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Next generation sequencing: Coping with rare genetic diseases in China

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Summary **With a population of 1.4 billion, China shares the largest burden of rare genetic diseases worldwide. Current estimates suggest that there are over ten million individuals afflicted with chromosome disease syndromes and well over one million individuals with monogenic disease. Care of patients with rare genetic diseases remains a largely unmet need due to the paucity of available and affordable treatments. Over recent years, there is increasing recognition of the need for affirmative action by government, health providers, clinicians and patients. The advent of new next generation sequencing (NGS) technologies such as whole genome/exome sequencing, offers an unprecedented opportunity to provide large-scale population screening of the Chinese population to identify the molecular causes of rare genetic diseases. As a surrogate for lack of effective treatments, recent development and implementation of noninvasive prenatal testing (NIPT) in China has the greatest potential, as a single technology, for reducing the number of children born with rare genetic diseases.**

Keywords: Next generation sequencing (NGS), noninvasive prenatal testing (NIPT), whole exome sequencing (WES), chromosome disease, monogenic disease

1. Introduction

Genetic diseases, categorized as either chromosomal or monogenic diseases, affect approximately 1% of all individuals globally (*1*). There are over 100 different clinically recognized chromosome disease syndromes. Chromosome diseases are believed to be caused by de novo abnormal chromosomal rearrangements in the early preimplantation period of human development. The most prevalent is Down Syndrome (DS) with an incidence of 1 in 700 births. In contrast, around 7,000 distinct monogenic diseases have been described (*2*). These monogenic diseases originate from mutations carried in parental genomes and are inherited in an autosomal recessive, dominant or X-linked manner. In the Caucasian population, the most prevalent monogenic disease is Cystic Fibrosis, with a carrier frequency of 1 in 25 and an incidence of 1 in 2,500 births. Based on the combined disease incidences of all chromosomal and monogenic diseases, genetic disease is considered relatively rare compared to more complex genetic diseases such as cancer. Being the most populated nation in the world, China has the largest share of patients with rare genetic diseases. It is generally believed that there are approximately 10 million such patients living among the 1.4 billion people (*3*), although this number could be grossly under-estimated due to patients with unrecognized or less severe disease.

Each year in China, we estimate that there are around 26,000 new DS patients added to the population. This estimate is based on an annual birth rate of 18 million newborn and a disease incidence of DS of 1 in 700 births. Factoring in the life expectancy of DS patients which today is generally over 50 years of age and, adjusting for population growth rate over the last 50 years, we estimate that DS patients alone currently account for around 1.4 million people. After DS, sex chromosomal diseases, such as Turner (45,X), Klinefelter (47,XXY), Triple X syndrome (47,XXX)

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and Jacob (47,XYY) syndromes have a combined incidence of 1 in 1,000 births (*4*). With a near normal life expectancy, the number of Chinese individuals with sex chromosome disease syndromes is estimated to exceed 2 million. In a review of chromosome disease incidence in the United Stated (US), it has been estimated that the combined number of patient's with other types of chromosome disease syndromes far exceeds that of DS and sex chromosome disease patients (*5*). On this basis, we estimate that the number of Chinese patients with rare genetic disease caused by chromosomal abnormalities alone is well over 10 million.

Although monogenic diseases are less prevalent than chromosome diseases, based on population size, they still represent a significant proportion of the overall genetic disease burden in China. On top of the list are blood disorders such as alpha- and beta- Thalassemia, Sickle Cell Anemia (SCA) and Hemophilia, the muscle disorders Duchenne Muscular Dystrophy (DMD) and Spinal Muscular Atrophy (SMA), the metabolic disease Phenylketonuria (PKU), the sensory disorder Hereditary Hearing Loss (HHL) and mental disabilities such as Fragile X Syndrome (FXS). In southern China, the incidence rates of alpha- and beta- thalassemia are among the highest in the world, with a combined carrier rate of 11% and 23% in Guangdong and Guangxi provinces respectively (*6,7*), making thalassemia the number one rare genetic disease in these regions. However, since the average life expectancy is less than 10 years, we estimate that the number of living thalassemia patients in these provinces would not exceed more than 25,000. For the other monogenic diseases mentioned, disease incidence is much lower, ranging from as high as 1 in 3,000 to as low as 1 in 10,000 individuals. Despite the low incidence rate of each individual single gene disease, their combined total still represents a large body of patients in China because of the sheer magnitude of the population size. We estimate that the number of existing patients afflicted with monogenic diseases exceeds well over a million in China.

2. Rare genetic disease management and treatment

Despite being increasingly recognized as an important health issue, management of rare genetic diseases in China still has long way to go. The main reason why China is currently lagging behind many other countries is primarily due to its large population size, with the majority of health service resources directed towards providing a good standard of general health care for all people. However, several other factors such as low economic status in the past, lack of ability to implement technological advances and a conservative cultural view of genetic disease have proven to be significant contributing factors that have impeded managing the burden of genetic disease.

Even though the number of patients with genetic disease in China is very high, most stay confined in the family home and do not participate in daily life. Except in special events dedicated to a particular disease or disability, patients with genetic diseases are rarely seen in public. As in many other cultures, traditional Chinese values discourage a family from allowing the affected individual to go out. Without understanding the causes of the diseases, the society in the past tended to blame the family for the disability. Also, within the family, when a child was born with a genetic disease, there was a strong tendency for one of the parents to blame the other for bringing disrepute to the family, causing shame and guilt.

Armed with modern communication tools and internet, attitudes are rapidly changing. More than ever before, Chinese society as a whole is more likely to accept a person with a rare genetic disease. Families of rare genetic disease patient's are openly sharing their experiences online. There are chat rooms created by internet companies to accommodate such needs though the regulations to protect patient privacy has not been put in place to safeguard information exchange. Recently, a public outcry against the Chinese internet giant Baidu's selling the hemophilia chat room information to a special interest group forced Baidu to abandon the practice.

This event also exposed lack of patient advocacy groups in China. During the event, it was the patient families, the public and the government agencies who were vocal and strongly demanded Baidu to make the correction, not only for the hemophilia chat room, but also for the chat rooms of the other rare genetic diseases. That being said, there is momentum in forming such advocacy groups. The Chinese Organization for Rare Diseases (CORD) represents one of such groups. Located in Beijing, CORD is visible in almost every major conference for genetic diseases. Further, since the first patient registry for genetic diseases was created in 2011 (*8*), registries are steadily growing.

In recent years, government sponsored programs for public awareness and screening of rare genetic diseases has played an important role in diseases prevention. In southern China, because of the prevalence of thalassemia, special educational programs were created to make the public aware of its existence, which led to a very high rate of acceptance for the thalassemia screening program. As a result, the birth rate of thalassemia patients has dropped substantially (*9*). To further such programs, in December of 2015, the National Health and Family Planning Commission (NHFPC) created a special committee consisting of eighteen prominent scientists and Chinese academy members, dedicated to research and advise the government in making policies for prevention and treatment of rare genetic diseases.

There is also a cultural shift in the way families

are coping with genetic disease in China. Now, more than ever, families and patients are presenting to clinics to seek the medical opinion of doctors regarding the disease and possible treatments to alleviate suffering. Medical doctors have become increasingly skilled at making the correct clinical diagnosis for most genetic diseases and some patients are willing to pay a small DNA testing fee to define the causative familial mutations. This is an important step if members of the families have married and are planning to have children, enabling the option of prenatal diagnosis. In cases where the type of disease is not obvious, sequencing based DNA testing of the likely genes can also be ordered to define the mutation(s) and provide a firm clinical diagnosis. Once parents have a better understanding of their child's disease and potential treatments, they are finding it much easier to come to terms with the disease in the family and cope better with the day to day impact of the disease burden.

For the vast majority of genetic diseases, there is no cure or effective treatments. Thus, once diagnosed, patients with rare genetic diseases have very limited treatment options. Patient care and support have improved substantially in recent years, but access to drugs is limited. As of 2014, the US FDA has approved seven enzyme replacement therapy drugs for lysosomal storage disorders, three drugs for hereditary engioedema, and one drug for cystic fibrosis (Kalydeco). The European Medicines Agency (EMA) has also approved one gene therapy drug (Glybera) for lipoprotein lipase deficiency. As listed in the clinical trial website (clinicaltrials.gov), some of these drugs are being evaluated under the guidelines of the Chinese Food and Drug Administration (CFDA). To receive treatments today, patients must be admitted to the trial cohorts.

Even if these drugs are approved by CFDA in the next few years, the cost of the drugs will prevent most patients from receiving treatment. On average, each drug costs about 300,000 US dollars per year per patient in the US and European Union (EU). The prices are set at such a high premium value because the number of patients in the US and EU is low, and because they are protected under the Orphan Drug Act. For some of these diseases, the number of patients in China is relatively high, making the pricing difficult to justify. The equivalent law underpinning the Orphan Drug Act has yet to be established in China. Such pricing will definitely render these drugs unaffordable for Chinese patients. The economy of rare genetic disease management in China will likely adopt a more cost-effective model for patients.

3. Next generation sequencing technologies for prevention of genetic disease

In China, prenatal diagnosis continues to play a small, but significant role in prevention of chromosome disease, allowing couples the option of terminating an affected pregnancy. In all major hospitals in China, maternal serum screening and fetal ultrasound services are available to assist in the detection of fetuses with chromosome diseases. Confirmation of positive results is usually performed by amniocentesis and fetal karyotyping (*10*), although high-resolution array based chromosome analysis methods have been available in the last five years to detect more subtle chromosome disease syndromes. Nonetheless, detection of sex chromosome diseases still remains problematic because these syndromes cannot be detected by maternal serum screening and, in general, do not show any overt clinical signs on ultrasound (*4*).

Prevention of monogenic diseases has largely been ineffective because most couples are not aware of their carrier status prior to conceiving a child. Further, the vast majority of couples with a family history of genetic disease, and therefore at high risk, have not pursued testing due to the cost and time required to identify the causative mutations. Therefore, with traditional methods, the overall penetrance of diagnostic testing remains very low. Further, the situation has been confounded by other factors unique to China, including the fact that 50% of the population lives in rural areas and do not have good access to genetic services and, the cost of testing is not subsidized (*11*). Further, available genetic services are stretched to full capacity and thus it is logistically impossible with current laboratory infrastructure, staff expertise and equipment for public hospital diagnostic laboratories to cope, even if more patients presented for testing.

The advent of NGS technologies (*12*) such as noninvasive prenatal testing (NIPT) and whole exome sequencing (WES) offers new hope for disease prevention in China. In fact, China is one of the leading countries to develop and implement NIPT of maternal blood for fetal chromosome abnormalities (*13,14*). Several major companies including Berry Genomics and Beijing Genomics Institute have fundamentally driven this opportunity, making the test available for all pregnant women over the last five years. Furthermore, the CFDA took an unprecedented step to mandate that all sequencing platforms and technologies were accredited for clinical application, giving the doctors and patients more confidence with the test. NIPT has proven to be reliable and accurate, demonstrating very high sensitivity and specificity for detecting common aneuploidies such as T21 (DS), T18 (Edward syndrome) and T13 (Patau syndrome) as well as sex chromosome aneuploidies, with low false positive and negative rates (*14*).

The introduction of NIPT in China is beginning to have an impact on reducing the incidence of common chromosome diseases. For example, during 2015 where 18 million births were expected, commercial

companies performed approximately one million tests for pregnant women with borderline fetal risk. In this screened population, the detection rates for T21, T18 and T13 fetuses were approximately 1 in 200, 1 in 1,000 and 1 in 6,000, respectively. Since the vast majority of couples elected to terminate these affected pregnancies, the contribution of NIPT alone has already started a downward trend in prevention of chromosome disease in children, particularly DS. Therefore, with government support, expanding the availability of NIPT in China to the majority of pregnant women should be a key objective of commercial companies and hospital laboratories empowered with the technology. This will also require government subsidies for NIPT testing, further patient and doctor education and importantly, the development of a more cost-effective test to encourage higher uptake by pregnant women. If this can be achieved, in time, the balance will eventually be tipped in favor of preventing more babies born with chromosome diseases than babies born with these diseases.

There is also hope on the horizon for preventing single gene disease in China. Carrier testing before pregnancy is the key to success. Recently, WES technology has been developed and validated for identifying deleterious mutations associated with rare monogenic diseases (*15*). In the US, the uptake of WES has been high and is currently offered by several companies as a commercial service. China is heading in the same direction and within the next few years, it is anticipated that a cost effective WES test will be available to Chinese couples. To be quickly effective and provide the greatest impact, the testing strategy needs to be focused initially on the top 10 diseases and targeted to provinces or regions where the carrier frequency is known to be high. Thus couples identified at high genetic risk of having an affected child can have traditional invasive molecular testing of the fetus, which is widely available in most diagnostic laboratories. Alternatively, these couples could choose assisted reproduction and preimplantation genetic diagnosis (PGD) to select disease-free embryos for transfer and implantation (*16*). Using this approach couples can commence their pregnancy knowing that their fetus does not have the familial genetic disease. However, there are only around 20 fertility centers in China that have a license to perform PGD, and therefore access to this technology is currently limited.

Active research using the power of NGS technologies and effective clinical translation is central to further reducing the total burden of rare monogenic diseases in China (*2,17-19*). Currently, only half the genes and mutations associated with the 7,000 known monogenic diseases have been identified (*2*). With the increasing availability of whole genome sequencing (WGS), it has been predicted that within the next ten years, we will have a complete database of all genes and mutation types causative of monogenic diseases. For the first time, this is bringing WGS closer to the clinic, providing valuable information to clinicians for making diagnoses of children with congenital conditions that were previously undiagnosable (*17,18*). One recent success story in China has been the application of NGS to unveil novel genes and mutations associated with hereditary hearing loss (*20*). This approach is now providing important information for genetic counseling and expanding the reproductive options for couples at genetic risk to prevent hearing loss in their offspring.

Current clinical research activities in China are now heavily focused on developing novel NIPT strategies for detecting the full spectrum of chromosome disease syndromes. NGS based NIPT methods for simultaneous detection of common aneuploidies as well as submicroscopic deletions and duplications are well advanced (*21*). At the clinical level, pilot studies are underway in several provinces to evaluate the reliability and accuracy of these new methodologies. In addition, promising NGS based technologies have been developed for NIPT of monogenic diseases. Haplotype based targeted sequencing of maternal plasma has been shown to be accurate for the diagnosis of HHL (*22*) and SMA (*23*). Further, an alternative NIPT method called circulating single molecule amplification and re-sequencing technology (cSMART) has also been demonstrated to accurately genotype the fetus in pregnancies at risk for Wilson Disease (*24*). In principle, even if there is no knowledge of the causative parental mutations, it is possible to develop these two technologies further for the diagnosis of a broader range of monogenic diseases. Thus, with a focused strategy to clinically implement these second-generation NIPT tests, it will be possible over time to substantially reduce the burden of chromosome and monogenic disease in China.

4. Conclusion

Management of the burden of rare genetic disease in China remains a challenging issue due to the sheer size of the population. With few treatment options, families cope remarkably well when caring for affected individuals. Government bodies, clinicians and patients are becoming increasing educated about the causes of genetic disease. The way forward for China is active disease prevention, which will serve as a surrogate for the lack of effective and affordable treatments. A plethora of new NGS technologies are now widely available for detection of chromosome disease and uptake is rapidly growing. With the development of more cost effective tests combined with Government subsidies, increased numbers of pregnant women will more likely undertake NIPT for chromosome disease, and eventually when available, also embrace NIPT for monogenic diseases. If this can be achieved in the short

term, the number of Chinese children born annually with rare genetic diseases will rapidly decline and, over a sustained period of time, the number of existing individuals with rare genetic diseases will begin to steadily decline.

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