Newborn screening for Duchenne muscular dystrophy in China: follow-up diagnosis and subsequent treatment

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Background: **Newborn screening for Duchenne muscular dystrophy (DMD) is currently being initiated in Zhejiang Province, China and is under consideration in other countries, including the United States. As China begins to implement DMD newborn screening (DMD-NBS), there is ongoing discussion regarding the steps forward for follow up care of positively identified patients as well as false positive and false negative results.**

Data sources: **Relevant papers related to DMD-NBS, and NBS in China were reviewed in PubMed.**

Results: **The current state of DMD-NBS is discussed, along with the steps needed to effectively screen infants for this disease in China, recommendations for establishment of follow up care in patients with positive and negative screens, and measurement of patient outcomes.**

Conclusions: **Zhejiang Province, China is ready to implement DMD-NBS. Future challenges that exist for this program, and other countries, include the ability to track patients, assist with access to care, and ensure adequate follow-up care according to evidence-based guidelines. In addition, China's large rural population, lack of specialty providers, and difficulty in educating patients regarding the benefits of treatment create challenges that will need to be addressed.**

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Review article

Introduction

Screening newborns for neuromuscular diseases such as Duchenne muscular dystrophy (DMD) has the potential to change the lifelong care of patients affected with this disease. In the case of DMD, early intervention with corticosteroids, often delayed and infrequently prescribed in many countries, including China, prolongs ambulation and improves quality of life.[1,2] Additionally, investigational genetic treatments (e.g., exon skipping) in boys enrolled over seven years of age, are in stage 3 clinical trials with outcomes suggesting a slower functional decline than expected.[3] Thus, newborn screening (NBS) to identify patients who qualify for these treatments much earlier in life will be important. In 2012, Mendell et al[4] reported a new 2-step approach for DMD-NBS, testing creatine kinase (CK) on newborn dried blood spots followed by direct *DMD* gene testing on samples with elevated CK. This method provides an opportunity for countries, such as China, to diagnose DMD patients using a CK marker without requiring the patient to return for repeat serum testing. Since 1976, there have been 10 DMD-NBS programs, which screened over 1.8 million newborns. With the exception of the recent Mendell et al study in

Ohio, USA,[4] all programs utilized a 3-step approach to diagnosis: testing CK on dried blood spots, followed by confirmatory serum CK testing, and then clinical follow up, muscle biopsy, or more recently *DMD* gene testing.[5] Currently, DMD-NBS pilot programs have begun in Australia and Hangzhou, China with the aim of identifying best practices for a standard CK assay utilizing the 2-step approach described by Mendell et al.[4] The timing of a DMD-NBS program in China correlates with a recent relaxation of the long standing Chinese population restriction from a "one-child" restriction to "two-child," which will not only add to an already large population of newborns, but also enhances the importance of establishing an effective and efficient framework for screening and follow-up to ensure the screening programs' success.[6]

Current state of NBS in China

NBS in China was initiated in 1981, with screening for phenylketonuria (PKU) in Shanghai, China. As of 2013, 202 Chinese screening laboratories were operational with most labs screening only for PKU, congenital hypothyroidism (CH), and glucose-6-phosphate dehydrogenase deficiency. Screening coverage and diseases screened varies by location; in 2013, screening for PKU and CH, including rural areas, was 85% (approx. 13 940 000 babies).[7] Screening is regulated at the province level as directed by the Chinese Ministry of Health. Within each province, there is a Newborn Screening Center that oversees each NBS laboratory. Due to the many large rural areas in China, with limited access to care and few trained physicians to discuss neonatal screening and its implications with parents, there are major geographic differences in screening; the Eastern region (the most densely populated areas) screens 50% more than the mid and 60% more than the Western regions.[8] NBS is primarily by informed consent and in most provinces relies on parents to pay the cost of screening in combination with government subsidies; exceptions include the cities of Shanghai and Guangzhou which require infants be screened for PKU with costs paid in full by government funds.[9] Additionally, in Hangzhou city, the largest city in Zhejiang Province, with a population of 5 million, screening for 46 disorders (15 amino acid, 14 organic acid, 12 fatty acid, and 5 other disorders) is financially covered.[7] As of 2009, the screening rate for PKU and CH in Zhejiang Province (population 49.80 million with 11 cities under its jurisdiction) was 97.75%.[7] Newborn screening in this province includes not only testing the blood samples, but also recalling the patients, diagnosing, and arranging follow up for patients with positive screens.[7] The shift of Chinese policy from population quantity to population quality with the modification of the "one child policy" could provide for widespread expansion of clinics for NBS and disease prevention.[6]

Challenges identified throughout the Chinese NBS process include the large rural and floating (migrant) populations, with a lack of understanding among parents about the seriousness of the diseases screened. Many low income parents wait until a child has severe disease before initiating or asking for treatment, despite suspecting the child has an underlying disease process.[10] Additionally, there continues to be a push for improvement in laboratory quality with more government support to make neonatal screening efficient with satisfactory control systems, shorter time to report, and greater range of diseases screened.[7]

Current state of DMD diagnosis and treatment in China

DMD is a neuromuscular X-linked disorder with an incidence of approximately 1:5000 male births.[4] The hallmark of DMD is progressive muscle weakness, with an average age of diagnosis in the United States of 5 years old.[11] However, the onset of symptoms and signs is much earlier, with neurocognitive delays documented in DMD patients as early as 1 year old.[12] In the United States, on average, there is a delay in DMD diagnosis of 2.5 years, and this delay has been unchanged for two decades.[11] Longer delays occur in other parts of the world with limited access to care and medical knowledge.[1,11,13] Untreated, patients with DMD typically lose ambulation in their early teens. Ambulation and life expectancy are greatly prolonged with corticosteroids and supportive measures such as mechanical ventilation; life expectancy can be anywhere from late teens to forties depending on the amount of supportive care.[2,14-16]

In 2011, a dystrophinopathy registry was established at Children's Hospital of Fudan University (CHFU) in Shanghai, China; CHFU database for dystrophinopathy.[1] Between 2011 and 2016, 569 patients with DMD have been registered, with most patients from East China.[1] In this registry, the majority of patients were >8 years of age when a DMD diagnosis was made, with a small number diagnosed at age 3-4 years, probably because liver enzymes are sometimes checked prior to entering kindergarten. Most of the boys in the older age groups showed clinical symptoms of muscle weakness but were from rural areas with difficult access to specialty care.[1] The most common genetic mutation in Chinese patients in this registry was an exon deletion (65.5%).[1] Only 26.3% of patients in the registry (this does not

even include patients with the disease who have never been diagnosed) have been treated with corticosteroids. This is a much lower percentage than other developed countries.[1,17] Seventy-four percent of patients/families refused the use of corticosteroids due to a lack of understanding regarding DMD treatment guidelines.[18,19] This is due, in part, to a lack of knowledge by Chinese physicians regarding the importance of corticosteroids, in combination with societal opinions that if treatment is not a cure, it is rejected.[1] There is also a concern of the Chinese population/physicians that administering corticosteroids to patients <4 years old will initiate an interaction with vaccines, administered around this age. Alongside improvements in initiating treatment with corticosteroids, a gap in other identified DMD care guidelines, including pulmonary and cardiac follow up care, has been identified in China: 88.4% of patients in the Fudan registry refused cardiac testing and none of the patients received ventilatory support.[1,20,21] Overall, there is a lack of physician knowledge regarding DMD care guidelines in China, inadequate financial support for medical care, and limited support for patient education on the use and impact of supportive measures, especially non-invasive and invasive ventilatory measures.[1] As patients become weaker and eventually wheelchair bound, due to these limited supportive services, the patients' family must pay for most treatment costs; many parents end up staying at home to take care of their children, sometimes even providing repetitive diaphragmatic pressure to maintain ventilation. Additionally, due to transportation issues and a societal feeling that DMD follow up care is not important, it has become exceedingly difficulty to bring more debilitated patients into a clinic for follow up medical evaluation.[1]

Initiating DMD-NBS in Zhejiang Province, China-the Hangzhou NBS program

The NBS laboratory in Hangzhou, China, where screening for DMD in China is being initiated, has established that it is capable of screening for a high number of diseases (46 currently), and reaching over 97% of the area population, while providing full financial coverage for screening. DMD-NBS will include a standardized creatine kinase-MM (CK-MM) isoform in blood spots with standardized reagents and laboratory analyzers, currently under development by PerkinElmer. A pilot study is ongoing in the province, and in the completed first part of the study, 18 424 neonatal dried blood spot specimens from boys [majority (94.5%) sampled between 3-5 days of age] were measured with the PerkinElmer GSP Creatine Kinase-MM (PerkiinElmer, Finland; research use only kit). The distribution of CK-MM concentration in the neonatal population was log-normal with a high degree of confidence (P<0.005). The median measured CK-MM concentration was observed to progressively decrease with age at sampling, up to 10 days (population median was 119 ng/mL at 3 days and 46 ng/mL at 10 days), staying approximately constant after that. This indicates that age of the newborn affects the CK-MM level to only a minor degree when sampling at day 3 or older. The presumptive CK-MM cut-off used (>700 ng/mL) corresponded to the 99.985th percentile of the population, and the presumptive DMD-cases were all clearly separated from the normal population distribution. In total, 13 specimens had CK-MM elevated above the putative cut-off (700 ng/mL in blood), of which 4 cases were confirmed by DNA mutation testing to be true DMD cases. This corresponds to 1 in 4560 boys and is similar to that reported elsewhere.[4] The pilot program in Australia is also working to identify the optimal time for DMD-NBS, comparing screening at 24-96 hours *vs*. 6-7 days, *vs*. 6-12 weeks of age. Based on preliminary data in Hangzhou, screening will likely continue to be initiated on blood spots obtained 3-7 days after birth, the time all newborn blood spots are collected at the Hangzhou NBS laboratory. A higher CK cut-off may be used once further data are collected. Importantly, there is a process in place to obtain parental consents for disorders that can be screened on dried blood spots using tandem mass spectrometry technology but that are not considered mandatory.[22] Given the nature of DMD-NBS follow-up, consent will likely be an important component of DMD-NBS.[23]

A major challenge for China's DMD-NBS program will be establishing the system for evaluating patients with positive screens, as well as eventually identifying any false negative results.[5,23] The NBS laboratory in Hangzhou, China has a current registry to document results of all screens and to arrange appropriate follow up for patients with positive screens. It will be vital to link this registry to care providers who follow published DMD guidelines and raise DMD awareness through organizations such as the Muscular Dystrophy Association.[20,21] Specifically, the province will need to establish a follow up protocol for patients with both positive *DMD* gene testing and patients with elevated repeat serum CK, suspicious for an underlying neuromuscular disorder, but negative *DMD* gene testing. These patients will be found to have other neuromuscular diseases: other dystrophinopathies, congenital muscular dystrophies, and limb girdle muscular dystrophies, or DMD potentially missed by *DMD* gene testing.[5] *DMD* gene testing is not 100% sensitive but can identify almost 99% of *DMD* mutations.[5,24] All patients will require further

neuromuscular evaluation. Next-Gen sequencing could be utilized on patients with negative *DMD* gene deletion mutation testing and elevated CK, or eventually, on all patients, however this testing comes with its own ethical issues and is still premature for routine newborn screening.[25]

DMD follow-up care in Chinese patients identified through DMD-NBS

A boy, who screens positive for a *DMD* genetic mutation through Hangzhou's DMD-NBS program, will require systematic follow up care. Ideally, after a positive screen is made, the boy's local doctor should be made aware and subsequent steps would include setting up an initial discussion through telemedicine with the family, the local doctor, a physician with expertise in neuromuscular disease, and a genetic counselor. The first formal medical visit would include a dedicated neuromuscular exam with a neuromuscular specialist. A social worker would also be appointed and available to provide family support. Further specialty visits with cardiology, pulmonology, and orthopedics could be arranged at this time. In the United States, this initial appointment can take over an hour. In China, where initial appointments are sometimes only a few minutes, even in specialty clinics, arranging such an appropriate neuromuscular consultation will be challenging. Additional challenges include identifying/training ancillary care specialists including genetic counselors, social workers, physical therapists, and respiratory therapists. A training program for genetic counselors has been initiated in Hangzhou and the first class of 158 trainees has graduated, with a goal of 1000 trainees in 3 years, but many more programs are needed to fulfill the large and diffuse population requirements. Maintaining follow up neuromuscular care will also be challenging since specialists for rare disease are usually located within hospitals, requiring some patients to travel hours for appointments.[1,26] Specialist care (pulmonology, cardiology, orthopedics) in some areas could even be at multiple different medical centers, which further hinders patient follow-up for these specialties.[1] Although there are clear benefits from direct, hands-on patient care, telemedicine in combination with community providers, could assist in coordinating neurological and supportive care. An application is in development for handheld devices where patients can enter data that can be reviewed by a team remotely. All patients diagnosed with DMD should be encouraged to start corticosteroids (prednisone or deflazacort), which have been shown to delay disease progression and prolong life.[18,19] Deflazacort, mainly used in Canada and in Europe, will likely be the first FDA approved treatment for DMD based on data recently published.[2,27] Supportive care is also essential as routine standard of care for patients with DMD. This includes routine physical therapy evaluations throughout life, dietary counseling for those patients on corticosteroids, pulmonary care (manual or mechanical cough assistance, non-invasive or invasive ventilation), and cardiac care (angiotensin converting enzyme inhibitor/angiotensin receptor blockers). Patients in China have limited access to these resources because of cost and lack of awareness/education of such services. Establishing a mobile supportive care network, which can potentially provide these services locally, may be feasible in China, and ultimately prolong and improve quality of life in these patients.

Conclusions

Zhejiang Province, China is ready to implement newborn screening for DMD. The challenges for China, and for other countries, will be: How to ensure that the boys identified through newborn screening receive the established DMD standard of care to include initiation of corticosteroids, follow-up with appropriate specialists, including neuromuscular, pulmonology, orthopedics, and cardiology, as well as providing ancillary and supportive care services. A protocol will also need to be in place to follow patients with elevated CK who test negative for *DMD* gene deletion mutation testing, as well as to track all screened patients for potential false negative results. The challenges that exist for China's DMD-NBS program are not unique to China. In the United States a unified DMD registry to track patients, assist with access to care, and ensure adequate follow-up care according to documented guidelines remains in development.[28,29] China's large rural population, lack of specialty providers, and difficulty in educating patients regarding the benefits of treatment create additional challenges that will need to be addressed as DMD-NBS moves forward in China.

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Sazani P, and Narayana A revised the article critically for important intellectual content. Griggs R contributed to the conceptualization, acquisition and interpretation of data, drafting/revising the article critically for important intellectual content. All authors approved the final version to be published.

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