Research Article

Complicated Relationship between Genetic Mutations and Phenotypic Characteristics in Transient and Permanent Congenital Hypothyroidism: Analysis of Pooled Literature Data

Wei Long, Lingna Zhou, Ying Wang, Jiaxuan Liu, Huaiyan Wang, and Bin Yu

1Department of Medical Genetics, Changzhou Maternal and Child Health Care Hospital Affiliated to Nanjing Medical University, Changzhou 213003, China
2Department of Pediatrics, Changzhou Maternal and Child Health Care Hospital Affiliated to Nanjing Medical University, Changzhou 213003, China
3Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA 02115, USA

Correspondence should be addressed to Bin Yu; binyu@njmu.edu.cn

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Purpose. Mutations and phenotypic characteristics remain unclear in patients with congenital hypothyroidism (CH), and no study concerning whether the outcome of transient CH (TCH) or permanent CH (PCH) is determined by mutations has been reported. Methods. We searched the literature up to April 2019. Eligible studies and data extraction were performed. We estimated the relationship between mutations and phenotypic characteristics in pooled patients with CH. Results. Two hundred forty-one cases were pooled from 41 eligible studies. The thyroid morphology, classification of mutated genes, and types of mutations were different between 94 patients with TCH and 147 patients with PCH. Heterozygous missense mutations prevailed in PAX8, TSHR, FOXE1, and NKX2-5, and patients with these mutated genes had a higher risk of PCH (OR = 37.38, 95% CI 5.04–277.21, \( P < 0.001 \)). TCH and PCH have equal shares in patients with mutated DUOX2 or DUOXA2. Dual-site and multisite mutations were frequently detected in DUOX2. High phenotypic heterogeneity was observed in mutated DUOX2 even in the same mutations. However, there was no relationship found between mutations and transient or permanent outcome in patients with mutated DUOX2. Conclusion. Transient or permanent outcomes were influenced by the biological function of mutated genes instead of types of mutations among patients with CH. Patients whose mutations were related to thyroid dysgenesis (TD) were more likely to have PCH. The relationship between mutations and phenotypic characteristics is complicated, and phenotypic characteristics may be affected by mutations and other factors.

1. Introduction

Congenital hypothyroidism (CH), the condition of thyroid hormone deficiency present at birth, is the most common neonatal endocrine disorder with an incidence of 1:2,000 to 1:4,000 live births [1]. Based on the final outcome of thyroid hormone deficiency, CH can be classified into transient CH (TCH) and permanent CH (PCH) [2].

Genetic causes were reported in patients with CH [3, 4]. Thyroid dysgenesis (TD), one of the most common etiologies of CH, was reported to be linked with mutations in genes including 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) [4]. Thyroid dysshormonogenesis (TDH) was reported to be linked with mutations in the thyroid oxidase 2 (DUOX2), dual-oxidase maturation factor 2 (DUOXA2), thyroglobulin (TG), thyroid peroxidase (TPO), solute carrier family 5 member 5 (SLC5A5), solute carrier family 26 member 4 (SLC26A4), and iodotyrosine deiodinase (IYD) genes [5, 6]. It has been reported that TD or TDH can cause
permanent or transient CH in unclear proportions [1]. However, the relationship between the underlying genotype of TD or TDH and CH outcome remains unclear.

Predicting the outcome of CH for patients is necessary to expeditiously determine whether he or she needs lifelong levothyroxine medication. Some potential indicators, such as the dose of levothyroxine and thyroid hormones at diagnosis or treatment, were studied to predict TCH or PCH [7, 8]. It is well established that the genotype plays an important role in thyroid morphology and the regulation of thyroid hormone levels [4]. However, little is known about whether mutations are related to CH outcomes. Previous case reports or small sample size studies reported patients with various mutations, while it is difficult to assess the relationship between genotype and phenotype resulting from the small sample size [9]. In the present study, we pooled the reported cases of the literature and performed an analysis, to examine (1) the characteristics of gene mutations in TCH and PCH populations and (2) the relationship between mutations and the outcome of PCH or TCH.

2. Materials and Methods

2.1. Literature Search and Inclusion Criteria. A literature search was performed by MEDLINE (via PubMed) and Web of Science (including Web of Science Core Collection, Chinese Science Citation Database, KCI-Korean Journal Database, MEDLINE, Russian Science Citation Index, and SciELO Citation Index) up to April 2019. The combined terms “congenital hypothyroidism OR thyroid dyshormogenesisis OR thyroid dysgenesis OR goiter OR ectopic OR ectopia” AND “mutation OR mutant OR variation OR variant” AND “permanent OR transient” were searched. Studies that fulfilled the following criteria were included: (1) published original studies, or studies that combined case reports and reviews; (2) clear final outcomes (TCH or PCH) could be inferred; and (3) complete information on the mutation.

2.2. Data Extraction. Eligible studies were reviewed by two reviewers, and selected articles were mutually agreed upon by the authors. The following information of the patients was collected: patients’ country or region (if not mentioned, the author’s region was replaced), sex, blood-spot TSH at newborn screening (bs-TSH), TSH at diagnosis (dTSH), FT3 or FT4 at newborn screening and diagnosis (the thyroid hormones data were not necessary due to the various detection methods and reference ranges), thyroid morphology, mutation information (including gene, position change of nucleotide and amino acid, and monoallelic or biallelic mutation), and clinical outcome (TCH or PCH, based on the thyroid function test results of the authors’ reevaluation when patients were over 3 years old [10]).

Studies about gene polymorphisms or patients with aneuploidy, chromosomal structural abnormalities, or undefined copy number variation were not included. For cases studied in multiple publications at different ages, the latest information was included. Repetitive cases in different studies were identified according to the information, including the same authors’ name or institution, the same levels of thyroid hormones at newborn screening or diagnosis, and the same mutation. Details of the assessment of CH outcome, the detection method of mutation, and the pathogenicity of mutations in the studies can be found in the original papers.

2.3. Statistical Analysis. All statistical analyses were performed by R (version. 3.4.3, http://www.r-project.org). We performed chi-square tests to compare the distribution of demographic and clinical characteristics as well as genetic mutation types between patients with TCH and those with PCH. Univariate analysis was performed to estimate the relationship between mutation and the final outcomes of patients with CH. Two-sided P values of less than 0.05 were considered statistically significant.

3. Results

3.1. Literature Search and Case Inclusion. A total of 41 articles fulfilled the inclusion criteria, and 72 family cases and 176 single cases were identified after data extraction (Supplementary Table 1). In the process of data analyses, we identified seven repeat cases in different reports according to the same thyroid hormones and similar authors or authors’ institution. Finally, 241 patients with clear final outcomes were identified, including 94 patients with TCH and 147 patients with PCH, and details about included cases are listed in Supplementary Table 1. A flow chart of the article selection process is shown in Figure 1.

3.2. Characteristics of Gene Mutations in Patients with CH. According to the data of the included articles, we analyzed the clinical data and mutations. Patients with mutations of DUOX2, DUOXA2, TG, TPO, FOXE1, NKX2-5, PAX8, and TSHR were identified in our analysis, and DUOX2 was the most frequent mutation in both patients with TCH and those with PCH. Novel mutations were identified in 34 studies. In 20 of 34 studies, the pathogenicity of novel mutations was evaluated by in vitro functional characterization. Eleven other studies evaluated the pathogenicity of novel mutations according to the strategy that mutations were not detected in the healthy control subjects on the basis of in silico prediction analysis.

The TCH/PCH proportion of different gene mutations was distinct. Forty-one patients were identified with gene mutation-related TD, including 23 with PAX8, 17 with TSHR, one with FOXE1, and one with NKX2-5. One heterozygous PAX8 mutation patient was TCH, while all of the others were diagnosed with PCH. The result was complicated for genes related to TDH. In our analysis, five TG and 34 TPO mutation patients were identified, and all of them had PCH, while TCH and PCH had equal shares among the patients with mutations in DUOX2 or DUOXA2. In patients who harbored mutations of two different genes, four patients harboring heterozygous DUOX2 and TSHR were diagnosed with PCH, while two combinational DUOX2 and TPO...
mutation patients were diagnosed with TCH. In addition, one patient with compound heterozygous c.565T>C of DUOX2 and 43kB deletion (encompassing DUOX2, DUOXA1, and DUOXA2) was TCH. The distributions of genotypes in the TCH and PCH groups are shown in Figure 2.

3.3. Characteristics of the Patients with TCH and with PCH. We compared the characteristics between patients with TCH and those with PCH, and the results are shown in Table 1. The proportion of area, thyroid morphology, gene classification, and type of mutation were different between patients with TCH and those with PCH, while no difference was observed in sex, mutation state, or location. We also evaluated the relationship between the patient data and the outcome. Compared to normal thyroid morphology, the abnormal morphology of TD, including small volume, hypoplasia, and ectopy, seemed to be a risk predictor of PCH (OR = 21.08, 95% CI: 2.70–164.37, P < 0.004). Similar results were obtained in the gene classification, and patients with mutations associated with TD were more likely to have PCH than patients with mutations of TDH (OR = 37.38, 95% CI: 5.04–277.21, P < 0.001). In terms of the types of mutations, patients with single non-missense mutations, dual-site mutations, or multisite mutations were less likely to have PCH than patients with single missense mutations.

According to the pathogenic mechanism and the distinct TCH/PCH proportion of gene mutations, we further divided genes into three subgroups, DUOX2/DUOXA2, TG/TPO,
Among those patients, 73.47% were goiter and 66.1% were TCH.

### 4. Discussion

To the best of our knowledge, this is the first study concerning the relationship between mutations and phenotype in previously reported patients with CH. We compared the mutation data between patients with TCH and patients with PCH, and the proportion of genes and mutation types between TCH and PCH was different. The outcome of patients with CH at reevaluation was influenced by the mutated gene. Compared to patients whose mutated gene is related to TDH, patients with mutations related to TD have a higher risk of developing PCH.

Recent evidence points to the possibility of a genetic component in patients with TD [4]. Transcription factors encoded by PAX8, FOXE1, and NKX2-5 are necessary for thyroid embryogenesis and are also expressed in other tissues of the developing fetus. Mutations in these genes lead to syndromes combined with TD [11, 12]. It is easy to understand that most patients with CH with mutations in PAX8, FOXE1, and NKX2-5 present with PCH and thyroid morphology of dysgenesis in our study. Characteristics of heterozygote or single missense mutations were obvious among those patients. However, some unusual cases were identified. Three PCH cases with normal thyroid but one TCH case with decreased thyroid were reported in the same study, and the authors found that the thyroid gland ranged from aplastic and hypoplastic to normal in patients with the same PAX8 mutation (p.R31H) and reported the first patient with PAX8 mutation with an enlarged thyroid gland [13]. TSHR, which is located on the surface of thyroid follicular cells and mediates the effects of TSH, is important for the development and function of the thyroid gland [14]. Although TSHR gene mutations were reported to be one of the causes of TD [15] and patients with mutations in TSHR all presented with PCH in our study, thyroid morphology was variable. Our results showed that eight of twelve patients with PCH with mutations in TSHR have eutopic thyroid glands. Previous studies demonstrated that inactivating TSHR mutations result in mild or severe thyroid hypoplasia [16, 17], and the heterogeneity was presumably due to different residual functions of TSHR mutations.

We concluded that patients with mutations of TPO or TG were permanent in our included cases. Both monoallelic and biallelic mutations of TPO were classically characterized by total iodide organification defect [18, 19]. Unlike TPO or TG, equal shares of TCH or PCH were found in the patients with mutations in DUOX2 or DUOXA2 in our review, and dual-site and multisite mutations were frequently detected in DUOX2. Mutation in DUOX2, which is responsible for TDH, was the most frequent mutation in both TCH and PCH patients, especially among Asian populations. Our previous study also demonstrated that DUOX2 was the most common genetic defect in Chinese patients with CH [20]. Most patients with DUOX2 pathogenic mutations have a thyroid gland with increased or normal size [21], but Fu et al. [22] reported one female PCH case with decreased thyroid
size. In the original study [22], mutations of DUOX2 were screened by Sanger sequencing, while other genes were not screened. We presumed that a further study of gene test is necessary to interpret this unusual case.

The characteristics of the DUOX2 mutation and the phenotype-genotype correlations have not yet been fully understood. Our results showed that dual-site or multisite mutations were frequently reported in DUOX2 but rarely in other genes. We also found that variable phenotypes were caused by DUOX2 mutations, and there was no relationship between the mutation from DUOX2 and the outcome of patients with CH at reevaluation, which was controversial in previous studies. Previous studies demonstrated that biallelic DUOX2 mutations cause severe, permanent hypothyroidism, while monoallelic mutations result in milder, transient hypothyroidism [23, 24]. However, subsequent studies have reported biallelic mutations in DUOX2 in patients with TCH [25, 26], and monoallelic mutations in patients with PCH were also reported [27, 28]. We found that TCH and PCH have equal shares in patients with DUOX2 mutations, which demonstrated that monoallelic and biallelic DUOX2 mutations can both cause transient or permanent CH, while there was no correlation between the final outcome and the mutation type. Although a study showed that patients with one or two DUOX2 mutations turned out to have subclinical or transient CH and that patients with three or more DUOX2 mutations were mostly associated with permanent CH [29], another study showed that disease severity, neurodevelopment, and prognosis were not correlated with DUOX2 mutation types or numbers [30].

<table>
<thead>
<tr>
<th>Continent</th>
<th>TCH (n = 94)</th>
<th>PCH (n = 147)</th>
<th>Total (n = 241)</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
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<tbody>
<tr>
<td>Africa</td>
<td>0 (0.00%)</td>
<td>1 (0.68%)</td>
<td>1</td>
<td>&lt;0.001</td>
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<td>Asia</td>
<td>85 (90.43%)</td>
<td>90 (61.22%)</td>
<td>175</td>
<td>§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europea</td>
<td>5 (5.32%)</td>
<td>50 (34.01%)</td>
<td>55</td>
<td>§</td>
<td></td>
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<tr>
<td>South America</td>
<td>4 (4.26%)</td>
<td>6 (4.08%)</td>
<td>10</td>
<td>§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
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<td></td>
<td></td>
<td>0.958</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>48 (52.17%)</td>
<td>62 (52.54%)</td>
<td>110</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>44 (47.83%)</td>
<td>56 (47.46%)</td>
<td>100</td>
<td>0.99</td>
<td>0.57–1.70</td>
<td>0.958</td>
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<tr>
<td>Unknownb</td>
<td>2 29</td>
<td>31</td>
<td>NA</td>
<td></td>
<td></td>
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<td>Thyroid morphology</td>
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<td>Normal</td>
<td>30 (34.48%)</td>
<td>37 (28.46%)</td>
<td>67</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goiter/enlarged</td>
<td>56 (64.37%)</td>
<td>67 (51.54%)</td>
<td>123</td>
<td>0.97</td>
<td>0.53–1.76</td>
<td>0.921</td>
<td></td>
</tr>
<tr>
<td>Small volume/ectopy</td>
<td>1 (1.15%)</td>
<td>26 (20.00%)</td>
<td>27</td>
<td>21.08</td>
<td>2.70–164.37</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Unknownb</td>
<td>7 17</td>
<td>24</td>
<td>NA</td>
<td></td>
<td></td>
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<tr>
<td>Gene classificationc</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gene relating to TDH</td>
<td>93 (98.94%)</td>
<td>102 (69.39%)</td>
<td>195</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene relating to TD</td>
<td>1 (1.06%)</td>
<td>41 (27.89%)</td>
<td>42</td>
<td>37.38</td>
<td>5.04–277.21</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Gene relating to TDH and TD</td>
<td>0 (0.00%)</td>
<td>4 (2.72%)</td>
<td>4</td>
<td>§</td>
<td></td>
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<tr>
<td>Type of mutationd</td>
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<td></td>
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</tr>
<tr>
<td>Single missense mutation</td>
<td>18 (19.15%)</td>
<td>53 (36.05%)</td>
<td>71</td>
<td>Reference</td>
<td></td>
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<tr>
<td>Single non-missense mutation</td>
<td>34 (36.17%)</td>
<td>41 (27.89%)</td>
<td>75</td>
<td>0.41</td>
<td>0.20–0.83</td>
<td>0.013</td>
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<tr>
<td>Dual-site mutations</td>
<td>34 (36.17%)</td>
<td>47 (31.97%)</td>
<td>81</td>
<td>0.47</td>
<td>0.23–0.94</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>Multisite mutations</td>
<td>8 (8.51%)</td>
<td>6 (4.08%)</td>
<td>14</td>
<td>0.25</td>
<td>0.08–0.83</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>Mutation statee</td>
<td>0.419</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygous</td>
<td>44 (46.81%)</td>
<td>74 (50.34%)</td>
<td>118</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homozygous</td>
<td>8 (8.51%)</td>
<td>20 (13.61%)</td>
<td>28</td>
<td>1.49</td>
<td>0.60–3.66</td>
<td>0.388</td>
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<tr>
<td>Compound heterozygous</td>
<td>40 (42.55%)</td>
<td>49 (33.33%)</td>
<td>89</td>
<td>0.73</td>
<td>0.42–1.28</td>
<td>0.267</td>
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<td>Combinational heterozygous</td>
<td>2 (2.13%)</td>
<td>4 (2.72%)</td>
<td>6</td>
<td>1.19</td>
<td>0.21–6.76</td>
<td>0.845</td>
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<tr>
<td>Mutation location</td>
<td>0.143</td>
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<tr>
<td>Monoallelic</td>
<td>44 (46.81%)</td>
<td>83 (56.46%)</td>
<td>127</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biallelic</td>
<td>50 (53.19%)</td>
<td>64 (43.54%)</td>
<td>114</td>
<td>0.68</td>
<td>0.40–1.14</td>
<td>0.144</td>
<td></td>
</tr>
</tbody>
</table>

*aOne French-Canadian case with TCH was included in the Europe group. bCases with unknown sex or thyroid morphology were not included in the comparison of TCH vs. PCH. cGene classification was based on the function of the gene according to previous studies. The TDH genes included DUOX2, DUOXA2, TG, and TPO; the TD genes included FOXE1, NKX2-5, PAX8, and TSHR; and the TDH and TD genes included DUOX2 and TSHR. dSingle non-missense mutations, including single nonsense, frameshift, splice site, or in-frame mutations. One TCH case with a 43kbp deletion of chromosome 15 (encompassing DUOX2, DUOXA1, and DUOXA2) was included in the group of multisite mutations. eCompound heterozygous cases harbored at least two mutations of one gene. Combinational heterozygous cases harbored mutations of two different genes. fThe odds ratio refers to TCH vs. PCH. §The analysis failed because of the small sample size. TCH: transient congenital hypothyroidism; PCH: permanent congenital hypothyroidism; TDH: thyroid dyshormonogenesis; TD: thyroid dysgenesis; NA: not analyzed.
patients, implying that the phenotype might be different in individuals with DUOX2 mutations, even in those with the same mutation. DUOX2 mutations result in variable phenotypes ranging from mild to severe CH [31], which could be explained by the compensation effect. The coexistence of two DUOX isoforms (DUOX1 and DUOX2) and their corresponding maturation factors could certainly constitute an efficient redundant mechanism to maintain sufficient H2O2 supply for thyroid hormone synthesis [32]. In addition, phenotypic heterogeneity could be explained by other mechanisms such as unidentified mutations in non-coding regions or other genes, nutrition, environment, or other epigenetic factors [33–36].

The genetic diagnosis of CH could provide great benefits not only to current patients but also in genetic counseling for the next birth or subsequent generations. In addition, it is also helpful to understand the genetic etiology of CH. Our study indicated that the transient or permanent outcome of patients with CH was influenced by the type of genes instead of the form of mutations and patients whose mutations related to TD were more likely to have PCH. However, the limitations of the present study need to be noticed. Although 41 studies were included in this study, the number of CH patients included in the final data analysis was relatively small. This limitation might affect the accurate analysis of relationship between mutations and phenotypes. In addition, it is difficult to predict the outcome by the mutation in patients with the DUOX2 mutation because of the variable phenotypes resulting from the DUOX2 mutation. Further study is necessary to understand the complicated relationship between the genetic etiology and phenotypic characteristics of CH.

5. Conclusions

The outcome of transient or permanent was influenced by the functional type of mutated genes instead of the form of mutations among patients with CH. Patients whose mutations were related to TD were more likely to have PCH. The relationship between mutations and phenotypic characteristics was complicated, and there was no relationship between mutations and transient or permanent outcome in patients with mutated DUOX2.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

Acknowledgments

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Supplementary Materials

Supplementary Table 1: clinical data and mutation of included cases. Note: bTSH: blood-spot TSH at newborn screening; dTSH: TSH at diagnosis; PCH: permanent CH; TCH: transient CH; fsX: frameshift with an early stop codon.

Supplementary Table 2: univariate analysis for mutation data associated with the final outcome in patients with DUOX2 mutation. Supplementary Table 3: phenotypic heterogeneity in the patients with the same-site mutation of DUOX2 gene.

References


