Decoding structural birth defects through genomic landscapes: Innovative frameworks for diagnosis (Review)

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Abstract. Structural birth defects (SBDs) represent a major subset of congenital malformations arising from abnormalities during organogenesis and subsequent tissue morphogenesis. The triad of congenital heart defects (CHDs), orofacial clefts (OFCs) and neural tube defects (NTDs) dominates the global epidemiology of SBDs, collectively contributing to considerable neonatal mortality while imposing profound clinical and socioeconomic burdens. Conventional genetic screening approaches, such as karyotype and non-invasive prenatal testing, remain limited in their capacity to decipher the complex genomic factors underlying these SBDs. The advent of advanced genomic technologies (including chromosomal microarray analysis and next-generation sequencing) and integrated genomic analysis methods [such as copy number variation analysis, single nucleotide variation/insertion and deletion analysis and genome-wide association studies (GWAS)] has enhanced the capacity to identify pathogenic genetic factors, thereby transforming the mode of prenatal diagnosis and genetic counseling. The application of these technologies, by virtue of more accurate diagnosis and finer disease classification, not only provides a more comprehensive basis for assessing disease severity and prognosis in clinical decision-making but also offers support for implementing targeted intervention and treatment. The present review systematically evaluates state-of-the-art genomic methodologies and computational approaches for detecting genomic aberrations in CHDs, OFCs and NTDs, and integrates insights from GWAS to elucidate the underlying genetic architecture, contributing to achieving precise predictive modeling and targeted therapeutic innovation for SBDs.

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1. Introduction

Structural birth defects (SBDs) are defined as congenital abnormalities in physical structure that arise during fetal development. Globally, these defects affect 3-6% of live-born infants (1). The most prevalent forms include congenital heart defects (CHDs) (0.33%), orofacial clefts (OFCs) (0.14%) and neural tube defects (NTDs) (0.13%) (2-4). These conditions are associated with substantial perinatal mortality and long-term morbidity, imposing notable burdens on affected families and society (5).

Current clinical screening for SBDs primarily relies on prenatal ultrasound, maternal peripheral blood testing and amniocentesis. While prenatal ultrasound can detect >80% of fetal structural abnormalities (6), its diagnostic accuracy is limited by operator expertise and equipment resolution, frequently failing to identify subtle or complex malformations (7). Peripheral blood biochemical analysis provides risk assessment for high-risk congenital anomalies; however, current methodologies exhibit relatively high rates of both false-positive and false-negative results (8-10). By contrast, genomics-based approaches, including amniotic fluid karyotyping and conventional non-invasive prenatal testing (NIPT) of peripheral blood, directly identify chromosomal aneuploidies and microdeletion/microduplication syndromes and address the diagnostic limitations of ultrasound and enhance structural anomaly detection rates (11,12). Nevertheless, these methods currently elucidate the genetic factors in merely 19.1% of SBDs cases, leaving 79.8% undiagnosed (13). Consequently, incorporating advanced,

comprehensive genomic technologies into both research and clinical practice is essential for deciphering the complex genetic architecture underlying SBDs.

Genomics, an interdisciplinary field investigating genomic structure, function, evolution and interactions within biological systems, seeks to elucidate fundamental biological principles through comprehensive analysis of genetic information (14,15). Recent advances in genomic technologies have notably enhanced both research and clinical applications for SBDs. These innovations facilitate not only more precise identification of pathogenic variants and the discovery of novel disease-associated genes, but also the refinement of prenatal diagnostic approaches, thereby strengthening the scientific basis for clinical decision-making and genetic counseling.

The present review discusses the genomic technologies currently prevalent in genetic research, as well as their application in prenatal diagnosis and candidate gene screening for SBDs (Fig. 1), while assessing the advantages and limitations of each method. Furthermore, the present review explores potential future directions within the context of reproductive health applications. The aim of the present review was to establish a comprehensive framework to equip clinicians with actionable technical and analytical guidelines for evaluating the clinical translation of these technologies as precision medicine modalities, with the ultimate goal of improving diagnostic accuracy and therapeutic strategies for SBDs.

2. Advanced genomic technologies in SBDs

In SBDs, genomic variations primarily present as copy number variations (CNVs) and single nucleotide variations (SNVs)/insertions-deletions (indels) (16,17). The precise detection of these variants within the vast human genome constitutes the foundation for elucidating the underlying genetic factors of SBDs. Advanced genomic screening approaches primarily rely on high-throughput technologies, including chromosomal microarray analysis (CMA) and next-generation sequencing (NGS). The NGS platform encompasses various methodologies such as CNV-sequencing (CNV-seq), whole-exome sequencing (WES), whole-genome sequencing (WGS) and targeted sequencing. These technologies have markedly improved the detection rate of pathogenic variations in SBDs, providing important evidence support for clinical diagnosis (Table I).

CMA. CMA is a high-throughput microarray-based technology. Through specifically designed probes that hybridize with sample DNA, CMA detects various genomic variations including CNVs, single nucleotide polymorphisms (SNPs), loss of heterozygosity and uniparental disomy through fluorescence signal intensity analysis (18). At present, CMA is recommended as the effective genomic test for fetuses with SBDs in clinical practice (19,20).

In fetuses with structurally abnormal findings detected by ultrasound, CMA can effectively enable high-resolution genomic screening to identify chromosomal abnormalities undetectable by conventional karyotyping, including clinically relevant microdeletions and microduplications (21-23). Besides, for fetuses with ultrasound-detected multi-system structural abnormalities, CMA demonstrates a notably higher detection rate of 41.2% for chromosomal abnormalities, markedly surpassing the rates observed in isolated system anomalies (such as 25.4% for cardiovascular system abnormalities and 18.6% for central nervous system abnormalities) (24). Furthermore, CMA offers particular diagnostic value by identifying ~1.2% of clinically relevant abnormalities in low-risk pregnancies with normal NIPT results (25). Furthermore, CMA technology is relatively mature and easy to operate, making it conducive to promoting prenatal genetic testing in regions with limited medical resources (26).

However, CMA has inherent technical limitations in clinical practice. The methodology carries detection risks for variants <50 kb and cannot identify balanced translocations or low-level mosaicism (26). Furthermore, CMA lacks diagnostic capability for balanced chromosomal rearrangements such as inversions and translocations (27).

CNV-seq. CNV-seq is an NGS technique that utilizes low-coverage WGS (0.1-1x depth) to analyze fetal DNA for chromosomal abnormalities and CNVs (28). With the development of NGS technology, CNV-seq is emerging as a viable alternative to CMA for the prenatal diagnosis (29).

CNV-seq demonstrates superior detection efficiency for both CNVs and chromosomal abnormalities compared with NIPT in SBDs (30,31). For fetuses with ultrasound-detected abnormalities, CNV-seq exhibits a 2-4% higher sensitivity compared with karyotyping in identifying chromosomal aberrations (32,33). Moreover, CNV-seq provides superior resolution compared with CMA, allowing for precise detection of microdeletions, microduplications and low-level mosaicism (<20%) (34,35). Notably, CNV-seq not only detects all chromosomal aneuploidies and large-scale genomic rearrangements identifiable by CMA but also uncovers an additional 34.88% of clinically relevant variants (34). In clinical practice, due to its faster detection speed and smaller sample requirement, CNV-seq can serve as a preliminary rapid test for early clinical intervention. Meanwhile, its high compatibility with other NGS platforms greatly simplifies testing workflows and reduces costs, making the combination of CNV-seq with other NGS technologies a preferred strategy in genetic testing for SBDs (36).

However, in clinical testing, CNV-seq cannot detect balanced chromosomal translocations or inversions and has restricted diagnostic utility in identifying polyploidy and uniparental disomy associated with syndromic SBDs (37). Furthermore, CNV-seq exhibits reduced sensitivity and undetermined specificity in CNV detection when benchmarked against CMA (30,38).

Targeted sequencing. Targeted sequencing represents a precision detection methodology that employs NGS technology. This hypothesis-driven approach utilizes customized probe or primer panels to selectively capture and enrich predefined genomic regions for high-throughput analysis, focusing primarily on functionally characterized regions with established or putative disease associations (39). Through target enrichment, targeted sequencing achieves notably enhance sequencing depth, enabling sensitive detection of rare pathogenic SNVs/indels and CNVs in known disease-related

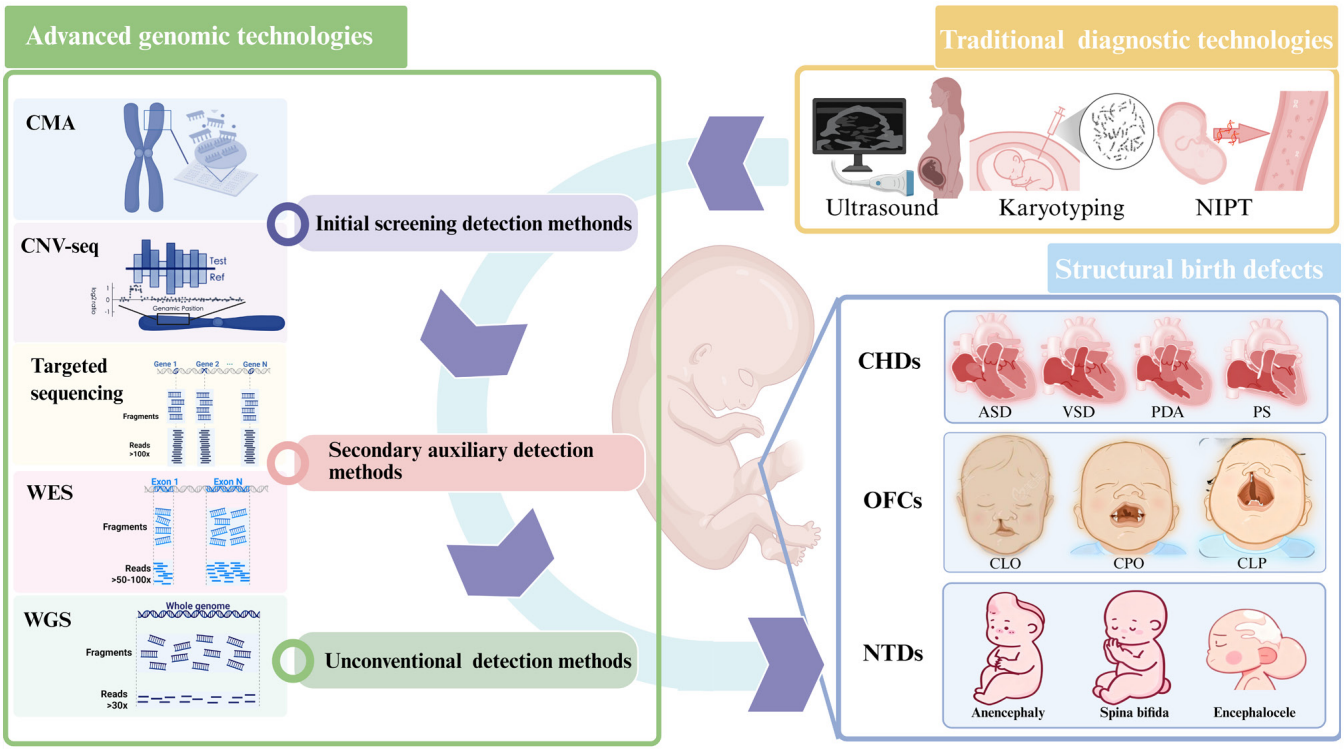

Figure 1. Advanced genomic technologies propel the diagnosis of SBDs. Research and diagnosis of SBDs have gradually shifted from traditional diagnostic technologies (such as ultrasound, karyotyping and NIPT) to advanced genomic technologies (such as CMA, CNV-seq, targeted-sequencing, WES and WGS). These advanced genomic technologies, with their distinct application scopes and clear clinical recommendations in clinical practice, not only deeply unravel the genomic landscape of structural birth defects but also provide critical support for genetic counseling and targeted therapy. SBDs, structural birth defects; NIPT, non-invasive prenatal testing; CMA, chromosomal microarray analysis; CNV-seq, copy number variation sequencing; WES, whole exome sequencing; WGS, whole genome sequencing; CHDs, congenital heart defects; ASD, atrial septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus; PS, pulmonary stenosis; OFCs, orofacial clefts; CLO, cleft lip only; CPO, cleft palate only; CLP, cleft lip and palate; NTDs, neural tube defects.

genes among fetuses with SBDs (40,41). Therefore, in clinical practice, specific genes or gene combinations can be selected based on fetal phenotypes to perform targeted deep sequencing analysis and diagnosis, while avoiding the blind use of WES or WGS (42).

In prenatal diagnosis, targeted sequencing serves as a supplementary diagnostic tool for CMA-negative cases and is particularly advantageous for fetuses with specific phenotypes, detecting ~13.6% of pathogenic variants in those with SBDs (42,43). Besides, the technology demonstrates particular utility in diagnosing genetically heterogeneous SBDs, allowing simultaneous analysis of multiple candidate genes (44). Targeted sequencing offers a cost-effective diagnostic solution for SBDs, with core advantages of lower cost and short turnaround time. When prenatal examinations suggest a fetus may have specific types of structural abnormalities, this method can quickly confirm relevant genetic subtypes and provide key evidence for genetic counseling and clinical decision-making (41,45,46).

However, targeted sequencing has notable limitations inherent to its design, which limits its application in certain clinical scenarios; its diagnostic scope remains constrained by current genomic knowledge, potentially missing novel pathogenic genes or functionally relevant non-coding variants beyond established disease associations (47). Furthermore, its capacity for systematic investigation of complex structural variations is intrinsically limited by restricted genomic coverage, which impedes comprehensive genome-wide analyses (48).

WES. WES employs an NGS technology coupled with probe-based hybridization capture systems to specifically target and enrich exonic regions and adjacent splice sites (±20 bp). Although these regions constitute merely 1-2% of the human genome, they harbor ~85% of known pathogenic variants. WES of these regions enables the identification of clinical relevance for the majority of SNVs/indels and CNVs in the human genome (49). Clinically, trio-WES is the most effective exome testing method, as it enhances diagnostic rates, elucidates variant origins and permits accurate recurrence risk assessment and early prenatal diagnosis (50).

In SBDs, WES serves as a supplementary diagnostic tool following inconclusive initial tests (such as CMA/CNV-seq) or in special cases such as recurrent or lethal fetal abnormalities, offering comprehensive exonic coverage that enhances the detection of pathogenic variants compared with these methods (50,51). For fetuses with isolated SBDs (such as CHDs or NTDs), WES offers an incremental diagnostic yield of 10-50%, increasing to ~33% in cases with multi-system SBDs (52-55). This approach is particularly advantageous for disorders exhibiting high genetic and phenotypic heterogeneity. Moreover, in fetuses with ultrasound-detected cardiac or central nervous system abnormalities but negative CMA and karyotyping results, WES effectively identifies

Table I. Genomic technologies in examining structural birth defects.

Detection technologies	Clinical application	Advantages	Limitations
CMA	Initial detection methods for fetuses with SBDs	CMA can detect genome-wide CNVs >50-100 kb, SNPs, uniparental disomy and triploidy; relatively mature and widespread use.	Inability to detect variants <50 kb; insensitive to balanced chromosomal rearrangements; limited capability in identifying low-level mosaicism.
CNV-seq	Initial detection methods for fetuses with SBDs; an alternative or supplementary approach to CMA.	CNV-seq can detect genome-wide CNVs >50-100 kb; faster detection speed; low sample requirement.	Ineffective for detecting balanced translocations and inversions; of limited value in diagnosing polyploidy and uniparental disomy; lower sensitivity in CNV detection and undefined specificity compared with CMA in clinical trial (30,38).
Targeted sequencing	Secondary auxiliary diagnostic tool; primarily conducts targeted testing based on fetal disease phenotypes.	Targeted-sequencing is cost-effective for focused panels with high clinical utility; achieves high sequencing depth for sensitive detection of targeted pathogenic genes.	Restricted to the investigation of known disease-associated genes; unable to systematically detect complex structural variations due to limited genomic coverage.
WES	Secondary auxiliary diagnostic tool; recommended under geneticist guidance when initial detection methods are negative, particularly in cases of complex malformations or a history of adverse pregnancies.	WES provides comprehensive coverage of exons and flanking intronic sequences; more cost-effective than WGS.	Lack of detection capability for non-coding region variants; susceptible to false-positive calls in regions of high sequence homology.
WGS	Non-standard use; reserved as an auxiliary tool following negative WES results, specifically in cases with complex malformations or a recurrent adverse pregnancy history.	WGS provides complete genome coverage, allowing for the identification of non-coding variants.	Reduced specificity (risk of false positives) in low-coverage regions; high overall sequencing and analytical costs.

CMA, chromosomal microarray analysis ; WES, whole-exome sequencing; WGS, whole-genome sequencing; CNV, copy number variation; SNP, single nucleotide polymorphism; SBDs, structural birth defects.

recessive or *de novo* dominant pathogenic mutations undetectable by conventional methods, demonstrating superior diagnostic sensitivity (56,57).

However, as WES relies on short-read sequencing platforms, it may lead to inaccurate alignment in genomic regions with high sequence homology, such as repetitive elements or pseudogenes, potentially generating false-positive variant calls (58,59). Additionally, emerging evidence implicates that non-coding variations (including deep intronic variants, long non-coding RNA regulatory elements and mitochondrial DNA structural alterations) are also involved in the occurrence and development of SBDs, while WES is mainly restricted to exonic regions and adjacent splice sites, exhibiting markedly reduced sensitivity for detecting these non-coding variants (60).

WGS. WGS shares technical principles with WES but extends beyond WES's limitation to exonic regions. By enabling systematic identification of SNVs/indels and CNVs across the entire genome, WGS provides comprehensive genome-wide detection (49).

In fetuses with SBDs, WGS enables detection of subtle structural variations (such as <10 kb) and yields more precise genetic characterization (61,62). Beyond structural variants, WGS identifies regulatory mechanisms underlying gene expression dysregulation, including promoter/enhancer

variants and deep intronic splice-altering mutations. Notably, WGS precisely maps complex genomic rearrangements such as intronic balanced translocation breakpoints that disrupt normal mRNA splicing (63,64). For fetuses exhibiting SBDs, WGS demonstrates superior diagnostic capability by identifying both variants detectable through CMA and WES, along with additional pathogenic variants (65). This comprehensive approach enhances diagnostic yield by 10-20% compared with conventional methods. Currently, WGS offers the most comprehensive diagnostic performance for SBDs, making it a potential 'one-stop' test that reduces the need for repeated testing (66).

Despite its superior sensitivity for structural variation detection, WGS presents technical challenges. First, genome-wide coverage generates extensive datasets with typically low per-base sequencing depth, increasing the risk of false-positive variant interpretation due to reduced confidence in mutation calling (67,68). Second, the substantial costs associated with per-sample sequencing and bioinformatic analysis create economic barriers to implementation WGS in routine prenatal screening programs (69). Thus, the application of WGS in prenatal diagnosis is restricted to supplementary roles in select cases such as fetuses with organ system abnormalities, a history of recurrent adverse pregnancies and negative CMA and WES results. However, with advancing sequencing technology and falling costs, WGS is expected to become a key tool in prenatal genetic diagnosis (70).

3. Genomic identification of candidate genes associated with SBDs

In genomic research, candidate genes are key genetic elements identified through integrated bioinformatic analyses of their potential associations with specific genetic disorders or phenotypes. The CNVs and SNVs/indels identified through advanced genomic technologies have precisely narrowed the search scope for pathogenic variants via CNV analysis and SNVs/indels analysis, while also advancing the development of genetic diagnostic methods. Furthermore, genome-wide association studies (GWAS) have expanded the candidate gene spectrum by further analyzing the polygenic inheritance effects of SBDs. These findings provide critical insights into deciphering the genetic basis of SBDs (Fig. 2).

Candidate genes related to SBDs identified through CNV analysis. CNVs represent a class of genomic structural variations involving DNA segment duplications or deletions typically >50 kb. These variations can influence gene expression through dosage effects, gene structure disruption or regulatory element interference (71,72). To uncover such candidate genes related to SBDs, comprehensive bioinformatic analysis of CNV regions and their functionally associated gene networks provides a crucial approach (73,74) (Table II).

CNV analysis of CHDs. CNVs contribute to 10-15% of CHD cases. Recurrent CNVs in specific genomic regions, such as 1q21.1, 22q11.2 and 16p11.2, are frequently observed in patients with a CHD (75). These regions harbor dosage-sensitive genes critical for cardiac development, underscoring their role as a major genetic etiology of CHDs. *TBX*1 haploinsufficiency caused by 22q11.2 microdeletions represents one of the well-characterized core genetic mechanisms (76). A WES study by Zhao *et al* (77) further revealed that chromatin regulatory genes within the 22q11.2 region, including *EP400*, *KAT6A, KMT2C, KMT2D*, *NST1*, *CHD7* and *PHF21A*, exhibit markedly higher mutation frequencies in patients with a CHD compared with controls, which may contribute to abnormal cardiac development through epigenetic regulatory mechanism. Additionally, a case-control study using CMA identified *SLC2A3* duplications in 5.0% (18/346) of 22q11.2 deletion syndrome patients with CHDs and aortic arch abnormalities, a frequency markedly higher than in deletion-positive individuals with normal cardiac anatomy. This finding implicates *SLC2A3* duplication as a potential genetic modifier of cardiac phenotypes in 22q11.2 deletion syndrome (78). CNV studies of other pathogenic regions have revealed additional candidate genes. For instance, Lin *et al* (79) performed CMA on a cohort of 1,118 Chinese fetuses with CHDs, identifying a notable association between proximal 16p11.2 deletions (BP4-BP5 region) and CHDs; moreover, this association may be mediated through *TBX6* gene dysfunction. Another investigation of 78 non-22q11.2 deletion patients identified 15q21.1 and 2p22.3 duplications as novel pathogenic CNVs, with multiple implicated genes (*IRX4, BMPR1A, SORBS2, ID2, ROCK2, E2F6, GATA4, SOX7, SEMAD6D, FBN1* and *LTBP1*) known to participate in cardiac development (80). Mak *et al* (81) performed a CMA analysis using a large control cohort comprising 3,987 Caucasian and 1,945 Singaporean Chinese subjects. The study identified 10 large rare CNVs, with further analysis revealing that nucleoredoxin in the 17p13.3 region, *COL4A1* and *COL4A2* in 13q33.3, and *ZEB2* in the 2q22.3 region were strongly associated with CHDs. Dasouki *et al* (82) conducted CNV-seq analysis in 134 Saudi Arabian patients with CHDs, detecting 21 copy number gains and 11 losses. The genomic variants were primarily clustered in chromosomal regions 17q21.31, 8p11.21, 22q11.23 and 16p11.2. Functional and network analyses identified *NPHP1, PLCB1, KANSL1* and *NR3C1* as potential candidate genes associated with CHD pathogenesis.

CNV analysis of OFCs. Genetic investigations of OFCs have demonstrated the crucial role of CNVs and their associated genes in disease pathogenesis. Lansdon *et al* (83) performed CMA on cohorts from the Philippines (n=869) and Europe (n=233) with non-syndromic cleft lip/palate (nsCL/P), identifying recurrent 2q31.1 duplications (11 cases), 22q11.2 CNVs (4 cases) and 3q29 deletions (2 cases). Further analyses implicated *COBLL1*, *RIC1* and *ARHGEF38* as candidate genes, all involved in Rho/Rab GTPase signaling pathways associated with OFCs and craniofacial anomalies. Additionally, a separate multi-center study involving 270 orofacial clefts cases identified a rare duplication variant at 19p13.12, suggesting its potential as a pathogenic locus. The variant affects *SYDE1*, *BRD4* and *AKAP8*, which exhibit spatiotemporally specific expression patterns in the branchial arches and frontonasal processes of E10.5 mouse embryos. These findings indicate that this gene cluster may represent a novel susceptibility factor for nsCL/P (84). Furthermore, in a CMA analysis of 467 OFC trios (comprising 1,375 subjects) and 391 control trios (902 subjects), Younkin *et al* (85) identified a genome-wide significant 62 kb non-coding region at

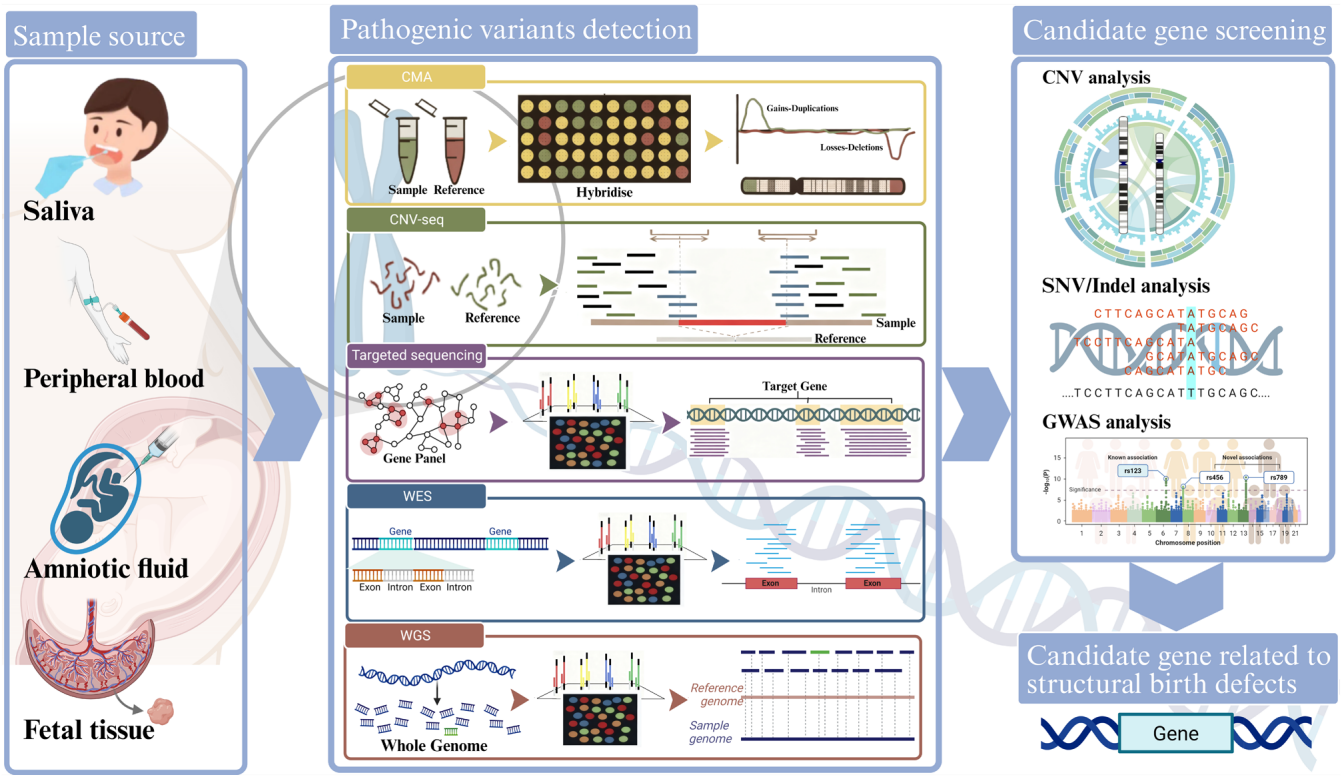

Figure 2. Overview of using genomics to screen the candidate genes of SBDs. The analysis of differential variation of various candidate genes in pregnant women, fetuses or patients with SBDs based on genomics of saliva, peripheral blood, amniotic fluid and fetal tissue samples provides crucial information for comprehending the genetics factors underlying SBDs. SBDs, structural birth defects; CMA, chromosomal microarray analysis; WES, whole exome sequencing; WGS, whole genome sequencing; CNV, copy number variation; SNV, single nucleotide variant; Indel, insertions-deletions; GWAS, genome-wide association study.

7p14.1 and proposed *TARP* as a candidate gene for the early prenatal diagnosis of OFCs, suggesting a potential role of T-cell receptors in OFC pathogenesis. A further study on this cohort identified two deletions: A 67 kb deletion in *MGAM* on chromosome 7q34 and a 206 kb deletion spanning *ADAM3A* and *ADAM5* in the 8p11 region. The frequency of these deletions was markedly higher in affected families compared with in unaffected controls, further supporting their potential role as candidate genes (86).

CNV analysis of NTDs. CNV analysis has been used to identify associations among common and rare genetic variants and NTD risks and has identified several promising candidate genes. A WES study analyzed patients with meningomyelocele and identified 6 cases harboring a 22q11.2 deletion, which included an LCR22C-D deletion within the low-copy repeat region. Furthermore, the study pinpointed *AIFM3*, *CRKL* and PI4KA as key candidate genes within this locus (87). Additionally, a WGS-based analysis involving cohorts from the United States and Qatar (comprising 140 patients with spina bifida and 183 controls) expanded the gene network associated with NTD-related CNVs. Rare coding CNVs were identified in pathways critical to NTD pathogenesis, including cell cycle regulation (*SH3GL3* and *PARD3*), mitochondrial metabolism (*DMGDH* and *FOXRED1*), transmembrane transport (*SLC44A2* and *SLC44A3*) and signal transduction (*VAV2* and *DOCK10*) (88). Tian *et al* (89) performed CNV analysis of all exonic regions in planar cell polarity (PCP) pathway-related genes (*VANGL1, VANGL2, CELSR1, SCRIB, DVL2, DVL3* and *PTK7*) in 11 NTD probands, identifying 16 CNVs. The CNVs were predominantly located in *DVL2*, *VANGL1* and *VANGL2*, suggesting these genes as strong candidates for NTD pathogenesis. These findings not only delineate the multi-pathway pathogenic framework of NTDs but also provide novel insights into the genetic association between NTDs and CNVs.

In summary, CNVs are notably associated with the incidence of SBDs. CNV analysis can elucidate the genetic effects of large-fragment deletion mutations, holding substantial relevance for identifying key pathogenic genes and exploring potential gene dosage-phenotype associations.

Candidate genes related to SBDs identified through SNV/indel analysis. Small variants typically encompass SNVs, short insertions and short deletions (<50 bp) (16). These variants can impact the function of critical genes, thereby contributing to the development of SBDs (90,91). The detection and analysis of SNVs/indels have notably enhanced the understanding of these subtle genetic alterations in SBDs and have effectively expanded the candidate gene spectrum for these conditions (Table III).

SNV/indel analysis of CHDs. Through SNV/indel analyses have served a key role in unraveling the complex genetic heterogeneity characteristic of CHDs. In a landmark study, Sevim *et al* (92) performed a systematic WES analysis on 559 families with CHDs, identifying 23 novel candidate pathogenic genes. Notably, *HSP90AA1*, *IQGAP1* and *TJP2* showed

Table II. Research on candidate gene screening in structural birth defects via CNV analysis.

Authors, year	Candidate genes	Disease phenotype	Number of cases	Genomic methods	Specimen type	(Refs.)
Zhao *et al*, 2023	*EP400, KAT6A, KMT2C, KMT2D, NSD1, CHD7* and *PHF21A*.	CHDs	645 cases and 537 controls	WGS	-	(77)
Mlynarski *et al*, 2015	*SLC2A3*	ICD; AAD	949 cases	CMA	Peripheral blood and saliva	(78)
Lin *et al*, 2024	*TBX1, TBX6, LZTR1, KMT2D, WAC, CHD7, RAF1, EP300, GDF1* and *PQBP1*.	Non-isolated CHDs	1,118 cases	CMA and WES	Amniotic fluid and umbilical cord blood	(79)
Molck *et al*, 2017	*IRX4, BMPR1A, SORBS2, ID2, ROCK2, E2F6, GATA4* and *SOX7*.	CHDs	78 cases	CMA	Peripheral blood	(80)
Mak *et al*, 2016	*ZEB2, COL4A1, COL4A2* and *NXN*.	CTD	116 cases	CMA	Peripheral blood	(81)
Dasouki *et al*, 2020	*NPHP1, PLCB1, KANSL1* and *NR3C1*.	CHDs	134 cases	CNV-seq	-	(82)
Lansdon *et al*, 2023	*COBLL1, RIC1* and *ARHGEF38*.	CL/P	1,102 cases	CMA	Peripheral blood	(83)
Cao *et al*, 2016	*CRKL, AKAP8, SYDE1* and *BRD4*.	OC	270 cases	CMA	Amniotic fluid	(84)
Younkin *et al*, 2014	*TARP*	OC	467 case families and 391 control families	CMA	Saliva and peripheral blood	(85)
Younkin *et al*, 2015	*MGAM, ADAM3A* and *ADAM5*.	OC	467 case families and 391 control families	CMA	Saliva and peripheral blood	(86)
Vong *et al*, 2024	*AIFM3, CRKL* and *PI4KA*	NTDs	715 case families	WES	-	(87)
Wolujewicz *et al*, 2021	*DMGDH, SLC44A2, SH3GL3, PARD3, FOXRED1, SLC44A3, VAV2, DOCK10* and *BHMT2*	SB	140 cases	WGS	Peripheral blood	(88)
Tian *et al*, 2020	*DVL2, VANGL1* and *VANGL2*	AN, SB and EC	175 cases and 101 controls	CMA	Umbilical cord tissue	(89)

CHDs, congenital heart diseases; CTD, conotruncal heart defect; ICD, intracardiac defects; AAD, aortic arch defects; CL/P, cleft lip with/without cleft palate; OC, orofacial clefts; NTDs, neural tube defects; SB, spina bifida; AN, anencephaly; EC, encephalocele.

strong associations with isolated CHDs, whereas *ROCK2*, *APBB1*, *KDM5A* and *CHD4* were primarily associated with non-isolated CHDs. Among these, *HSP90AA1*, *ROCK2*, *IQGAP1* and *CHD4* exhibited the highest relevance in pathogenicity prediction models, underscoring their central roles in disease pathogenesis. An additional WES study involving 52 Qatari families (178 individuals) identified four pathogenic or likely pathogenic variants: *ROBO1, SLC2A10, SMAD6* and *CHD7*. Notably, *ROBO1* was found to functionally interact with classical CHD-related genes such as *TBX1, TBX5, NOTCH1* and *NKX2-5* in interaction networks. Mechanistic studies using a chick heart development model demonstrated that *SMAD6* contributes to CHD pathogenesis through synergistic regulation with *NKX2-5* (93). Substantial progress has also been made in subtype-specific CHD research. Shi *et al* (94) analyzed WES data from 100 patients with pulmonary artery atresia

Table III. Research on candidate gene screening in structural birth defects via SNV/indel analysis.

Authors, year	Candidate genes	Disease phenotype	Number of cases	Genomic methods	Specimen type	(Refs.)
Mangold *et al*, 2016	*GRHL3*	nsCL/P and nsCPO	672 cases	Targeted sequencing	-	(24)
Sevim *et al*, 2020	*HSP90AA1, IQGAP1, TJP2, ROCK2, APBB1, KDM5A, CHD4, KDM5A* and *PHIP*.	Isolated/non-isolated CHDs	559 case families	WES	Peripheral blood and saliva	(92)
Okashah *et al*, 2022	*ROBO1, SMAD6, SLC2A10* and *CHD7*.	CHDs	52 case families	WES	Peripheral blood	(93)
Shi *et al*, 2020	*DNAH10, DST, FAT1, HMCN1, HNRNPC, TEP1* and *TYK2*.	PA/VSD, PA/IVS and TOF/PA	100 cases and 100 controls	WES	Peripheral blood	(94)
Shi *et al*, 2018	*CLTCL1, CST3, GXYLT1, VAV2, HMGA2, SNAI1* and *ZDHHC8*.	TAPVC	78 cases and 100 controls	WES	Peripheral blood	(95)
Basha *et al*, 2018	*TP63, TBX1, LRP6* and *GRHL3*.	nsCL/P	84 cases	WES	Peripheral blood	(96)
Hoebel *et al*, 2017	*ACACB, PTPRS, MIB1, GRHL3* and *CREBBP*	nsCPO	132 cases and 623 controls	WES	Peripheral blood	(97)
Awotoye *et al*, 2024	*AFDN*	nsCL/P	130 case families	WES	Peripheral blood	(98)
Fu *et al*, 2023	*LAMA5*	nsCL/P	30 cases	WES	Peripheral blood	(99)
Kumari *et al*, 2019	*TGFβ3, MSX1* and *MMP3*.	nsCL/P	245 cases and 201 controls	WES	Peripheral blood	(100)
Renard *et al*, 2019	*LRP2, MMAA, TCN2, FPGS, BHMT* and *GLI3*	MMC	23 cases	WES	Umbilical cord blood	(101)
Han *et al*, 2022	*CIC*	Isolated SB	140 cases	WGS	Peripheral blood	(102)
Lemay *et al*, 2015	*SHROOM3, PAX3, GRHL3, PTPRS, WBSCR28, MFAP1* and *DDX3X*.	MMC and AN	43 cases	WES	Abortive tissue and peripheral blood	(103)
Chen *et al*, 2018	*SHROOM2*	NTDs	343 cases and 206 controls	Targeted sequencing	Peripheral blood	(104)

CHDs, congenital heart diseases; PA, pulmonary atresia; VSD, ventricular septal defect; IVS intact ventricular septum; TOF, tetralogy of Fallot; TAPVC, total anomalous pulmonary venous connection; nsCPO, non-syndromic cleft palate only; nsCL/P, non-syndromic cleft lip with/without cleft palate; MMC, myelomeningocele; AN, anencephaly; NTDs, neural tube defects; SB, spina bifida.

and 100 healthy controls, integrating network analysis and gene expression validation to establish associations between *DNAH10, DST, FAT1, HMCN1, HNRNPC, TEP1* and *TYK2* with the pathological mechanisms of pulmonary artery atresia for the first time. In the research of total anomalous pulmonary venous connection, WES analysis of 78 sporadic cases and 100 controls identified seven candidate genes: *CLTCL1, CST3, GXYLT1, HMGA2, SNAI1, VAV2* and *ZDHHC8*. Functional verification and zebrafish model interaction network analysis further validated *SNAI1, HMGA2* and *VAV2* as key candidate genes driving total anomalous pulmonary venous connection pathogenesis (95).

SNV/indel analysis in OFCs. By examining protein-altering variations such as missense and frameshift mutations, SNV/indel analysis has revealed how coding sequence modifications induce functional changes (loss/gain-of-function), thereby offering novel perspectives on OFC candidate genes. WES of 84 affected individuals from 46 families with nsCL/P identified rare deleterious variants in *TP63, TBX1, LRP6* and *GRHL3,* genes previously associated with syndromic

forms (96). Mangold *et al* (24) performed targeted sequencing in a cohort of 576 European patients with nsCL/P and 96 non-syndromic cleft palate only cases, identifying four novel truncating *GRHL3* mutations. Notably, all nine mutation carriers exclusively presented with non-syndromic cleft palate only, providing compelling evidence for the notable pathogenic contribution of *GRHL3* to non-syndromic cleft palate only. A subsequent WES study of 132 non-syndromic cleft palate only cases and 623 multiethnic controls identified three novel candidate genes (*ACACB, PTPRS* and *MIB1*), along with *de novo* variants in *GRHL3* and *CREBBP*, genes associated with Van der Woude syndrome and Rubinstein-Taybi syndrome, respectively (97). A trio-WES analysis of 130 African nsCL/P families identified pathogenic *AFDN* missense mutations (p.E485K and p.G1703R) that may disrupt connexin binding, potentially impairing cell polarity regulation and mesenchymal migration during facial morphogenesis (98). A WES study from Eastern Chinese cohorts demonstrated *LAMA5* mutations in nsCL/P cases, with further investigations revealing the crucial role of *LAMA5* in palatal development, establishing it as a strong candidate gene (99). Kumari *et al* (100) identified variants in *TGFβ3, MSX1* and *MMP3* through WES analysis of 245 Indian nsCL/P cases and validated these genes as strong candidates for OFCs.

SNV/indel analysis of NTDs. The application of SNV/indel analysis has facilitated the identification of candidate genes within the underlying pathways of NTDs. WES performed on 23 myelomeningocele cases in France revealed that *de novo* variants in crucial genes of the vitamin B12 metabolism pathway (*LRP2, MMAA* and *TCN2*), genes related to folate metabolism (*FPGS*), the core regulator of choline metabolism (*BHMT*) and *GLI3*, a key regulatory element of the Sonic Hedgehog signaling pathway, were notably linked to genetic susceptibility to NTDs (101). A WGS study involving 140 isolated spina bifida samples further identified eight rare missense variants in the *CIC* gene, which were validated through functional analysis. These *CIC* missense variants found in NTD cases notably suppressed folate receptor 1 protein expression levels and disrupted the transduction of the PCP signaling pathway (102). Additionally, analysis of trio-WES in 43 cases of sporadic myelomeningocele/anencephaly and their parents identified seven core candidate genes: *SHROOM3, PAX3, GRHL3, PTPRS, WBSCR28, MFAP1* and *DDX3X*. Among them, *SHROOM3* is noted for its high degree of evolutionary conservation, the knockout of its double isoform resulted in 100% incidence of exencephaly and a 23% penetrance rate of spina bifida phenotypes in mouse embryos. As a key regulator of neural crest differentiation, rare variants c.218C>A in *PAX3* can potentially lead to NTD phenotypes by disrupting neural crest cell migration during neural tube closure (103). Building on this understanding, Chen *et al* (104) employed targeted sequencing technology to perform a comprehensive analysis of the *SHROOM* gene family in a cohort of 343 individuals with NTDs and 206 control subjects in China. The findings revealed a markedly higher frequency of damaging missense variants in the *SHROOM2* gene among the case group compared with the control group. These mutations lead to the failure of neural tube closure by disrupting the interaction between SHROOM2 and ROCK1 proteins, thereby interfering with the cytoskeletal remodeling mediated by the planar cell polarity pathway.

SNV/indel analysis systematically investigates subtle genetic alterations overlooked in CNV analysis, precisely mapping associated loci of SBDs and revealing the impact of nucleotide-level modifications on gene function. This approach notably establishes a crucial molecular foundation for optimizing precision prevention strategies of SBDs and exploring molecular pathways for targeted therapies.

Candidate genes related to SBDs identified through GWAS analysis. The genetic basis of most SBDs stems from the cumulative effects of multiple minor-effect genes rather than single-gene mutations (105). Consequently, identifying candidate genes involved in polygenic synergistic pathogenesis is crucial for elucidating the genetic factors underlying these complex disorders. GWAS analysis represent a pivotal approach in contemporary genomics research, employing SNP arrays or NGS technologies to systematically examine genome-wide genetic variations. Through multivariate data analysis, GWAS analysis elucidates associations between these variations and phenotypic traits, thereby revealing the genetic architecture of complex traits (106,107). Advances in GWAS applications for SBDs have led to the discovery of numerous potential candidate genes, representing a notable breakthrough in understanding the molecular pathogenesis of these conditions (Table IV).

GWAS analysis of CHDs. GWAS analysis has provided crucial insights into the complex genetic architecture of CHDs. A large-scale GWAS involving 40,000 UK Biobank samples identified 130 loci notably associated with right heart phenotypes, a number of which are located near known regulatory genes such as *NKX2-4, TBX5/TBX3, WNT9B* and *GATA4*. The pivotal role of these genes in cardiac morphogenesis has been further substantiated (108). Additionally, a single-trait GWAS analysis of 29,506 individuals with right ventricular structural abnormalities revealed 12 candidate genes shared across different right ventricular phenotypes, including *TTN, ATXN2, PTPN11* and *ACTN4*. Notably, *FHOD3*, *MYH6*, *MYL4* and *TMEM43* exhibit functional overlap with Mendelian cardiomyopathy genes, with their encoded proteins primarily involved in myocardial contraction and cell adhesion processes (109). Lahm *et al* (110) identified *MACROD2, GOSR2, WNT3* and *MSX1* as critical genes in embryonic cardiac development, which were significantly associated with SNPs based on CMA and GWAS analyses of 4,034 Caucasian patients with CHDs and 8,486 healthy controls. Jin *et al* (111) expanded this research by performing WES combined with GWAS on 2,871 CHD probands. The findings demonstrated that *MYH6* mutations were notably associated with an elevated risk of ventricular dysfunction, whereas dominant mutations in *FLT4* were specifically linked to tetralogy of Fallot. Furthermore, rare inherited and *de novo* heterozygous variants were markedly enriched in genes such as *CHD7, KMT2D, PTPN11, RBFOX2, FLT4, SMAD6* and *NOTCH1* among patients with complex CHDs, highlighting novel candidate targets for further investigation.

GWAS analysis of OFCs. GWAS analysis has identified multiple candidate genes potentially involved in OFCs. A GWAS of a Maya population (149 patients with nsCL/P and 303 controls) revealed notable associations between nsCL/P and genetic variants in *IRF6*, as well as loci at 8q24, 10q25

Table IV. Research on candidate genes screening in structural birth defects via GWAS analysis.

Authors, year	Candidate genes	Disease phenotype	Number of cases	Genomic methods	Specimen type	(Refs.)
Pirruccello *et al*, 2022	*NKX2-5, TBX5, TBX3, WNT9B* and *GATA4*.	RHD	40,000 cases	CMA	-	(108)
Aung *et al*, 2022	*TTN, ATXN2, PTPN11, ACTN4, RBL2, LUC7L2, AK097794, BAG3, GOSR2, SLC6A6, OBSCN* and *FHOD3*.	RHD	29,506 cases	CMA	-	(109)
Lahm *et al*, 2021	*MSX1, WNT3, MACROD2* and *GOSR2*.	CHDs	4034 cases and 8486 controls	CMA	-	(110)
Jin *et al*, 2017	*CHD7, KMT2D, PTPN11, RBFOX2, FLT4, SMAD6* and *NOTCH1*	CHDs	2,871 cases	WES	Peripheral blood, saliva and cardiovascular tissue	(111)
Rojas-Martinez *et al*, 2010	*IRF6*	nsCL/P	149 cases and 303 controls	CMA	Peripheral blood	(112)
Alade *et al*, 2024	*MAP3k7, MDN1, CASP8AP2, BACH2, BCL9L, H2AFX, HINFP, KMT2A, RPS25, UPK2, VPS11, HYOU1, CCDC84, PHLDB1, VADC2, ARCN1, ATP5L, DDX6* and *MPZ12*.	nsCL/P and nsCPO	1,019 cases and 2,159 controls	CMA	Saliva	(113)
Gaczkowska *et al*, 2019	*PAX7*	nsCL/P	247 cases and 445 controls	CMA	-	(114)

CHDs, congenital heart diseases; RHD, right heart defect; nsCL/P, non-syndromic cleft lip with/without cleft palate; nsCPO, non-syndromic cleft palate only.

and 17q22. Single-marker analysis further confirmed these associations, particularly for *IRF6*, 8q24 and 10q25 (112). A pleiotropy-informed conditional false discovery rate analysis, incorporating 814 nsCL/P cases, 205 nsCPO cases and 2,159 controls from an African population GWAS, along with mouse facial development expression data, identified 19 potential candidate genes. Among these, *MDN1, MAP3K7, KMT2A* and *ARCN1* emerged as top candidates, supported by evidence from mouse models or individuals harboring pathogenic variants in these genes (113). In a separate GWAS of oral clefts in a Polish cohort, eight single-nucleotide polymorphisms in *PAX7* were strongly associated with nsCL/P, with the etiological role of the gene independently validated in a cohort of 247 patients and 445 controls (114). These findings not only expand the repertoire of candidate genes implicated in OFCs but also provide a molecular framework for understanding how genetic background influences craniofacial development across diverse populations. The application of GWAS has notably advanced the identification of candidate genes for SBDs, with large sample sizes enhancing result accuracy.

GWAS analysis in NTDs. In genetic investigations of NTDs, a GWAS performed by Tindula *et al* (115) involving 321 Bangladeshi NTD cases and 177 controls failed to identify genome-wide significant variants. This negative finding may reflect insufficient statistical power due to the limited sample size. Notably, such challenges are pervasive in SBD research: Constrained by clinical sample availability, certain malformation subtypes lack large-scale GWAS implementations, while existing studies often fail to detect biologically meaningful susceptibility genes due to inadequate cohort sizes.

GWAS analyses enhance the understanding of SBDs with polygenic inheritance patterns by analyzing genome-wide associations between genetic variants and these defects

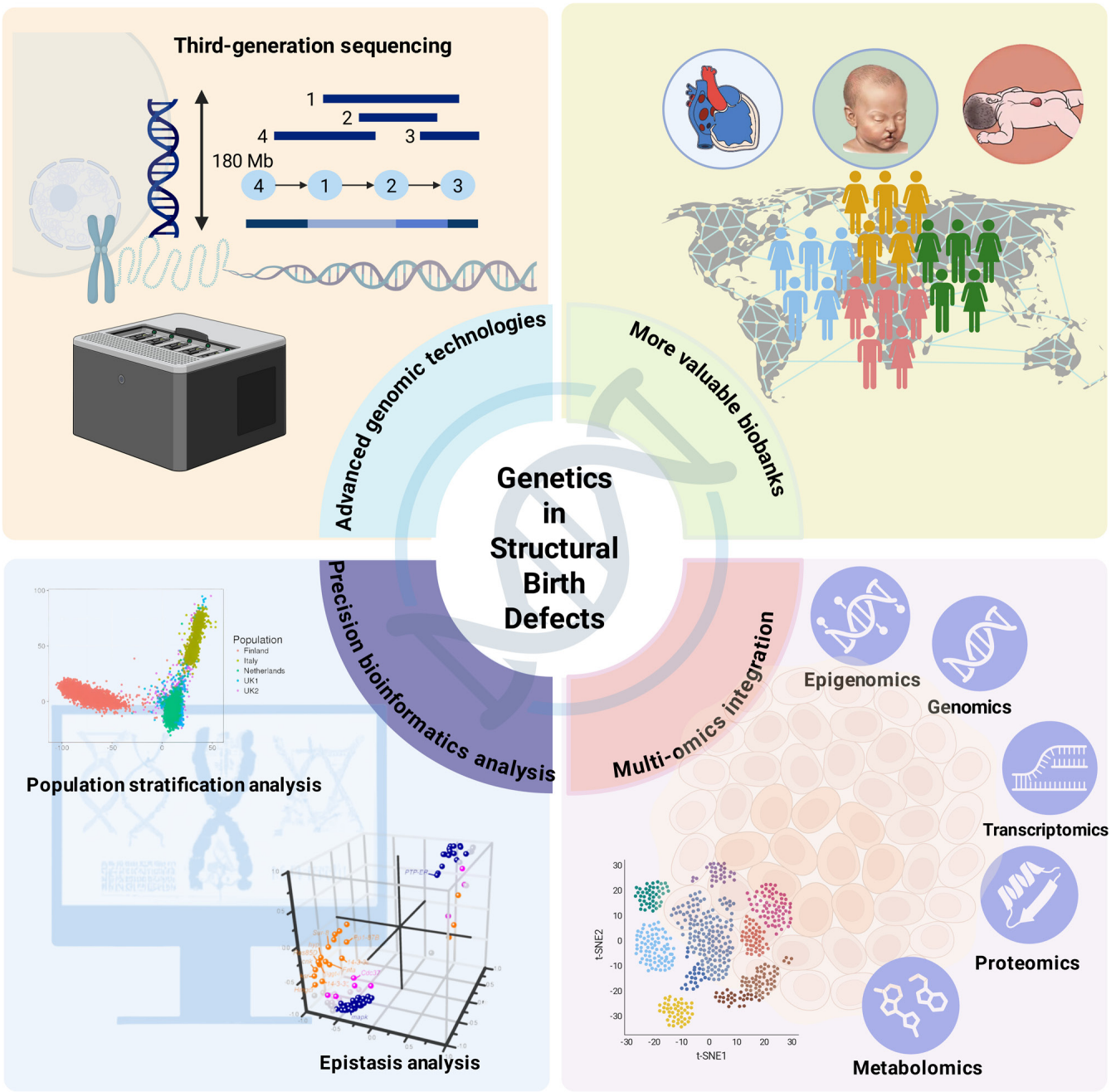

Figure 3. Future approaches for investigating the genetics factor in SBDs. The interdisciplinary effort of probing into the genetic factors of SBDs will require the combination of several disciplines, including advanced genomics technologies, valuable multi-center and large-population biobanks, precise bioinformatics analysis and the integration of multi-omics data. The fusion of these fields will contribute to improving the detection ability of pathogenic variations and optimizing the screening strategy of candidate genes in SBDs. SBDs, structural birth defects.

while quantifying the cumulative effects of multiple variants. Furthermore, GWAS analysis provides a comprehensive assessment of gene-gene interactions throughout the genome, filling the research gap in the study of polygenic effects in CNV analysis or SNV/indel analysis, and greatly expanding the potential candidate gene pool associated with SBDs.

4. Conclusions and perspectives

The clinical evaluation of SBDs has been revolutionized by continuous innovations in genomic technologies, establishing a tiered diagnostic pathway. For fetuses with ultrasound anomalies, CMA or CNV-seq serves as first-tier testing. In cases with negative results, particularly those with a complex phenotype or adverse pregnancy history, NGS methodologies such as WES or targeted sequencing provide a subsequent high-yield option. This evolving diagnostic paradigm enhances variant detection, facilitates precise genetic diagnosis and ultimately informs prenatal counseling, prognosis and recurrence risk assessment. WGS, through comprehensive detection of the entire genome, further improves the detection rate of pathogenic genes; although its clinical application is still limited due to technical constraints and cost factors, WGS has the potential to become the 'ultimate' prenatal diagnostic method.

Notwithstanding notable progress in genomics that has advanced research on SBDs, numerous challenges remain in pathogenic variant detection and candidate genes screening strategies. Current genomic approaches exhibit technical limitations in sensitivity and specificity when detecting specific genetic variants (61,116,117). CMA fails to identify certain complex structural variations, while NGS inherently misaligns sequences in GC-rich regions, resulting in undetected mutations within these GC-rich areas and consequent false-negative findings (118,119). Additionally, the short-read (<300 bp) nature of NGS presents inherent limitations, demonstrating particularly poor mapping quality for homologous region sequences, with frequent read misalignments from homologous segments leading to insufficient coverage. For repetitive genomic regions, reliable read mapping requires the presence of unique flanking sequences adjacent to the repetitive elements (120). Single-omics-based candidate gene screening strategies impose additional limitations on genetic dissection of SBDs. Genomics-dependent analyses cannot authentically validate the functional disruption and regulatory network imbalance caused by pathogenic variants. Moreover, numerous detected genetic variants remain classified as variants of uncertain relevance, substantially constraining variant interpretation and accurate classification (121,122). Furthermore, insufficient cohort sizes and a lack of ethnic diversity in current studies not only constrain the discovery of pathogenic variants and analysis of candidate genes, but also compromise the generalizability of diagnostic findings, particularly hindering the clinical translation of candidate gene research outcomes across diverse populations (105). Therefore, it is necessary to utilize large-scale, multi-ethnic biobanks established through international collaboration, which provide statistically robust cohorts for screening and validating candidate genes while reducing the impact of population representation bias.

Therefore, improving the identification of pathogenic variants and candidate gene screening strategies represents a critical challenge for advancing the understanding of the genetics of SBDs (Fig. 3). In recent years, high-precision genomic technologies, particularly third-generation sequencing (TGS) and optical genome mapping (OGM), have emerged as powerful tools in genetic disorder exploration (120). TGS platforms, with their long-read (>10 kb) sequencing capabilities, effectively address short-read sequencing limitations by enabling precise characterization of structurally complex genomic regions, including telomeres and centromeres (123-125). TGS has demonstrated promising application prospects in neonatal genetic disorders but has not yet been applied to research on SBDs (126). OGM demonstrates exceptional capabilities in detecting cryptic structural variations through fluorescent labeling of DNA fragments >150 kb (123,127). In the field of NTDs research, Sahajpal *et al* (128) pioneered the application of OGM technology to perform comprehensive analysis of genome-wide structural variations in 104 patient samples with NTD. The findings not only identified notable associations between NTDs and genes including *RMND5A, HNRNPC, FOXD4* and *RBBP4*, but also extended for the first time the phenotypic spectrum of *AMER1* and *TGIF1* genes to encompass NTDs. This groundbreaking study demonstrated the crucial role of genomic structural variations in the pathogenesis of such SBDs, providing essential molecular genetic evidence for subsequent research in this field. Furthermore, the rapid advancement of bioinformatics has notably optimized the genomic-based analysis pipeline for pathogenic variants. For instance, epistasis analysis enables the examination of joint genetic effects across multiple loci, helping to elucidate a portion of the 'missing heritability' that encompasses marginal genetic effects undetectable by conventional GWAS (129,130). Population stratification methods, such as principal component analysis or linear mixed models with random effects, can effectively mitigate confounding effects from population stratification and structure in GWAS (131,132). Multi-omics approaches enable mechanistic investigations into how candidate genes disrupt cellular pathways, proving particularly valuable for elucidating the functions of regulatory elements in non-coding regions that were previously overlooked in genomic studies (133). Therefore, it has further refined candidate gene screening strategies, providing novel perspectives for deciphering the genetic basis of SBDs (134).

In summary, the present review delineates current advanced genomic technologies applied in SBD research and diagnostics, while providing a comparative analysis of their respective advantages and limitations. The present review further consolidates candidate genes identified through these technological approaches, with these clinically-relevant candidates being derived from human clinical specimens that more faithfully recapitulate disease pathogenesis compared with animal or cellular models. The investigation of genetic contributors to SBDs has now entered a transformative era marked by multi-omics convergence and technological synergy. Through the implementation of higher-resolution platforms, expanded cohort sizes, refined bioinformatics analyses and sophisticated multi-omics integration, researchers are now empowered to more systematically and precisely identify pathogenic variants associated with SBDs, thereby uncovering clinically actionable candidate genes.

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Authors' contributions

RX and WH conceived the idea; RX contributed to writing the initial draft and preparing the figures; HR designed the tables; WH and HG contributed to the improvement of the initial draft

and revision; RX, ZY and HR helped proofread and improved the manuscript; RX, WH, ZY and HG critically reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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