Newborn Screening and Molecular Profile of Congenital Hypothyroidism in a Chinese Population

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To review the characteristics of newborn screening of congenital hypothyroidism (CH), we reviewed the newborn screening data, including the levels of blood spot thyroid-stimulating hormone (TSH), and serum TSH and free thyroxine (FT4), of all newborn infants who accepted the newborn screening program during the last 14 years. In total, 437,342 newborn infants underwent CH screening and 192 infants were diagnosed with CH and the incidence of CH was 1:2278. The positive rate of the initial screening was 0.96%, and the positive predictive value was 4.8%. We also designed a target sequencing panel including 13 causative genes: DUOX2, TG, TPO, TSHR, TTF1, TTF2, PAX8, NKX2-5, GNAS, THRA, TSHB, IYD and SLC5A5, to identify the spectrum and prevalence of disease-causing gene mutations in Chinese CH patients. CH-causing genes were detected by targeted next-generation sequencing in 106 CH infants. A total of 132 mutations were identified in 69 cases (65.1%). Of these 132 mutations, 92 (69.70%), 28 (21.21%), and 12 (9.09%) were related to thyroid dyshormonogenesis, thyroid dysgenesis, and thyrotropin resistance, respectively. Mutations in CH-causing genes were found mainly in DUOX2, TG and TSHR, and DUOX2 is the most gene mutation in Chinese CH patients.

Keywords: newborn screening, congenital hypothyroidism, thyroid-stimulating hormone, molecular diagnosis, gene mutation

INTRODUCTION

Congenital hypothyroidism (CH), which is defined by inadequate thyroid hormone production in newborn infants, is the most common neonatal metabolic disorder worldwide, with an incidence of 1 in 2000–4000 live births (Rastogi and LaFranchi, 2010). Most neonates born with CH have a normal appearance and no detectable physical signs. In the past, we have often overlooked the harmfulness of hypothyroidism during the newborn period. Patients with the disease suffer from delayed diagnosis and treatment, and severe CH can lead to growth retardation and permanent intellectual disability. CH screening is an important component of the newborn screening (NBS) program, which is widely used as the third prevention intervention of birth defects (Keskinkılıç, 2014; Berry, 2015). Using the NBS program, we can detect symptomless children with CH early. Children can receive a definitive diagnosis, and the proper treatment can be applied in time to prevent further complications and sequelae. The NBS program was established in Changzhou city in 2004, and approximately 430,000 infants have since been tested.
Congenital hypothyroidism screening has been carried out all over the world for nearly 50 years, but the pathogenesis of CH remains unclear. An increasing amount of evidence suggests that genetic mutations are an important factor of CH (Nettore et al., 2013). At present, more than 600 genomic variations have been recorded in the ClinVar database. CH is divided into two main types: thyroid dysgenesis and thyroid dyshormonogenesis. According to previous reports, the cause of CH in approximately 80–85% of patients is thyroid dysgenesis (including agenesis, ectopy, and hypoplasia), which is related to gene mutations in thyroid-stimulating hormone receptor (TSHR), paired box gene 8 (PAX8), thyroid transcription factor 1 (TTF1/NKX2-1), thyroid transcription factor 2 (TTF2/FOXE1), and NK2 transcription factor related locus 5 (NKX2-5). Otherwise, 10–15% of cases are caused by thyroid dyshormonogenesis, which is associated with mutations in thyroid oxidase 2 (DUOX2), dual-oxidase maturation factor 2 (DUOX2A), thyroglobulin (TG), thyroid peroxidase (TPO), solute carrier family 5 member 5 (SLC5A5), solute carrier family 26 member 4 (SLC26A4) and iodotyrosine deiodinase (IYD) (Nettore et al., 2013; Cherella and Wassner, 2017). These genes play important roles in the growth and development of the thyroid gland. Genomic variations can prevent or destroy normal development of the gland or disturb the production of thyroid hormones. However, most previous studies have focused on Western populations. Few similar studies have been reported in a Chinese population and have focused on one or two pathogenicity genes (Fu et al., 2016b; Hu et al., 2016; Kizys et al., 2017). There are few reports on the mutation spectrum of CH-causing genes in the Chinese population.

In the present study, we retrospectively analyzed the clinical data of CH screening over the last 14 years and performed mutation screening of CH-causing genes in CH infants using next-generation sequencing (NGS). We hope to improve CH neonatal screening and better characterize the mutations of CH-causing genes in a Chinese population.

**MATERIALS AND METHODS**

**Patients and Design**

From January 2004 to December 2016, all newborn infants who accepted the NBS program in Changzhou Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University were recruited for this study. All subjects received CH screening via collection of dried blood spots (DBSs). In 2012, we began our search for CH-causing genes. One hundred and six non-consanguineous patients diagnosed with CH consented to undergoing the gene mutation test.

The study design and protocol were reviewed and approved by the ethics committee of Changzhou Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University.

**NBS Program**

The methods of screening, diagnosis, and treatment were carried out according to the “Subspecialty Group of Endocrinologic
damaging effects were selected. We also searched the selected mutations in other published studies to evaluate their potential pathogenicity.

**Statistical Analysis**

Data that were not normally distributed are expressed as medians (M), 25th percentiles (P25), and 75th percentiles (P75). All data were analyzed using EmpowerStats x64 software (Wu et al., 2015).

**RESULTS**

A total of 437,342 newborns, including 236,820 males and 200,522 females, underwent CH screening. In total, 3,931 infants had positive results, and their NTSH levels were in the range of 9.0–20.0 mIU/L. After being recalled, 3,768 infants underwent the second DBS test. Otherwise, 289 infants were considered as positive because their NTSH levels were >20.0 mIU/L. The positive rate of initial screening was 0.96% (4220/437342); 181 cases were missing in the first recall. The positive recall rate of initial screening was 95.7% (4039/4220). The NTSH levels of 99.04% of the normal infants was 2.39 mIU/L (P25–P75: 1.37–3.93). The NTSH level of the CH infants was 75.0 mIU/L (P25–P75: 75.00–75.00), and that of FT4 was 5.14 pmol/L (P25–P75: 2.83–8.39). In serum TSH level of CH infants was 46.10 mIU/L (P25–P75: 17.90–120.00). The NTSH level of the CH infants = 192 (100) 46.10 17.90–120.00.

**DISCUSSION**

The NBS program for CH is a major method used in preventive medicine. In this study, we retrospectively analyzed the clinical data from NBS over the last 14 years with the goal of improving CH neonatal screening. According to our results, the incidence...
### TABLE 2 | Top 10 of genes mutations in our study.

| Gene_symbol | CytoBand | Exon position | Nucleotide position | Amino acid position | Mutation types | Number | RS ID     |
|-------------|----------|---------------|---------------------|---------------------|----------------|--------|-----------|
| TG          | 8q24.22  | Exon45        | c.7847A > T         | p.N2616I            | Non-synonymous | 11     | rs10091530 |
| DUOX2       | 15q21.1  | Exon14        | c.1588A > T         | p.K530X             | Stopgain       | 6      | rs180671269|
| DUOX2       | 15q21.1  | Exon30        | c.4027C > T         | p.L1343F            | Non-synonymous | 5      | rs147945181|
| DUOX2       | 15q21.1  | Exon25        | c.3329G > A         | p.R1110Q            | Non-synonymous | 5      | rs368488511|
| DUOX2       | 15q21.1  | Exon28        | c.3632G > A         | p.R1211H            | Non-synonymous | 4      | rs141763307|
| TG          | 8q24.22  | Exon42        | c.7364G > A         | p.R2455H            | Non-synonymous | 4      | rs2272707  |
| DUOX2       | 15q21.1  | Exon4         | c.227C > T          | p.P76L              | Non-synonymous | 5      | rs767705906|
| DUOX2       | 15q21.1  | Exon26        | c.3478_3480del      | p.1160_1160del      | Non-frameshift | 3      | rs758318135|
| DUOX2       | 15q21.1  | Exon6         | c.605_621del        | p.Q202fs            | Frameshift     | 3      | rs769318570|
| TSHR        | 14q31.1  | Exon10        | c.1349G > A         | p.R450H             | Non-synonymous | 3      | rs189261858|

NCBI Reference Sequence: TG (NM_003235), DUOX2 (NM_014080), TSHR (NM_000369).

### TABLE 3 | Novel mutations in our study.

| Gene_symbol | CytoBand | Exon position | Nucleotide position | Amino acid position | Mutation types | Number |
|-------------|----------|---------------|---------------------|---------------------|----------------|--------|
| TG (NM_003235) | 8q24.22  | Exon34        | c.6185G > A         | p.W2062X            | Stopgain       | 2      |
|             | 8q24.22  | Exon8         | c.976C > T          | p.Q236X             | Stopgain       | 1      |
|             | 8q24.22  | Exon10        | c.1000delG          | p.Q334fs            | Frameshift     | 1      |
|             | 8q24.22  | Exon16        | c.3457A > T         | p.K1153X            | Stopgain       | 1      |
|             | 8q24.22  | Exon16        | c.3538C > T         | p.Q1180X            | Stopgain       | 1      |
|             | 8q24.22  | Exon18        | c.3994C > T         | p.Q132X             | Stopgain       | 1      |
|             | 8q24.22  | Exon25        | c.5020C > A         | p.P1674T            | Non-synonymous | 1      |
|             | 8q24.22  | Exon45        | c.7799G > A         | p.W2600X            | Stopgain       | 1      |
| DUOX2 (NM_014080) | 15q21.1  | Exon9         | c.1007_1009del      | p.336_337del        | Non-frameshift | 1      |
|             | 15q21.1  | Exon12        | c.1300_1320del      | p.434_440del        | Non-frameshift | 1      |
|             | 15q21.1  | Exon25        | c.332delC           | p.T1107fs           | Frameshift     | 1      |
|             | 15q21.1  | Exon29        | c.3721A > T         | p.I1241F            | Non-synonymous | 1      |
| TSHR (NM_000369) | 14q31.1  | Exon1         | c.152C > A          | p.P51Q              | Non-synonymous | 1      |
|             | 14q31.1  | Exon6         | c.501C > G          | p.I167M             | Non-synonymous | 1      |
|             | 14q31.1  | Exon9         | c.700T > C          | p.S234P             | Non-synonymous | 1      |
|             | 14q31.1  | Exon10        | c.1384T > C         | p.C462R             | Non-synonymous | 1      |
| TTF1 (NM_007344) | 9q34.13  | Exon2         | c.269Q > A          | p.R90K              | Non-synonymous | 2      |
|             | 9q34.13  | Exon2         | c.515A > G          | p.Q172R             | Non-synonymous | 1      |
|             | 9q34.13  | Exon4         | c.1598C > T         | p.A533V             | Non-synonymous | 1      |
| GNAS (NM_000516) | 20q13.32 | Exon4         | c.308T > C          | p.I103T             | Non-synonymous | 2      |
|             | 20q13.32 | Exon6         | c.478C > T          | p.R160C             | Non-synonymous | 1      |
|             | 20q13.32 | Exon12        | c.1018T > C         | p.F340L             | Non-synonymous | 1      |
| PAX8 (NM_013953) | 2q13     | Exon4         | c.275T > C          | p.I92T              | Non-synonymous | 1      |
|             | 2q13     | Exon6         | c.398G > A          | p.R133Q             | Non-synonymous | 1      |
| TPO (NM_175722) | 2p25.3   | Exon13        | c.2080T > C         | p.S694P             | Non-synonymous | 1      |
| NKX2-5 (NM_001166176) | 5q35.1   | Exon2         | c.416G > A          | p.S139N             | Non-synonymous | 1      |

of CH in Changzhou is 1:2278, which is the average level in China (Zhong et al., 2016). The National Centre for Clinical Laboratories reported an incidence of CH was 1:2281 based on the data of the 202 laboratories around China. Wassner and
Brown (2015) reported that the apparent incidence of CH has more than doubled in recent years ranging from 1:2800 to 1:1400. Meanwhile, countries, such as the United States (Mitchell et al., 2011), Canada (Deladédy et al., 2011), New Zealand (Heather et al., 2017), Scotland (Mansour et al., 2017), Brazil (Silvestrin et al., 2017), reported slight differences. In our study, there was no significant difference in the incidence of CH between males and females, consistent with the study by Zhao et al. (2016).

In the present study, we detected mutations of CH-causing genes in a Chinese population by targeted NGS and found that the abnormal rates of these related genes in Chinese CH patients was 65.1%. A total of 132 gene mutations were detected (69.70, 21.2, and 9.09% mutations were related to thyroid dyshormonogenesis, thyroid dysgenesis, and thyrotropin resistance, respectively). This result was quite different from previous reports. According to research in Western countries, the primary pathology of CH is thyroid dyshormonogenesis (Nettore et al., 2013; Cherella and Wassner, 2017). The pathogenic factors of CH in our Chinese population may differ from those in Western populations. It is very important to screen the pathogenic genes and pathogenic factors of CH in this region. In addition, the current study indicated that a considerable proportion of Chinese CH patients had mutations at multiple sites or in multiple genes. Multiple mutations may cause a more serious phenotype in CH patients. Recent studies have also revealed that a significant proportion of CH patients have multiple gene variations in more than one thyroid-specific gene (de Filippis et al., 2017). Moreover, heritable variations were found in more than half of our CH patients, as well as in the general population, albeit at a significantly lower prevalence. Together, these studies indicate that the pathogenesis of CH may be due to the sum effect of rare alleles (Persani et al., 2018). A previous study also indicated that patients with one or two DUOX2 pathogenic mutations developed subclinical or transient CH, whereas patients with three or more DUOX2 pathogenic mutations were associated with permanent CH (Matsuo et al., 2016). The coexistence of multiple pathogenic mutations may contribute to the severity of the hypothyroid condition, and mutations in multiple genes may lead to genotype-phenotype variability (Moreno et al., 2002; O’Neill et al., 2015; Zheng et al., 2016). Therefore, further studies are needed to enlarge the mutation spectrum of CH and to verify the functions of the associated mutations, which may provide more profound insight into the etiology of CH.

In the present study, DUOX2 was the most commonly mutated gene in Chinese CH infants. According to previous studies, mutations in DUOX2 are responsible for thyroid dyshormonogenesis (Moreno and Visser, 2007). Most patients with DUOX2 pathogenic mutations have an ectopic thyroid gland with an increased or normal size (Kizys et al., 2017). However, the mutational spectrum of the DUOX2 gene and the correlations between phenotype and genotype have not yet been fully established. The c.1588A > T mutation in DUOX2, which is responsible for thyroid dyshormonogenesis, was highly recurrent, with a prevalence of 1/40,000. The c.1588A > T mutation is population specific and has been reported mainly in Asian populations, including Chinese (Fu et al., 2015, 2016a; Tan et al., 2016), Japanese (Maruo et al., 2008, 2016), and Malaysian (Chow et al., 2017) populations. The c.4027C > T (Chen et al., 2018), c.3329G > A (Fu et al., 2015; Park et al., 2016), c.3632G > A (Chai et al., 2015), c.2335G > A (Jiang et al., 2016; Maruo et al., 2016), and c.2654G > A (Zheng et al., 2016) mutations are also predominant in Asians, mostly in the Chinese Han population. c.1883delA (Maruo et al., 2008, 2016; Park et al., 2016; Tan et al., 2016), c.3478_3480del (Narumi et al., 2011; Fu et al., 2016a; Park et al., 2016), and c.605_621del (Jin et al., 2014; Matsuo et al., 2016; Tan et al., 2016) show a scattered distribution in Asian populations, including China, Japan, and South Korea. Six other mutations, including c.2048G > T (Fu et al., 2016a), c.227C > T (Lv et al., 2011), c.2894C > T (Jiang et al., 2016), c.3391G > T (Wang et al., 2014; Fu et al., 2016a), c.2202G > A (Wang et al., 2014) and c.2104_2106del (Fu et al., 2016a), were reported only in China, and the missense mutation of c.4405G > A, which was previous reported in Korean (Park et al., 2016), was first identified among Chinese population in our study. c.1873C > T mutation was identified as a novel pathogenic mutation by the qCarrier test in a reproductive carrier-testing program (Abulí et al., 2016), but no direct evidence has shown that the mutation is related to CH. In addition, c.1265G > A, c.2413G > A, c.1717C > T, c.3721A > T, c.3321delC, c.1300_1320del, and c.1007_1009del mutations, which may be related to CH, were identified in our study for the first time.

CONCLUSION

The incidence of CH in Changzhou city is 1:2278. Some related quality control indicators indicate that the NBS program of CH in Changzhou is effective. Meanwhile, we preliminarily identified the pathogenic genes in infants with CH by targeted NGS. The rate of abnormal gene mutations was 65.1%, and most mutations were related to thyroid dyshormonogenesis, which differs from that observed in Western populations. A considerable proportion of the population had mutations at multiple sites, and DUOX2 was the most common gene mutation in Chinese CH infants.

AUTHOR CONTRIBUTIONS

BY, HW, and WL carried out the assays and participated in designing the study. HW and YW carried out clinical consultations. BY, YY, WL, and LJ carried out laboratory tests and performed the statistical analysis. ZC conceived the study, participated in its design and coordination, and helped draft the manuscript.

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Practical significance and molecular mechanisms of DUOX2 in the etiology of congenital hypothyroidism

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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