Review

DOI: 10.5582/irdr.2023.01052

Diagnosis, treatment, and research status of rare diseases related to birth defects

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SUMMARY Rare diseases are diseases that occur at low prevalence, and most of them are chronic and serious diseases that are often life-threatening. Currently, there is no unified definition for rare diseases. The diagnosis, treatment, and research of rare diseases have become the focus of medicine and biopharmacology, as well as the breakthrough point of clinical and basic research. Birth defects are the hard-hit area of rare diseases and the frontiers of its research. Since most of these defects have a genetic basis, early screening and diagnosis have important scientific value and social significance for the prevention and control of such diseases. At present, there is no effective treatment for most rare diseases, but progress in prenatal diagnosis and screening can prevent the occurrence of diseases and help prevent and treat rare diseases. This article discusses the progress in genetic-related birth defects and rare diseases.

Keywords rare diseases, birth defects, diagnosis, treatment, prevention and control

1. Introduction

Rare disease is defined as a disease with a prevalence of less than 1/2,000 with a total number of patients less than 200,000 by The European Organization for Rare Diseases (EURORDIS) and the United States, respectively. The World Health Organization (WHO) defines a rare disease as one affecting between 0.065% and 0.1% of the population. It is estimated that there are 300 million rare disease patients worldwide, and more than 7,000 rare diseases account for 10% of all human diseases. Of these, 80% are inherited, and approximately 50% develop at birth or during childhood. Rare diseases often progress rapidly and have a high mortality rate; and less than 1% of rare diseases have specific drugs for treatment (*1*). An epidemiological survey in Ireland in 2020 showed that rare diseases were the main cause of death in children, accounting for 58.6% of the deaths in children aged 14 years and younger (*2*).

The medical and social needs of individuals with rare diseases are not met despite the large number of patients and families affected by them internationally. A general lack of public awareness and limitations in expertise have left rare disease patient populations overlooked and marginalized in healthcare systems and policies. EURORDIS, the International Society for Rare Diseases, and the NGO Committee for Rare Disease called for a UN resolution on Rare Diseases in 2019 and urged the 193 Member States of the UN General Assembly to adopt a resolution on December 16, 2021 (*3*). R&D in rare diseases is necessary to advance the UN commitment to achieve the 2030 Sustainable Development Goals of "leaving no one behind".

In recent years, many countries have carried out research on registering rare diseases as single or multiple diseases; 13,703 articles on this topic existed until April 2, 2023. The Orphan Drug Act of the United States in 1983 established the criteria for rare diseases, with other countries introducing similar criteria. On June 25, 2018, the First List of Rare Diseases was issued jointly by five institutions in China, including 121 common rare diseases that have become the standard for defining rare diseases in China (*4*).

Worldwide, serious birth defects occur in 3–6% of newborns, and on average, a child with a birth defect is born every 4.5 minutes. Due to the large population, rare diseases are not uncommon in China, and it is estimated that approximately 20 million people are affected. Genetic defects account for 71.9% of these rare diseases (*5*).According to WHO estimates, about a quarter of birth defects in the world are related to genetic factors. In the "First List of rare Diseases", a considerable number of metabolic and genetic diseases were included. The

research scope of rare diseases is also increasing, from neural tube malformation to congenital heart disease.

With the development of maternal-fetal medicine, fetal surgery, non-invasive prenatal testing, including cell-free fetal DNA, and next-generation sequencing technology (NGS), prenatal diagnosis and screening of these diseases are progressing, which provides help for the prevention and treatment of rare diseases.

2. Importance of rare diseases associated with birth defects

Birth defects and rare diseases, including structural abnormalities, functional abnormalities, and metabolic abnormalities, have become public health problems that affect life quality and community health. The related incidence are shown in Table 1.

2.1. Genetic factors associated with birth defects

According to the WHO, about a quarter of birth defects in the world are due to genetic factors. The "First List of Rare Diseases", published in 2018, includes a considerable number of metabolic and genetic diseases. Genetic etiology research is the basis for the prevention and treatment of birth defects. Genetic factors include mostly gene mutations and a small number of chromosomal abnormalities (*6*).

2.1.1. Monogenic genetic diseases and treatment strategies

Rare genetic diseases are usually monogenic (*7*), and include more than 6,000 types with complex clinical and genetic heterogeneity. Gene mutations include point mutation and dynamic mutation, as well as deletion or repetition of fragments and genes, which increase the difficulty in diagnosing such diseases (*8*). Currently, many monogenic diseases. Currently, many monogenic diseases such as cystic fibrosis, spinal muscular atrophy, thalassemia, Duchenne muscular dystrophy, hemophilia, osteogenesis imperfecta, and phenylketonuria are included on the list of rare diseases.

As far as diagnosis is concerned, monogenic genetic diseases involve many disciplines, and the clinical symptoms are complex. Because of the diversity of gene mutations, each mutation may lead to different clinical symptoms, and the clinical significance of mutations is quite complex, with high genetic heterogeneity and clinical heterogeneity, so clinical diagnosis is difficult. Nowadays, with the sharp drop in the cost of gene sequencing, the emergence of gene big data is followed. In this situation, it is an easy and effective way to establish an auxiliary diagnosis system and clinical knowledge base based on the relationship between genotype and phenotype.

Currently, there is a lack of effective treatments for most monogenic diseases. Mutated alleles revealed by sequencing with aneuploidy and linkage analyses (MARSALA) strategy for the combined diagnosis of monogenic and chromosomal diseases, along with preimplantation genetic testing (PGT) and NGS, play an important role in reducing the occurrence of birth defects and rare genetic diseases (*9*).

2.1.2. Polygenic genetic disease

Polygenic diseases have genetic heterogeneity but the same clinical phenotype, and there are several pathogenic genes. For example, deafness is associated

Table 1. Newborn incidence, incidence, and prevalence of rare diseases associated with birth defects

Diseases	No.	Disease	Newborn incidence / 100,000 persons	Incidence/ 100,000 persons	Prevalence/ 100,000 persons
Rare birth defects associated with structural abnormalities	1	Osteogenesis imperfecta (*4*)	7.14	/	0.089
	2	Crouzon syndrome (*28*)	16.5	/	/
	3	Saethre-Chotzen syndrome (*25*)	1	/	/
	4	Goldenhar syndrome (*20*)	/	3.79–17.86	/
	5	Beckwith-Wiedemann syndrome (*17*)	7.29–10	/	/
Rare birth defects associated with abnormal function	6	non-syndromic hearing loss (*30*)	186	/	/
	7	Retinoblastoma (*39*)	5–6.67	/	1-9
	8	Progressive muscular dystrophy (*4*)	16.67–27.78	25.30	/
	9	Leber hereditary optic neuropathy (*4*)	/	/	1.092
	10	Mitochondrial encephalomyopathy	/	/	/
	11	Parkinson's disease (*4*)	/	/	7.39
	12	Branchio-oto-renal syndrome (*35*)	2.5	/	/
Metabolic abnormalities associated with rare birth defects	13	Phenylketonuria (*4*)	/	8.48	1.17
Others	14	Ocular albinism (*4*)	/	5.56	/
	15	Epidermolysis bullosa (*54*)	/	/	0.30
	16	Hemophilia (*4*)	/	/	2.73
	17	Stickler syndrome (*68*)	/	11.11–13.33	2.7

with as many as 400 genetic syndromes, corresponding to at least 140 gene loci (*8*). Most birth defects are the result of multifactorial gene-environment interactions. Robert *et al.* discussed the various models, with their strengths and weaknesses, on the etiology of multifactorial birth defects and compared them with other diseases caused by gene-environment interactions, including primary immunodeficiency and cancer (*10*). They proposed that multi-factor gene-environment interactions will be an important research direction in the future.

The combination of fifth-generation mobile networks (5G) and blockchain technology has provided a new direction for PGT to accurately control rare genetic diseases. Hefeng Huang's team (*11*) revealed for the first time the precise mechanism of epigenetic methylation in intergenerational transmission of exogenous diabetes. by analyzing the lack of insulin secretion in the offspring of individuals with TET3 deficiency. This provides new scientific perspective for understanding and preventing chronic diseases such as diabetes from childhood. The world's first Preimplantation Genetic Testing for Polygenic Disease (PGT-P) optimized low-risk diabetic test-tube baby was born in Shanghai on August 2, 2022. The construction idea of building a genetic diagnosis cloud service platform based on 5G+ blockchain, a remote collaborative diagnosis platform, and a China population genome mutation database based on the current development status of PGT in China allowed prevention and control of rare genetic diseases in China at the international frontier level.

The Chinese human phenotype ontology (CHPO) database launched in 2016 aims to establish standard Chinese clinical phenotype terminology to better guide clinicians and research on genetic diseases. The fifth update was completed on May 8, 2023 (*https://www.chinahpo.net/*), corresponding to HPO version V2022-10-05, with 16,691 entries after this update.

2.2. Rare birth defects associated with structural abnormalities

Structural abnormalities are usually manifested as changes in physical structure, such as osteogenesis imperfecta (OI), congenital achondroplasia, polycystic kidney disease, and congenital heart disease.

2.2.1. Osteogenesis imperfecta

OI, also known as fragile bone disease, is a monogenic hereditary bone disease with a prevalence of 1/15,000 to 1/20,000 (*12*). Based on phenotypic classification, OI can be classified into 18 subtypes (*13*). Clinically, autosomal recessive inheritance is rare, whereas autosomal dominant inheritance is most common. OI type I, caused by mutations in the *COL1A1/2* gene encoding collagen type I, accounts for 85–90% of the cases.

Currently, there is no standardized treatment for OI. In clinical practice, bisphosphonates are used to treat OI. Teriparatide and transforming growth factor-β monoclonal antibodies are expected to increase bone density (*14*). To date, only two haploinsufficient (HI) mouse models of mild OI, Col1a1 +/Mov13 (Mov13) and Col1a1 +/-365, have been established. To facilitate OI research and the testing of new therapies, Claeys *et al.* found that the expression of collagen and bone metabolism markers was significantly increased in Mov13 mice compared with WT, although there was no significant change in Col1a1 expression, bone structure, or strength (*15*). However, in adult Mov13 mice, bone phenotype variability and severe lymphoma appear. Therefore, new HI OI mouse models and long-term drug studies are required. In addition, a recent case report of an 11-year-old patient with OI showed that physical therapy rehabilitation plays an important role in the management of OI and in improving life quality (*16*).

2.2.2. Beckwith–Wiedemann syndrome (BWS)

BWS is a rare disease associated with the aberrant expression of an imprinted gene cluster located on chromosome 11, region 15.5. The incidence of live births is approximately 1/10,000–1/13,700. There was no ethnic specificity, and the male-to-female ratio was approximately 1:1 (*17*). Without intervention, children may die of difficulty breathing and eating, as well as hypoglycemia, electrolyte disturbances, or tumors. Therefore, early diagnosis and treatment are important for children with BWS. In 2018, the European Congenital Imprinting Disease Network formulated an expert consensus on the diagnosis and scoring of BWS (*18*). BWS is usually diagnosed after birth, specifically during the neonatal period; however, prenatal screening is recommended for those with a positive family history or BWS suggested on prenatal ultrasound. The prenatal ultrasound detection rate for BWS is 64.1%, and one-year survival rates were over 90% (*19*). Clinicians should strengthen their understanding of BWS and make timely diagnoses. BWS should be highly suspected in infants with special manifestations such as omphalocele, hypoglycemia, macroglossia, and excessive weight gain after birth. Genetic testing should be performed early to assist in diagnosis and identifying the molecular subtypes, which can help with subsequent treatment and prognosis.

2.2.3. Goldenhar syndrome (GS)

GS is a rare congenital malformation that manifests as abnormal organ system development originating from the first and second branchial arches. The prevalence of GS is approximately 1/5,600–1/26,370 (*20*), and is more common in males than in females. Due to the lack of clear diagnostic criteria and large-scale clinical

research, it is difficult to accurately calculate the prevalence of GS. The etiology of GS is complex and diverse, involving genetic and environmental factors, and research on the pathogenesis of GS is still in its infancy. GS is usually sporadic, although familial cases have occasionally been reported. In addition, mutations in chromosomes 1, 4, 5, 7, 6, 9, 10, 12, 14–18, 22, and X have been reported (*21*). Lopez *et al.* proved that *MYT1* is a candidate gene locus for GS by performing whole-exome sequencing and single nucleotide polymorphism sequence analysis (*22*). Tingaud-Sequeira *et al.* found a nonsense mutation in *ZYG11B* in a patient with GS after whole-exome sequencing and confirmed the role of this gene in craniofacial cartilage structure and notochord development in cell and animal models (*23*). They demonstrated the involvement of the *EYA3* gene in the occurrence of GS at the cellular and animal levels (*24*). In many cases, no significant chromosomal or genetic abnormalities have been identified. Most of candidate pathogenic genes are involved in the migration and differentiation of neural crest cells. *EPAS1* is closely related to the formation of blood vessels, providing the basis for the neural crest cell hypothesis. However, the specific mechanism must be verified with further studies.

Even though familial GS cases are rare, genetic counseling is important. With the improvement in prenatal diagnostic technologies, the severity of GS has decreased in recent years. The ocular manifestations of GS are diverse and require individualized treatment plans. Surgery is the primary treatment for ocular abnormalities. Considering the impact on visual development and mental health, intervention at a young age is recommended.

2.2.4. Saethre–Chotzen syndrome (SCS)

Craniosynostosis is a disease characterized by the premature closure of one or more cranial sutures, resulting in skull deformity and brain dysfunction. It occurs in one in 100,000 live births (*25*). Craniosynostosis can be divided into comprehensive and non-syndromic craniosynostosis-based anomalies in other organs such as the heart, limbs, and respiratory system. Non-syndromic craniosynostosis accounts for approximately 85% of all craniosynostoses and is sporadically present in approximately 92% of patients. Syndromic craniosynostosis accounts for approximately 15% of the total incidence of craniosynostosis, and the most common genetic causes are mutations in *FGFR2*, *FGFR3,* and *TWIST1* (*26*). Heterozygous *TWIST1* mutations cause Saethreo-Chotzen syndrome, which is characterized by hypertelorism, microtia, low-set ears, and a low hairline. The cranial and facial deformities vary, and require personalized treatment strategies (*27*). Current research mainly focuses on craniofacial plastic surgery and ophthalmic correction.

2.2.5. Crouzon syndrome (CS)

CS is a rare autosomal dominant genetic disorder characterized by craniosynostosis resulting in skull deformity, facial dysmorphism, the incidence in live births is about 1.65/100,000. CS is associated with various mutations in fibroblast growth factor receptor 2 (*FGFR2*). *FGFR2* and *FGFR3*-related craniosynostosis shows obvious mandibular morphological changes in the early stages, which confirms the hypothesis of a genotype-phenotype correlation related to mandibular morphology (*28*). CS is usually diagnosed prenatally or during delivery through clinical and physical evaluations, as well as various targeted laboratory tests. The diagnosis of craniostenosis craniostosis requires amniocentesis, chorionic villus sampling, or preimplantation diagnostic studies of *FGFR1*, *FGFR2*, and *FGFR3* mutations. Children with CS require multidisciplinary cooperation and long-term management from various specialties. The appearance of children with CS can significantly improve after a series of surgical and orthodontic procedures. Many studies on drug therapy for pathogenic genes, such as the role of tyrosine kinase inhibitors in mutated FGFR-related craniostenosis, exist; however, use of these in a clinical setting requires time (*29*).

2.3. Rare birth defects associated with abnormal function

2.3.1. Hearing impairment related diseases

Non-syndromic hearing loss (NSHL): Deafness is the most common sensory disorder, with an incidence of 1.86% in newborns worldwide. Currently, more than 60% of deafness cases are caused by genetic factors, of which approximately 70% are NSHL. Its clinical manifestation is auditory dysfunction without other organ or system abnormalities (*30*). Wang *et al.* studied the effectiveness of combined methods to screen newborns for hearing impairment, and the results showed that compared with physical screening, genetic testing could identify an additional 13% of missed patients and 0.23% of newborns carrying drug-induced deafness susceptibility gene variants (*31*).

Currently, 124 genes for hereditary non-syndromic hearing loss have been reported (*https://hereditaryhearingloss.org/*), among which *GJB2*, mitochondrial 12 s rRNA, *SLC26A4* deaf, and *GJB3* were reported in the Chinese population. In addition, 3299C>A (p.Ser1100Tyr) and 5185-2A>G were found in a family with non-syndromic hearing loss. Among these, 5185-2A>G is a newly discovered intronic variant of the *TRIOBP* gene and expands the spectrum of *TRIOBP* deaf-causing variants. In NSHL, race seems to play a role in determining the genetic burden of *LOXHD1*, NSHL is mainly diagnosed through tertiary prevention, neonatal hearing, and deafness gene screening. Most patients with NSHL present with congenital or delayed

sensorineural hearing loss. The treatment of NSHL mainly focuses on avoiding predisposing factors and cochlear implants. Several studies have demonstrated successful gene therapy in mouse models (*32,33*). Patient-derived induced pluripotent stem cells are also being investigated because of the unique properties of stem cells that are pluripotent and self-regenerating (*34*). Therefore, determining the etiology of NSHL will improve management and treatment strategies. Genetic variation data from different populations worldwide are expected to be available for genetic counseling and prenatal diagnosis.

Branchio-oto-renal syndrome (BOR): BOR is a rare autosomal dominant genetic disease with a neonatal incidence of 1/40,000, accounting for 2% of severely deaf children (*35*). Previous studies have shown that 88% of patients with BOR have hearing impairments; 73% have preauricular fistulas; 60% have branchial cleft cysts or fistulas and sinus tracts; and 10% have hypoplastic renal function (*36*). The *EYA1* (8q13.3) gene abnormality is the most common cause of BOR, with approximately 40% of patients carrying this gene mutation. Approximately 10% of the patients have *SIX1* (14q23.1) and *SIX5* (19q13.32) gene mutations (*37*). There are few reports on BOR in China. Approximately 50% of patients with BOR do not have pathogenic variants of *EYA1*, *SIX1*, or *SIX5*, which may be related to the existence of other unexplored regions and novel gene variants.

The low incidence and diverse clinical manifestations of BOR have brought great difficulties and challenges to its clinical diagnosis and treatment, especially prenatal diagnosis, which can easily lead to missed diagnoses. A case of BOR diagnosed prenatally due to renal dysplasia was detected by ultrasound at 33 weeks of gestation, at which point the pregnant woman finally chose induced labor (*38*). The BOR phenotype is highly heterogeneous and can cause severe deafness and a complete loss of renal function. Prenatal diagnosis can help families understand the fetal prognosis and make prudent pregnancy decisions.

2.3.2. Neonatal eye disease

Retinoblastoma (RB) is the most common primary intraocular malignant tumor in infants and young children and originates from primitive retinal stem cells or cone precursor cells. The incidence in newborns is 5-5.57/100,000, with 85% of cases occurring before the age of 3, and the prevalence rate is 1-9/100,000. With approximately 9,000 new cases worldwide each year, retinoblastoma is considered a curable cancer in high-income countries, with a disease-free survival rate of approximately 100% (*39*). However, the prognosis tends to be poorer in low- and middle-income countries, where more than 80% of global cases occur (*40*). RB can be classified into genetic and nongenetic types. Gene therapy has become a new therapeutic approach in recent years. Molecular and genetic studies have shown that species-specific intrinsic genetic redundancy and compensation among RB family members can prevent retinoblastoma in mice (*41*). Genetically engineered mouse models have provided important insights into RB biology, but there are differences between species, with the human tumor epigenome localized at a later stage of development than mouse tumors (*42*). Based on this, a human cancer model derived from induced pluripotent stem cells provides valuable insights into tumor cell origin and tumorigenesis following *RB1* inactivation (*43*). Individual differences exist in the diagnosis and treatment of RB.

2.3.3. Neuromuscular-related diseases

Progressive muscular dystrophy (PMD): PMD includes Duchenne and Becker muscular dystrophy (DMD/BMD). It is a common form of muscular dystrophy in childhood, and DMD is the most common disease with a poor prognosis. DMD/BMD is an X-linked, recessive, degenerative muscular disease. The incidence of DMD/BMD does not differ significantly between countries, regions, or races. The incidence of DMD/BMD is about 1/3,853 in China, and it is estimated that there are approximately 70,000 patients nationwide. PMD is caused by a dystrophin deficiency. The gene encoding dystrophin is located at Xp21.2 and contains 79 exons. Mutations in the dystrophin gene lead to the loss or disruption of its expression product, dystrophin (*44*). In addition to basic clinical manifestations and electromyography, the detection of DMD gene deletions or duplications has become an important diagnostic method. To date, there is no radical cure for DMD, and glucocorticoids remain the first choice of treatment. With a deeper understanding of DMD gene mutations, targeted therapy and targeted therapy for certain mutation sites are new directions for DMD treatment in the future.

Leber hereditary optic neuropathy (LHON): LHON is a maternally inherited optic nerve disease caused by mitochondrial DNA (mtDNA) mutations and is one of the most common blinding diseases in adolescents worldwide (*45*). The incidence of the disease is between 1/31,000 and 1/526,000, with racial differences. It generally manifests as painless vision loss in both eyes, successively or simultaneously, and the best-corrected visual acuity (BCVA) is usually below 0.1. The prognosis for visual acuity is poor, and a few patients show mild spontaneous visual improvement. At present, the treatment of this disease is limited, and early symptomatic, supportive, and drug treatment may improve the vision of patients (*46*). China's first ophthalmic gene therapy drug, NR082, has been officially approved by the State Medical Products Administration for clinical research on LHON treatment. Idebenone, a short-chain coenzyme Q10 analog, was

approved by the European Medicines Agency for the treatment of LHON in 2015. Several studies have shown that oral idebenone (900 mg/day) is helpful for the improvement of vision in LHON patients, especially in patients with the m.11778G>A mutation (*47*). Challenges such as drug dose, side effects, and the high price of gene therapy remain. Currently, gene therapy for LHON is only available for patients with the m.11778G>A mutation. Using the same principle, rAAV2-ND1 and rAAV2-ND6 can also be used to treat the other two primary mutations; however, their specific feasibility needs to be verified.

2.3.4. Mitochondria-related diseases

Mitochondrial encephalomyopathy (MELAS): MELA is a multisystem metabolic disease caused by mutations in mitochondrial DNA or nuclear DNA (*48*). It is usually caused by abnormal oxidative phosphorylation of the electron respiratory chain. MELAS is a hereditary disease, including maternal inheritance, autosomal inheritance, and X chromosome concomitant inheritance. In 2016, a cohort study of North-East England MELAS says the prevalence of the disease is 2.9/100,000 (nDNA), 9.6/100,000 (mtDNA). A total of 10.8/100,000 individuals carrying pathogenic mutations develop clinical symptoms. The prevalence of onset in children (<16 years of age) is 5-15/100,000 (*49*). Many types of MELAS exist, with diverse clinical manifestations, difficult diagnoses, high misdiagnosis rates, and second-generation sequencing. Currently, there is no specific treatment method, and the characteristics of multisystem damage make it difficult to implement gene or stem cell therapy. Clinical trials of empirical drug therapy should be combined with specific clinical manifestations and possible pathogeneses in patients.

Parkinson's disease (PD): Parkinson's disease (PD) is a complex neurodegenerative disorder. The onset age of < 50 years is defined as early onset parkinsonism (EOPD). The prevalence of young-onset Parkinson's disease (YOPD) between 21 and 50 years of age and juvenile Parkinsonism (JP) before 21 years of age is 7.39/100,000. EOPD is less common, accounting for 5–10% of all cases, approximately 5% in European and American countries, and 10% in Japan, and its incidence increases with age. Genetics may soon provide personalized predictions of heterogeneous characteristics that affect disease-related disabilities, such as dementia risk and PD subtypes. In addition to conventional physical and drug therapies, achievements have been made in the development of remission therapies and biomarker treatment strategies for PD. The most common and advanced genetically linked targets are α-synuclein (SNCA), leucine-rich repeat kinase 2 (LRRK2), and glucoseencephalosidase (GBA1) (*50*). The convergence of proteins at the lysosomal degradation system level provides further targets for new therapeutic candidates as well as for biomarker development, a key component of drug discovery efforts. PD is a complex, multifactorial disease for which precision medicine, personalized diagnosis, and targeted therapy are particularly suitable. Genetic testing should be combined with other biomarkers such as sleep or smell testing and neuroimaging to obtain useful predictive and/or diagnostic capabilities.

2.4. Metabolic abnormalities associated with rare birth defects

Rare birth defects related to metabolic disorders include phenylketonuria, congenital hypothyroidism, congenital adrenal hyperplasia, glucose-6-phosphate dehydrogenase deficiency, *etc*.; however, phenylketonuria (PKU), one of the most well-known diseases, is rare.

PKU is most commonly caused by an autosomal recessive defect in the phenylalanine hydroxylase (PAH) gene (OMIM 612,349), resulting in an elevated blood phenylalanine concentration. PKU is equally prevalent among men and women. The prevalence of PKU varies in different races and regions, and varies by race and geographic region. Approximately 1/24,000 people worldwide are affected by PKU. Its prevalence in China is 1/15,924, which is relatively high in Asia (*51*). Prenatal diagnosis of BH4 deficiency can also be made by evaluating the concentrations of biopterin and neopterin in the amniotic fluid.

In 2020, the Food and Drug Administration (FDA) granted an orphan drug designation to a candidate for the treatment of PKU, APR-OD031, a slow-release amino acid mixture edited with proprietary drug-delivery technology, to ensure physiological absorption of the delivered amino acids. In addition, gene therapy with daily subcutaneous injections of PEGylated Phe ammonialase is expected in recent clinical trials, and mRNA approaches are under investigation (*52*).

2.5. Other rare birth defects

2.5.1. Hereditary skin diseases

Ocular albinism (OA): The overall incidence of albinism worldwide is approximately 1/20,000, and the incidence in some African countries can be as high as 1 <B->5,000 (*53*). The overall incidence of albinism in the Chinese population is 1/18,000. OA is diagnosed based on clinical manifestations and routine auxiliary examinations, and genetic diagnosis is crucial for the treatment of patients with albinism.

Albinism has a high degree of genetic heterogeneity, and 20 genes have been found to be related to different clinical manifestations of albinism, such as GPR143, AROA, the pathogenic genes of ocular albinism in non-comprehensive albinism; The main pathogenic genes of oculocutaneous albinism (OCA) are TYR, P gene,

TYRP1, SLC45A2, OCA5, SLC24A5, and C10ORF11. LYST is the only pathogenic gene in the CHS of albinism syndrome, whereas there are up to 10 pathogenic genes in HPS, including HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, BLOC1S3, BLOC1S6, and AP3D1. Albinism is a single-gene genetic disease caused mainly by point mutations, and there is currently no effective treatment. Surgery or drug treatment can be used to optimize vision, and regular preventive examinations or symptomatic treatments can be performed to detect skin tumors. For many people with albinism, social and psychological burdens, such as low self-esteem and relatively poor social life abilities, may be greater than medical problems and deserve more attention.

Epidermolysis bullosa (EB): EB is a group of genetic disorders leading to recurrent skin blistering, the most common cause of which is infection. The overall incidence and prevalence of EBV in the United States are approximately 20 cases per million live births and 11 cases per million people, respectively (*54*). Epidermolysis bullosa simplex (EBS) is the most common type of EB, and its specific mode of inheritance depends on the pathogenic variant. The EXPH5 or TGM5 mutation is an autosomal recessive inheritance, whereas the dominant negative missense mutation of the KRT5 or KRT14 gene is an autosomal dominant inheritance (*55*). EB is a skin disease, and most complications are skin-related. The most life-threatening complication is an infection. To date, there is no cure for EB, and optimal management must be multidisciplinary, involving wound care, pain control, infection control, nutritional support, and the prevention and treatment of complications. There are many new treatment methods for EB, such as gene editing, gene replacement (*56*), reverse mutation therapy (*57*), exon skipping (*58*), protein supplementation (*59*), read-through therapy (*60,61*) and some small-molecule drugs (*62*), *etc.* All the countries are actively exploring and conducting various preclinical and clinical tests. This provides new hope for better patient care.

2.5.2. Inherited blood diseases

Hemophilia is an X-chromosome-linked recessive hemorrhagic disease, mainly divided into hemophilia A and B, of which hemophilia A (HA) accounts for 80–85%. Hemophilia B (HB) accounts for 15–20%. The pathogenic genes F8 and F9 are located on Xq28 and Xq27.1, respectively. Based on the large number of mutations detected in thousands of hemophilia patients worldwide, the molecular basis of mutations in hemophilia is extremely diverse. The main mutation types of hemophilia A are inversion/recombination, point mutation, and insertion/deletion (*63,64*), and the main mutation types of hemophilia B are slight mutation, deletion/insertion (*65,66*). Hemophilia treatment is primarily based on replacement therapy, including on-demand and preventive treatments. Recently, gene therapy has achieved breakthroughs in both animal and clinical trials. A new generation of lentiviral vectors designed for the efficient delivery of transgenes to the liver offers the possibility of curing patients with hemophilia (*67*). PGT is an effective reproductive option for preventing the birth of children with hemophilia.

2.5.3 Stickler syndrome (SS)

Stickler syndrome is a rare inherited collagenous connective tissue disease mainly associated with mutations in the gene encoding collagen. The incidence rate is between 1/9,000 and 1/7,500 (*68*). The disease primarily involves ocular, joint, maxillofacial, and auditory abnormalities. Stickler syndrome can be divided into six types based on the presence of ocular abnormalities, vitreous phenotypes, and molecular genetic characteristics. Among them, type 1 is autosomal dominant inheritance, type 2 is autosomal recessive inheritance, and type 3 is autosomal recessive inheritance. They are caused by mutations in COL2A1, COL11A1, COL11A2, COL9A1, COL9A2, and COL9A3, respectively. Whole-exome sequencing (WES) detection covers the coding regions and exon-intron boundaries of approximately 20,000 genes and is of great value for the diagnosis of families with genetic diseases (*69*).

Genetic testing can improve the diagnostic rate of a disease and facilitate accurate typing. Prenatal diagnosis is the best strategy for avoiding birth. CMA, gene panel, or exome/genome sequencing can all be used for the genetic evaluation of Stickler syndrome, and genetic test results can be used to help guide treatment and management, help detect other high-risk individuals, and provide the risk of recurrence for the patient's offspring. Currently, there are few reports on the treatment of this disease, and variable case selection, lack of molecular genetic subtypes, and inconsistent treatment strategies have led to historical uncertainty regarding the safety and efficacy of preventive treatment.

3. Related technologies for diagnosis of rare diseases

With the progress of molecular genetics, molecular diagnosis technology, gene sequencing technology and genomics technology, the diagnosis of rare diseases has made great progress. For the diagnosis of rare diseases, although the traditional enzymatic detection technology still occupies an important position, it can no longer meet the demand. The rise of protein's genomics and metabonomics makes it possible to accurately diagnose a variety of rare diseases. At the same time, combining molecular imaging technology and bioinformatics technology, computer-aided diagnosis also shows a wide application prospect.

3.1. Classical gene detection methods

Classical genetic testing methods, such as karyotype analysis, fluorescence in situ hybridization (FISH), array comparative genomic hybridization (aCGH), multiplex ligation-dependent probe amplification (MLPA), *etc.*, are considered to be effective in detecting copy number variations of fragments > 400bp, which play an important role in the genetic diagnosis of rare diseases, but cannot detect a smaller range of gene mutations, and cannot detect pathogenic variations with constant copy number, such as balanced translocation or inversion.

3.2. Next-generation sequencing technology

The diagnostic test of NGS is not affected by genetic mode, and the sequencing results can not only provide us with the location information of pathogenic genes, but also provide important information such as the type of pathogenic mutations. In recent years, next-generation sequencing technology has rapidly risen with its accurate and efficient characteristics, and has become one of the important methods for auxiliary diagnosis of rare diseases. Next-generation sequencing technology can be divided into genome sequencing, transcriptome sequencing and chromatin immunoprecipitation-sequencing (ChIP-seq). Genome sequencing includes whole genome sequencing (WGS), WES, single-cell genome sequencing, amplicon sequencing, *etc.*

3.3. Genome Sequencing

The application of next-generation sequencing technology in the diagnosis of rare diseases can be divided into WGS, WES and targeted gene sequencing. Although exons only account for 1% of the whole genome, it is inferred that about 85% of pathogenic mutations occur in this region. WES obtains DNA sequence information of the whole genome exon region through next-generation sequencing technology, and combined with clinical phenotype and bioinformatics analysis, it can infer possible pathogenic genes. Compared with WGS, WES eliminates non-coding sequences in genes, requires relatively small sample size, and is more economical. Compared with targeted gene sequencing, WES does not need to predict pathogenic genes in advance, and is more suitable for patients with rare diseases that have difficulty in diagnosis, so it is favored by many clinicians.

In 2020, the American College of Medical Genetics and Genomics (ACMG) published guidelines for the application of fetal exome sequencing technology in prenatal diagnosis. It is pointed out that fetal exome sequencing technology is suitable for cases with abnormal ultrasound findings but negative karyotype and chromosomal microarray results (*70*). WES can effectively improve the detection rate and accuracy of prenatal diagnosis, and pathogenic genetic variations can be detected in approximately 10% of cases with negative gene chip results.

WGS uses NGS technology to obtain the sequence information of the whole genome, which is more effective than other gene sequencing technologies in identifying single base mutation, cleavage site mutation, intron mutation and multiple copy number mutation. Compared with WES, WGS has better consistency and less bias in measurement results; however, due to its high price, its clinical application is not as wide as WES.

Targeted target gene sequencing is a method of sequencing a specific target gene, which is an economically feasible method for the detection of single gene genetic diseases with known pathogenic genes. For example, Usher syndrome is a group of autosomal recessive single gene diseases. Despite the genetic heterogeneity, the method of targeted target gene sequencing can detect pathogenic mutations caused by different mutation modes such as single base or sequence rearrangement at a time, improving the diagnostic efficiency (*71*).

3.4. RNA sequencing

RNA sequencing can not only be used to diagnose some rare diseases involving specific RNA pathogenesis, but also to identify rare diseases caused by abnormal protein transcriptional levels caused by splice site variations. Abnormalities regulated by miRNA may be one look at the pathogenesis of rare diseases, and detection of the variation types of miRNA may provide a reliable basis for clinical diagnosis. The use of RNA sequencing to diagnose rare muscle diseases from a genetic perspective, and the detection of skeletal muscle sample RNA, indicates that RNA sequencing can effectively identify harmful splicing sites located in exon or deep intron regions, and the overall diagnostic rate can reach 35% (*72*). The application of RNA sequencing in peripheral blood RNA analysis can improve the clinical diagnostic rate, and reduce the interference of mutations of unknown significance in the diagnosis of rare diseases. Splice analysis combined with prediction software such as SpliceAI can determine the important splicing abnormalities at the diagnostic level, and clarify the functional effects of some mutations of unknown significance (*73*). In addition, by detecting disease-related miRNAs and tracing them to find the mRNAs and coding genes regulated by them, it is also helpful to study rare diseases with unknown pathogenic genes.

3.5. Third-generation sequencing technology (TGS)

TGS, namely single molecule sequencing, abandons the sample amplification step that is prone to error, realizes the sequencing of a single DNA molecule, and the measurement results can be exported immediately,

further shortening the sequencing time from several days to several hours or even several minutes, which is a faster and more efficient sequencing method than second generation sequencing. Merker *et al*. (*74*) used TGS to detect structural variation of the PRKAR1A gene, and thus diagnosed a case of Carney syndrome. Despite many advantages, TGS has a high error rate, SMRT can reach 15% (*75*). Although it is a random error that can be overcome by sequencing multiple times, it also faces problems in information storage and result interpretation and ethical issues related to genetic information.

3.6. Proteinomics

At present, the detection technology of proteinomics is mainly based on mass spectrometry and affinity-based protein analysis, which has shown outstanding advantages in the diagnosis of some rare diseases. For example, GM2 ganglioside deposition in neural stem cells of Tay-Sachs patients was successfully detected by liquid chromatography-tandem mass spectrometry (*76*). Guo *et al.* (*77*) established a method based on two-dimensional nanometer ultra-high performance liquid chromatography and mass spectrometry analysis, which can detect the down-regulation of frataxin protein level from platelets of Friedrich's ataxia patients, and this method can be used for auxiliary diagnosis of diseases or judgment of intervention effect. Protein level detection is an important part of the diagnosis of rare diseases, and the results of proteinomics analysis may be directly related to disease phenotype.

3.7. Metabonomics

Metabonomics is a method to systematically study the metabolites of small molecules (< 1,500 Da) produced by biochemical reactions, which can monitor cell pathways in real time and reflect the metabolic state of cells, tissues and even organs. The study of metabolites can also reveal the hidden biochemical mechanism of diseases and provide new ideas for the diagnosis of some rare genetic metabolic diseases. Graham *et al.* (*78*) evaluated and integrated WGS data and liquid chromatography-mass spectrometry non-targeted metabolomics data, and determined the variation and priority of congenital metabolic diseases, suggesting that metabolomics detection is of great significance for the screening and diagnosis of rare diseases, especially hereditary metabolic diseases.

3.8. Other auxiliary diagnosis methods

It is necessary to attach importance to the application of information technology in the diagnosis of rare diseases while developing various biological technologies for diagnosing rare diseases. Creutzfeldt-Jacob disease is characterized by high signal intensity in brain gyrus and striatum on FLAIR sequence on MRI, which has high diagnostic value and is listed as one of the diagnostic criteria of CJD. Serum ceruloplasmin < 80 mg/L is strong evidence for the diagnosis of Wilson's disease. SpliceAI software developed by Illumina Company in the United States can predict the location of genome splicing sites, and analyze high-throughput sequencing technical data to effectively identify non-coding gene mutations that can cause abnormal splicing events (*79*). Face2Gene system developed by American digital medical company can identify facial features related to diseases through photos, thus assisting in identifying rare diseases (*80*).

4. Prevention and control of birth defects and rare diseases in the era of genomic medicine

As mentioned above, in recent years, with the rapid development of genomic detection technologies such as high-throughput sequencing, the level of screening and diagnosis of hereditary birth defects and rare diseases has greatly improved, and genomics plays an important role.

4.1. PGT and preconception carrier screening to prevent birth defects: primary prevention and control

PGT can be used for molecular genetic screening and diagnosis of embryos or gametes, which involve fluorescence in situ hybridization, microarray analysis, and second-generation sequencing.

Indications for PGT-M include monogenic genetic diseases with clear pathogenic genes, such as achondroplasia, osteogenesis imperfecta, thalassemia, hemophilia, Duchenne muscular dystrophy, and hereditary polycystic kidneys. Continuous breakthroughs in new detection technologies have also opened new prospects for the prevention and control of rare genetic diseases. For example, single-cell whole-genome sequencing, including single sperm/egg whole-genome and transcriptome sequencing, can detect chromosomal abnormalities and base mutations in a single cell. Owing to its technical advantages of high accuracy and high genome coverage, it has been gradually applied in the field of reproductive medicine (*81*). The combination of single-cell genome amplification technology and deep sequencing will not only elucidate the pathogenesis of more genetic diseases but also facilitate high-throughput screening and diagnosis of birth defects and rare diseases.

4.2. Prenatal screening and diagnosis to reduce the birth of severe defects: secondary prevention and control

Noninvasive prenatal testing (NIPT) sequencing of fetal DNA in maternal blood has better sensitivity and specificity while avoiding unnecessary invasive prenatal examinations and has become an important means of screening for fetal aneuploidy diseases. The detection range of NIPT ranges from the early stage, which

mainly involves trisomy 21,18,13 to the screening of chromosomal microdeletion, microduplication, and other syndrome diseases (*82*). With the continuous development and application of sequencing methods and trophoblastic separation of fetal-derived cells, the sensitivity of NIPT in the detection of fetal sex chromosome abnormalities, microdeletions, and microduplications has greatly improved, and the detection time window has advanced, allowing sufficient time for clinical diagnosis and decision-making.

Genomic abnormalities, including chromosome numerical abnormalities, large duplications, and pathogenic copy number variations (pCNVs), are important causes of birth defects. Compared to other techniques such as karyotype analysis and chromosomal microarray analysis, CNV-seq has the advantages of a wide detection range, high throughput, simple operation, good compatibility, and low DNA sample volume. CNV-seq technology has been gradually applied in clinical practice as a first-line prenatal diagnostic tool.

4.3. Postpartum newborn screening to reduce the risk of disability: Three-level prevention and control

Neonatal disease screening refers to the mass screening of diseases with serious consequences in the neonatal period; early diagnosis and treatment can avoid or reduce harm to the greatest extent. Currently, newborn heel blood screening, tandem mass spectrometry screening, and newborn hearing screening are routinely performed. With the rapid development of gene sequencing technology, the prospects of using WES and WGS as new auxiliary methods have attracted considerable attention (*83*).

5. Conclusions

Prenatal diagnosis and maternal-fetal medicine, as "pioneers" in the diagnosis and treatment of rare diseases, play an extremely important role in the clinical and scientific research systems of rare diseases. Prenatal screening and diagnosis and their corresponding management, promotion, and establishment of norms have become "urgent matters". However, implementation and achievement of precision, safety, economy, and ethics are the difficulties that need to be overcome.

The development of genomic technology has advanced the diagnosis, prevention, and control of hereditary birth defects and rare diseases to new heights. The United Nations launched the 100,000 Genomes Project in 2013, which achieved great success and provided the basis for the National Health Service genomic medicine service, becoming the first national healthcare system to provide genomic medicine services at the WGS. To fully realize the great potential of genomics in scientific research and clinical practice, it is necessary to continuously update and improve interdisciplinary collaboration, cross-population, policies, and regulations to ensure a more comprehensive understanding of the pathogenesis of diseases and the correlation between phenotypes and genotypes, which will help improve the equity of global access and the return to genomics. To promote the establishment of new, more standardized, and individualized prevention and treatment strategies to block the occurrence of the disease from the root to the greatest extent and benefit more patients.

Funding: This work was supported by a grant from Shandong province traditional Chinese medicine science and technology project(2021Q127), Inner Mongolia Grassland Talents Innovation Team Project (2022), Translational medicine project of Inner Mongolia Medical University, Inner Mongolia Autonomous Region Science and Technology Plan Project (2022YFSH0067).

Conflict of Interest: The authors have no conflicts of interest to disclose.

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Received June 29, 2023; Revised August 17, 2023; Accepted August 22, 2023.

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Released online in J-STAGE as advance publication August 25, 2023.