cases was approximately 10%. One study of isolated cleft lip cases found a chromosomal defect in 1.8% (2/110) cases (both having a 22q11.2 deletion). Overall, the diagnostic yield of screening for chromosomal defects in cases of cleft lip with or without cleft palate was 9.5% range (0.5–12.6%) (Maarse et al., 2012).

5. SINGLE-CANDIDATE GENE AND PANEL-BASED TESTING

PCR generates of thousands to millions of copies of a particular DNA sequence (Saiki et al., 1985). Efforts to develop tools utilize PCR led to other molecular techniques such as restriction fragment length polymorphism (RFLP), single-strand confirmation polymorphism (SSCP), and improvement in targeted classical chain-termination sequencing-based methods (Sanger sequencing). Up until the development of NGS, SSCP, and RFLP were the most widely used techniques for mutation screening but were not able to detect every mutation (Durmaz et al., 2015).

Most recognizable craniofacial syndromes are monogenic Mendelian disorders, but even within the same condition there is often *allelic heterogeneity* with most individuals having different mutations in the same gene. The exceptions to this are the recognizable craniosynostosis syndromes involving the *FGFR* genes (*FGFR1, FGFR2, FGFR3*) (Aleck, 2004). In craniofacial medicine, there are a growing number of clinically indistinguishable or overlapping phenotypes that may be caused by mutations in different genes (*locus heterogeneity*). Examples include rasopathies, cohesinopathies, mandibulofacial dysostoses, and Stickler syndrome (Alzahrani, Al Hazzaa, Tayeb, & Alkuraya, 2015; Bezniakow, Gos, & Obersztyn, 2014; Parenti et al., 2015; Robin, Moran, & Ala-Kokko, 1993; Vincent et al., 2015). When using a major malformation as the only search criterion, the number of genes involved can range from just a few to more than a hundred. For many genetically heterogeneous craniofacial disorders, the full complement of causal genes is yet to be established (Beaty et al., 2013; Wilkie et al., 2010).

Determining genetic causality for a particular disease and establishing a molecular diagnosis in clinical practice can be challenging. In recent years, exome and genome sequencing have increased the rate of gene discovery for single-gene disorders among patients with suspected, but previously undiagnosed, genetic disorders. Although exome and genome sequencing are becoming more readily available, the value of molecular diagnosis should be viewed from a clinical perspective as similar to other diagnostic tests. The decision to proceed with molecular testing must integrate many factors specific to clinical status of the affected individual, such as probability of diagnostic yield and the patient's/families personal preference.

The ability to confirm a clinical diagnosis and stop a diagnostic odyssey has historically occurred without any molecular testing, yet the growing

number of clinically indistinguishable craniofacial conditions that have mutations in different genes (*locus heterogeneity*) has spread uncertainty in the clinical setting and prompted more confirmatory molecular testing. A craniofacial condition such as Treacher Collins syndrome, which had initially been reported to be monogenic and autosomal dominant, has subsequently been found to be multigenic with autosomal dominant (*TCOF1, POLR1D*) and recessive forms (*POLR1C)* (Vincent et al., 2015). Stickler syndrome is another common condition with a growing number of genes related to an overlapping phenotype with autosomal dominant (*COL2A1, COL11A1, COL11A2, VCAN*) and autosomal recessive inheritance (*COL9A1, COL9A2, COL9A3, LOXL3*) (Acke et al., 2014; Alzahrani et al., 2015).

As of today, Sanger sequencing remains the gold standard molecular diagnostic tool used to screen DNA for unknown point mutations in defined genes; this may change as the confidence and quality of newer technologies improve (Neveling et al., 2013). Up until recently, some larger genes had remained inaccessible to clinical testing because the older methods were too burdensome on laboratory staff, or the condition was too rare for a test to be commercially viable.

6. NEXT-GENERATION SEQUENCING

In 2001, a new era of rapid gene identification emerged after the first draft of the human genome sequence was released. Large families with multiple affected members could easily have their condition identified and mapped using a whole-genome linkage approach. With the introduction of massively parallel sequencing in 2005, the technology also known as *NGS* or "Next Gen" became a cost-effective tool with advantages in detecting genetic variations ranging from single nucleotides to larger structural rearrangements. *Whole-exome sequencing* (WES) was rapidly developed to perform NGS on exon-enriched DNA. Companion software was developed to code the sequences and quickly identify protein-coding mutations. Using WES in Mendelian disorders has expedited identification of causative genes from a small number of individuals within a family (Zhang, 2014). In 2010, Miller syndrome (a condition with severe micrognathia, cleft lip and/or palate, hypoplasia or aplasia of the posterior elements of the limbs, coloboma of the eyelids, and supernumerary nipples) became the first condition for which WES facilitated the discovery of the underlying causative gene (Ng et al., 2010).

Broad molecular screening techniques have traditionally been reserved for disorders that are difficult to diagnose because of their nonspecific presentation, which is too subtle or has too many overlapping phenotypes to confidently determine which gene to sequence based on clinical features alone. Yet with the dramatic drop in NGS sequencing costs, many centers have begun considering a targeted-gene panel as their first approach.

Limitations of NGS for gene panels or WES include uneven efficiency in exon pull down or capture leading to missing exons or gaps in coverage. Structural variants such as large deletions, duplications, and rearrangements are not routinely detected or assessed outside of a research setting. Nucleotide triplet-repeat disorders cannot be detected nor quantified. Noncoding regulatory sequence mutations in the promoter, intronic sequences, and deep intronic splice site mutations will also not be detected. Signs of uniparental disomy or epigenetic changes are also not detected.

7. WHOLE-EXOME SEQUENCING AND WHOLE-GENOME SEQUENCING

WES has the advantages of speed and efficiency relative to Sanger sequencing of multiple genes, delivering results in weeks to months. Candidate variants can be significantly reduced in number (from an initial 20,000 to 30,000 per individual exome) using clinically validated bioinformatic pipelines. Targeted Sanger sequencing is often used to confirm the presence or absence of the potential variants in the individual being diagnosed, and in affected and unaffected family members. Outside of the fairly recognizable conditions and clearly pathogenic mutations, there remains a need to standardize bioinformatic pipelines as well as expand the lists of clinically relevant genes with associated phenotypes. Despite this, there are a growing number of centers that are jumping directly to WES when the diagnosis is unclear, but a genetic etiology is clearly suspected (Pengelly et al., 2014).

An average human exome contains between 20,000 and 25,000 coding variants, of which ~10,000 variants are nonsynonymous, and can potentially consist of the genetic factors that cause a Mendelian disease (Gonzaga-Jauregui, Lupski, & Gibbs, 2012). An individual with phenotypic manifestations would likely carry 40 to 100 deleterious variants simultaneously.

Despite the promise of ending the diagnostic odyssey, several limitations of NGS impede the complete explanation of an underlying genetic diagnosis. First, although WES has made great progress in the diagnosis of monogenetic diseases, the human genome is highly variable, and rare variants

outside coding exons can also confer disease. Approximately 85% of such diseases are caused by the coding exons and therefore by default >15% lie outside the exons, including single nucleotide variations (SNVs) and copy number variants (CNVs), in the noncoding, conserved, or regulatory regions. The latter data cannot be assayed with the use of WES but may be assessed by using whole-genome sequencing (WGS) (Gonzaga-Jauregui et al., 2012)

Even if the causative variant lies within the exome, WES relying on NGS technologies is not without challenges and limitations. In the human exome, about 1 in every 1,000 nucleotides will vary. Bioinformatic filtering can eliminate most variants that are presumed irrelevant to the condition of concern. The final steps of variant filtering involve manual research and review. This requires expertise, including knowledge of the patient's phenotype and family history, current knowledge, and exploration of published variant-disease associations, and judgment, all of which can contribute to variability in the final variant interpretation.

Reproducibility at the level of final manual variant interpretation has not been fully characterized; comparisons of exome capture platforms using detection of known medically relevant variants as the sequencing result for assessment indicate small differences in detection, gaps in capture platforms, and regions of poor coverage that are unlikely to benefit from improved capture or greater depth of sequencing. Tools to improve the computational and variant analysis pipelines are available and more are under development. Like Sanger sequencing, current standard review of NGS data cannot detect large deletions or duplications of DNA or nucleotide repeats that can cause disease, although this barrier may soon be surmounted. Error rates due to uneven sequencing coverage, gaps in exon capture prior to sequencing, difficulties with narrowing the large initial number of variants to manageable numbers without losing likely candidate mutations, poorly annotated variant databases, lack of standardized procedures, and identifying mutations in unrelated genes or unknown genes are all issues. Detailed guidance from regulatory or professional organizations is under development, and the variability contributed by the different platforms and procedures used by clinical laboratories offering exome sequencing as a clinical service is currently unknown.

There are also unresolved ethical questions that arise as a result of genetic testing. Some of these questions concern the reporting of incidental findings, such as identifying medically relevant mutations in genes unrelated to the diagnostic question and sex chromosome abnormalities. Although the cost of data storage is decreasing, there remains a question about what to do with

remaining data and who has ownership of and rights to this information. There is also a large amount of ancillary data with thousands of variants of uncertain significance detected some of which may be reinterpreted at a future date as being pathogenic and disease-associated. Further social and ethical issues can arise when family studies are performed such as when there is a discovery of nonpaternity or of incidental off-target actionable variants in parents.

WGS enables the identification of the majority of variant types including single-nucleotide variants (SNVs), insertions and deletions both in coding regions and noncoding regions, and also has the advantage of detecting copy number variations (CNVs) and structural variations.

WES is gradually being optimized to identify mutations in increasing proportions of the protein-coding exome, but WGS is currently touted as a superior alternative, especially as cost decreases. The distribution of quality parameters for single-nucleotide variants (SNVs) and insertions/deletions has been shown to be more uniform for WGS than for WES. Therefore WGS may be slightly more efficient than WES for detecting mutations in the targeted exome (Belkadi et al., 2015).

8. FUTURE PERSPECTIVES

As the cost of WES and WGS drops, this type of targeted approach to genetically heterogeneous conditions will become more useful and accessible to clinical practice. Large datasets of both genotypic and phenotypic information will eventually be mined to discover and characterize the clinical significance of low penetrant variants. Future research will also explore how environmental factors interact with the genome, and contribute to abnormal craniofacial development and human health.

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