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Analysis of clinical characteristics of thyroid phenotype in Pendred syndrome based on multiple databases

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Abstract. – OBJECTIVE: This study aimed to provide statistical data support for the development of thyroid phenotype-related follow-up and reference for follow-up duration and project selection by analyzing the clinical characteristics of thyroid phenotype in Pendred syndrome (PDS) based on multiple databases.

MATERIALS AND METHODS: PDS-related pathogenic or possibly/pathogenic mutations were searched by Deafness Variation Database (DVD), ClinVar, and PubMed databases, the mutation sites were counted and the characteristics and thyroid phenotypes were analyzed.

RESULTS: The median age of hearing phenotype onset in PDS cases reported in multiple databases was 1.0 (1.0, 2.0) years, the median age of thyroid phenotype onset was 14.5 (5.8, 21.0) years, and the median age that thyroid phenotype was more delayed than hearing phenotype was 10.0 (4.0, 17.0) years. There were significant differences in the distribution of onset time between the two phenotypes (Z=-4.560, $p<0.01$). In these patients, the positive rates of goiter, thyroid nodules, abnormal thyroid function, and perchlorate discharge test (PDT) were 78%, 78%, 69%, and 78%, respectively. Moreover, the number of thyroid phenotype-positive items in the genotype group with frameshift mutation was not significantly higher than that in the group without frameshift mutation (Z=-1.452, $p=0.147$).

CONCLUSIONS: The early missed diagnosis of PDS may be due to the late onset of thyroid phenotype and the non-100% positive rate of examination items. Therefore, multi-item follow-up of the thyroid gland into adulthood will benefit patients. At present, the relationship between genotype and phenotype is still unclear, and prognosis cannot be determined according to genotype.

Key Words:
Pendred syndrome, *SLC26A4*, Enlarged Vestibular Aqueduct, Goiter, Age of onset.

Introduction

Pendred syndrome (PDS, OMIM 274600) is an autosomal recessive genetic disorder, mainly characterized by sensorineural hearing loss and goiter[1]. The Enlarged Vestibular Aqueduct (EVA) combination of Modini deformities can be detected in the temporal bone CT of the cases[2,3]. The disease is mainly caused by mutations in the *SLC26A4* gene (OMIM 605646) encoding Pendrin protein[4,5]. Up to now, nearly 500 pathogenic mutations of this gene have been reported, of which 290 are associated with PDS and the rest are associated with Non-Syndromic Enlarged Vestibular Aqueduct (NSEVA).

The difference between PDS and NSEVA is only the presence or absence of a thyroid phenotype, and there has been controversy about whether PDS and NSEVA are two stages of the same disease[6,7]. Limited by the unclear genotype-phenotype relationship, only some scholars suggested routine thyroid examination for NSEVA patients with gene mutations[8], and there was a lack of specific statistical data as supporting evidence for thyroid-related follow-up. In this study, PDS cases caused by *SLC26A4* gene were retrieved from multiple databases, and the clinical characteristics of thyroid phenotype were statistically analyzed, to find the statistical basis for follow-up, and try to reveal a certain genotype-phenotype relationship.

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Materials and Methods

Retrieval of PDS Cases

Deafness Variation Database (DVD) database(https://deafnessvariationdatabase.org/) and ClinVar database (available at: https://www.ncbi.nlm.nih.gov/clinvar/) were used to retrieve the pathogenic/potentially pathogenic sites of *SLC26A4* gene in PDS phenotype, PubMed database (https://pubmed.ncbi.nlm.nih.gov/) was used to retrieve recently published (five years) pathogenic or new possible pathogenic sites of PDS as a supplement, and the last update retrieval time was March 24, 2022. After reprocessing, 292 mutation sites were obtained. The sources from the literature were read and included to simultaneously record the hearing phenotype and four items [goiter, thyroid nodule, thyroid function, perchlorate discharge test (PDT)] of thyroid phenotype with at least one outcome, and 110 PDS cases with *SLC26A4* biallelic mutation were analyzed for the clinical characteristics of thyroid phenotype (Figure 1).

Statistical Analysis

The paired Chi-square test was used to analyze the correlation between various thyroid phenotypes, and the correlation was calculated and judged according to the coefficient of contingency (r)[10]. Spearman's correlation was used to test the correlation between age and PDT release rate. Two independent sample rank sum test was used for comparison between the two groups of quantitative data containing rank data or not meeting the normality test. SPSS 26.0 (IBM Corp., Armonk, NY, USA) statistical software was used in this study, and a p-value lower than 0.05 was considered statistically significant.

Results

Genotypic Characteristics of Included PDS Cases

A total of 110 PDS cases with biallelic genotype and thyroid phenotype information were retrieved from 98 families from 13 countries, of

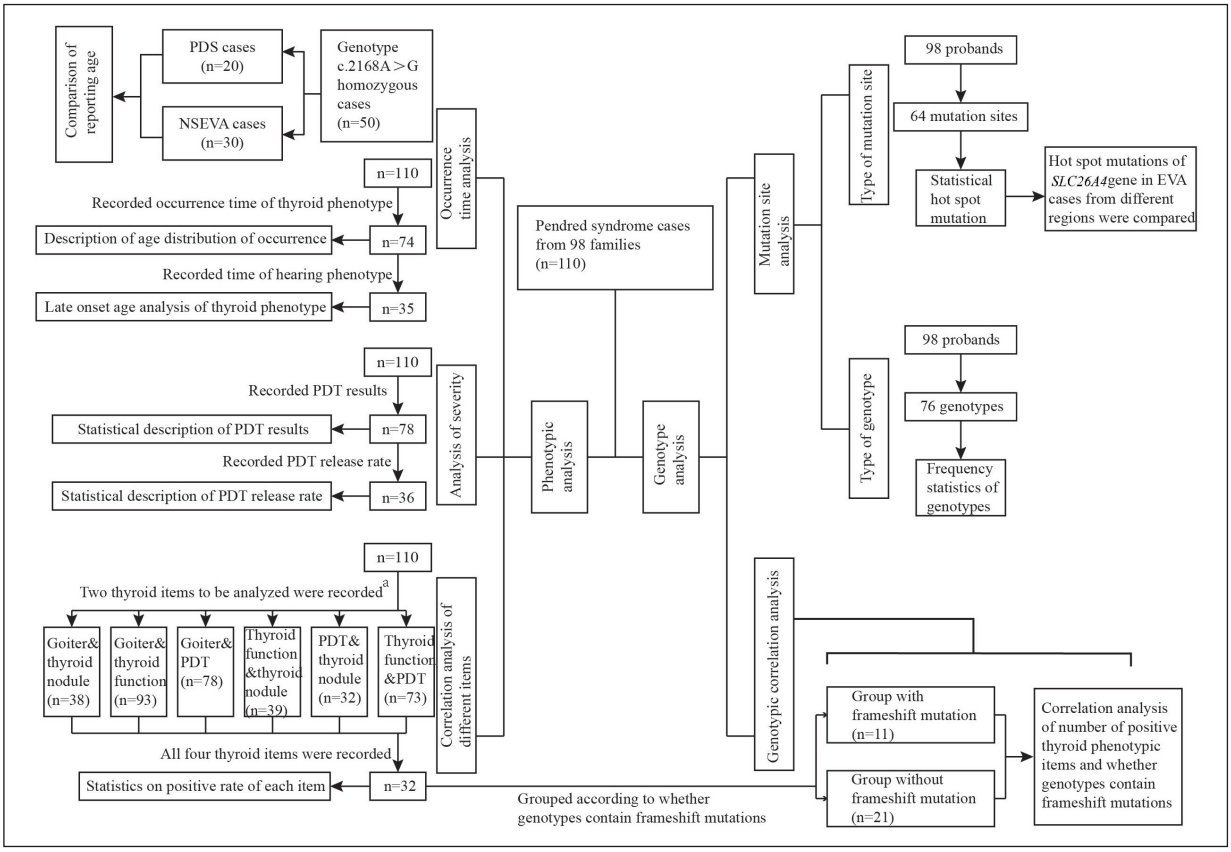

Figure 1. Retrieval of PDS cases and analysis methods. Thyroid phenotype items: goiter: goiter; thyroid nodule: thyroid nodule; thyroid function: thyroid function. PDT: Perchlorate discharge test, the release rate ≤10% was considered normal, >10% and ≤50% was considered mild iodine organization disorder, and >50% was considered severe iodine organization disorder[9].

which 85% (94/110) were European (non-Finnish), 13% (14/110) were East Asian, and 2% (2/110) were Latino/mixed American. Sixty-four mutation sites were involved, most of which were located in exon 10 (Figure 2). The most frequent mutation sites were c.2168A>G (21/196). Nine of the top 10 mutation sites had been reported as *SLC26A4* hotspot mutations in EVA cases in different regions. The c.2162C>T was found in EVA case screening in both East Asian and Caucasian races[11-14], but it was not a hotspot mutation. Seventy-six genotypes were involved, and the highest frequency was c.2168A>G/c.2168A>G (9/98).

Characteristics of Thyroid Phenotype in PDS Cases

The median age of hearing phenotype onset in PDS cases reported in multiple databases was 1.0 (1.0, 2.0) years, the median age of thyroid phenotype onset was 14.5 (5.8, 21.0) years (Figure 3a), and the median age of thyroid phenotype was more delayed than hearing phenotype was 10.0 (4.0, 17.0) years. Among c.2168A >G homozygous cases, the reported age of NSEVA cases was significantly lower than that of PDS cases (Z=-4.560, p<0.01) (Figure 3b).

The positive rates of each examination item of thyroid phenotypes were similar, but individual differences were noticeable. The positive rate of

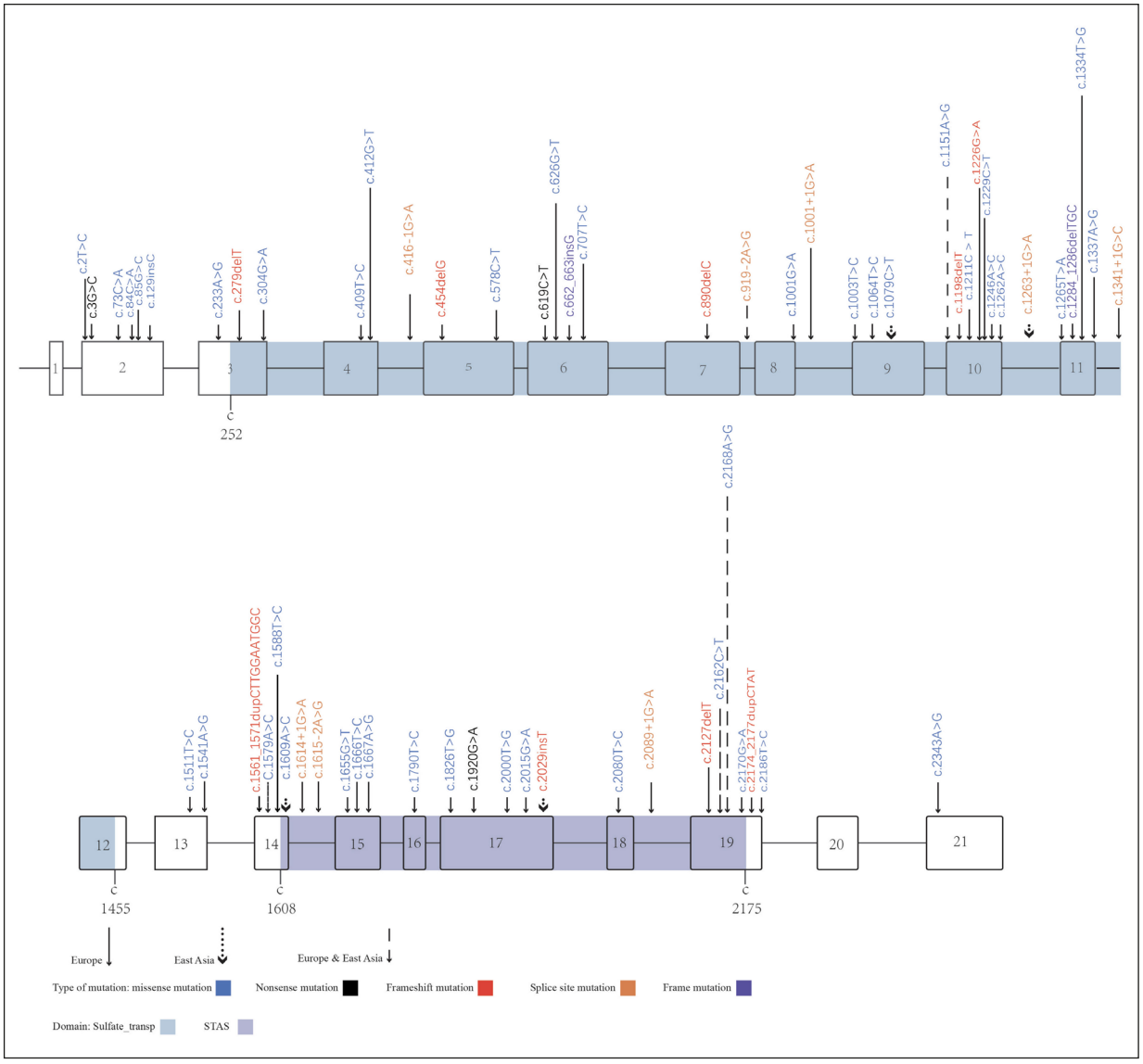

Figure 2. PDS-related *SLC26A4* mutation sites. The length of the arrows represents the frequency of mutations in the enrolled probands.

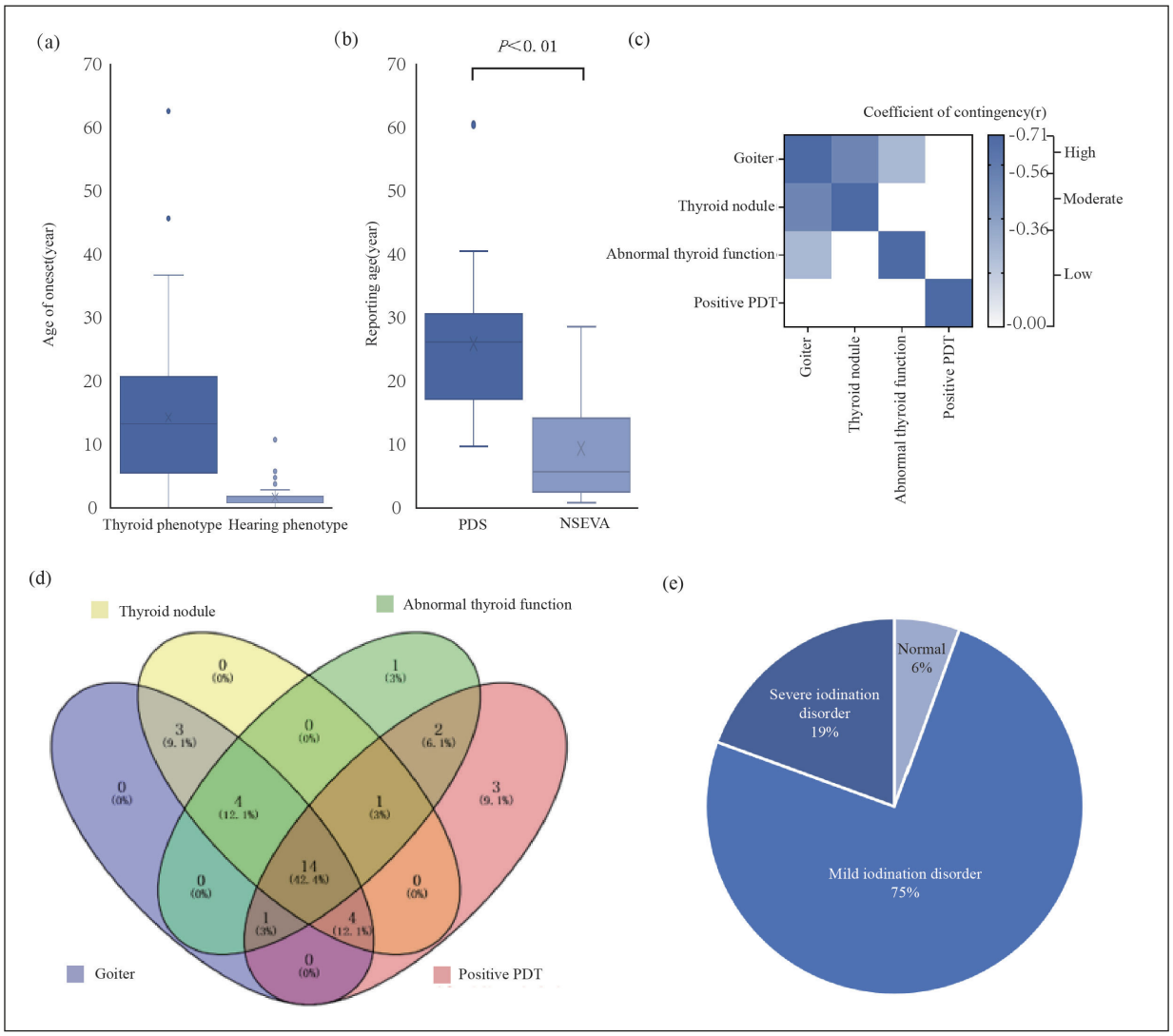

Figure 3. Characteristics of thyroid phenotype in PDS cases. **a**, Onset age of thyroid phenotype and hearing phenotype; (**b**) reported age of genotype NSEVA cases and PDS cases with c.2168A > G homozygous; (**c**) Correlation of thyroid phenotype items; (**d**) Positive results of thyroid phenotype items; (**e**) Distribution of severity of PDT.

abnormal thyroid function was 69% (22/32), and the positive rates of thyroid nodules, goiter, and PDT were 78% (25/32). All four items were positive in 44% (14/32) cases, and only some items were positive in the remaining cases (Figure 3d). The degree of iodination disorder was mostly mild (Figure 3e). There was no linear correlation between PDT release rate and age at examination (r=-0.19, p=0.34). There was a moderate correlation between goiter and thyroid nodules (r=0.54, p<0.01), a low correlation between goiter and thyroid dysfunction (r=0.278, p<0.01), and no correlation between other thyroid phenotype items (p>0.05) (Figure 3c).

Relationship Between Genotype and Number of Thyroid Phenotypes in PDS Cases

The number of positive thyroid phenotypes in the genotype group with frameshift mutation was 2-4, and that in the genotype group without frameshift mutation was 1-4 (Figure 4). There was no significant difference in the number of positives between the two groups (Z=-1.452, p=0.147).

The inner loop was genotyped: not containing non-frameshift mutation (NFSM), containing frameshift mutations (FSM). The outer ring was the proportion of cases with positive thyroid phenotype in each group, positive phenotype (PP).

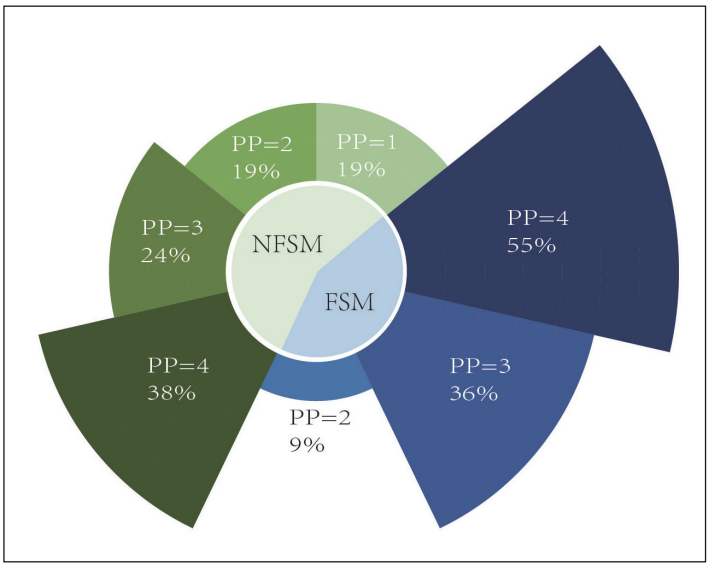

Figure 4. Positive numbers of thyroid phenotypes for PDS cases in the genotype groups with frameshift mutation and without frameshift mutation.

Discussion

There are some cases of missed diagnosis of PDS in clinical practice. Some cases only showed NSEVA in childhood, and PDS was diagnosed with a thyroid phenotype over time. Some studies[15,16] have found that thyroid phenotype appeared in long-term follow-up of NSEVA cases. In recent years, there have also been cases of poor surgical treatment of goiter, and PDS was diagnosed only after retrospective history[17]. In the literature, the age of NSEVA was significantly lower than that of PDS in cases with homozygous mutation of c.2168A>G genotype, suggesting that the thyroid phenotype had not yet appeared in the cases due to their young age, and early diagnosis of NSEVA could not be excluded as a potential PDS case. Therefore, as the relevant statistical evidence is scarce, it is necessary to carry out long-term thyroid-related follow-ups for such cases.

To further confirm the difficulties in early diagnosis of PDS and to find the basis for thyroid follow-up, the characteristics of thyroid phenotype in PDS were analyzed. It was found that the thyroid phenotype of PDS cases mainly appeared in adolescence and was significantly later than the hearing phenotype, which was consistent with previous findings[18,19]. However, the average first diagnosis time of EVA cases was 17 months old[20], so there was the possibility of missing PDS diagnosis due to the absence of thyroid phenotype in the early stage. On the other hand, individual differences in thyroid phenotype also made the diagnosis difficult. We found that the number and degree of thyroid phenotype positivity in PDS cases were different, only 44% of cases were positive for all four thyroid phenotypes, and the degree of iodination disorder was mild to moderate. Previous studies[21] also found that differences in thyroid phenotype still existed even among the same genotype cases from the same family. In addition, the lack of sensitivity of the examination is also the reason for the difficulty of diagnosis. Although some studies[22-24] have mentioned PDT as the gold standard of PDS[22], not all cases are positive[23,24]. Our study found that the positive rate of PDT was only 78%, it was not correlated with the occurrence of other positive items of thyroid phenotype, and PDT results alone may make a part of the cases a missed diagnosis.

Genetic testing plays an increasingly important role in the early diagnosis and prognosis of PDS. Unfortunately, the genotype-phenotype relationship of PDS is still unclear and prognostic inference is difficult. In terms of predicting the occurrence of thyroid phenotype, it was found that hot spot mutations of PDS and EVA in *SLC26A4* gene were essentially coincident and failed to find mutations that were only highly related to PDS. In terms of predicting the severity of thyroid phenotype, the number of positive thyroid phenotypes in the genotype group with frameshift mutation was higher than that in the group without frameshift mutation, but the difference was not statistically significant, which may be related to the small sample size included. Sugiura et al[25] summarized the clinical characteristics of 13 PDS cases carrying c.2168A>G and proposed that the thyroid pheno-

type of the cases carrying this mutation was mild and generally did not develop goiter. However, other studies[26-28] showed that all the cases carrying this mutation developed goiter, revealing the complexity of the genotype-phenotype relationship. Therefore, it is not possible to predict the occurrence of thyroid phenotype by using genetic testing technology, and it is not possible to make an early diagnosis or exclude the diagnosis of PDS in NSEVA cases according to the genotype. Therefore, it is necessary to carry out long-term thyroid-related follow-ups until adulthood, and the examination items should cover thyroid ultrasound, thyroid function, and PDT as much as possible to avoid delay in diagnosis and treatment.

Conclusions

The clinical characteristics of thyroid phenotype in PDS cases based on multiple databases were analyzed in this study, suggesting that the thyroid phenotype of PDS occurred late and early diagnosis was difficult, which provided a basis for the duration of thyroid phenotype follow-up and the selection of items. Genetic testing plays an increasingly prominent role in the diagnosis and treatment of PDS. However, large samples and basic experimental studies on the relationship between genotype and phenotype are still to be carried out. Adult cases should be included as much as possible in the study to avoid incomplete case phenotypes recorded because the thyroid phenotype has not yet occurred.

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Conflict of Interest
The authors declare that they have no conflicts of interest regarding the publication of this study.

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Availability of Data and Materials
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval
Not applicable.

Authors' Contributions
C.-C. Wang and Z.-G. Jin: designed research. Y.-L. Li and F.-Y. Gong: made contributions to the acquisition of data, analyzed the data, and drafted the article. Z.-Y. Dang, W. Xiong, M.-D. Zhang, and Y.-X. Wang searched for relevant articles. All authors read and approved the final manuscript.

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