DUOX defects: Genotype-phenotype correlations

Déficit en dual oxydase (DUOX) : corrélation génotype-phénotype

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Résumé

L’hypothyroïdie congénitale (HC) est la plus fréquente des désordres endocriniens congénitaux observée annuellement chez plus de 1/1500 nouveau-nés. L’HC peut être rapportée à des altérations à la fois de la formation et de la migration de la glande (dysgénésie) ou de la synthèse hormonale (dyshormonogenèse). La pathogénie de l’HC par dysgénésie est encore largement inconnue. Par contraste plusieurs mutations ont été découvertes au niveau des différents gènes impliqués dans la dyshormonogenèse, la pendrine, la thyroperoxydase (TPO), la thyroglobuline. Récemment ont été identifiés de nouveaux gènes impliqués dans l’étiologie de la dyshormonogenèse : dual oxydase 2 (DUOX 2) et le facteur 2 de maturation de la dual oxydase (DUOXA2). Ce sont les principaux facteurs contribuant à la génération du peroxyde d’hydrogène, nécessaire à la fonction de la thyroperoxydase. Des mutations de ce gène ont été associées à l’HC transitoire ou permanente, avec un haut degré de variabilité intra et interfamiliale. On a fondé plusieurs hypothèses rendant compte de la variabilité du phénotype DUOX2/A2. Parmi celles-ci, l’existence d’autres systèmes de génération d’H2O2, les différences de besoin en hormones thyroïdiennes en fonction de l’âge, de l’origine ethnique, de l’apport en iode. Dans cet article sont présentées les données génétiques et cliniques de l’HC liée aux altérations du système générateur de l’ion peroxyde.

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Mots clés : DUOX ; DUOX A ; Hypothyroïdie congénitale ; Dyshormonogenèse

Abstract

Congenital hypothyroidism (CH) is the most common congenital endocrine disorder, accounting for up to 1:1500 newborns per year. CH can be related to defects in either formation and migration of the thyroid gland (dysgenesis) or thyroid hormone synthesis. The pathogenesis of dyssgenic CH is still largely unknown. On the contrary, several mutations have been found in different genes involved in thyroid dyshormonogenesis (such as pendrin, thyroperoxiase-TPO, thyroglobulin). Recently, new genes involved in the etiology of dyshormonogenesis have been identified: dual oxidase 2 (DUOX2) and dual oxidase maturation factor 2 (DUOXA2). They are the principal elements generating the hydrogen peroxide needed for TPO function. Mutations in these genes have been associated to transient or permanent CH, with a high intra and interfamilial phenotypic variability. Some hypotheses have been drawn to explain the variability of the DUOX2/A2 phenotype. Among them, the existence of other H2O2 generating systems, the different requirements for thyroid hormones according to age, the ethnicity, the intake of iodine. In the present paper, the genetic and clinical features of CH caused by defects in the peroxide generator system will be revised.

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Keywords: DUOX; DUOX A; Congenital hypothyroidism; Dyshormonogenesis

1. Introduction

Congenital hypothyroidism (CH) is a frequent inherited disease, potentially leading to mental retardation if not promptly recognised and treated. In the last years, CH incidence has progressively increased in Lombardy currently reported as 1:1500...
newborn per year [1]. It can be related to defects in the formation of the thyroid gland (agenesis), to defects in the migration (ectopy) or to an altered development of the thyroid. The pathogenesis of thyroid dysgenesis is still largely unknown and monogenic mutations have been identified only in few cases. Indeed, recent hypotheses strongly indicate the possibility that several dysgenetic cases could recognize a multigenic origin [2]. Nevertheless, the majority of CH cases are associated with a gland in situ of normal or increased volume. In these cases, the hypothyroidism is due to defects in the hormonogenic process which leads from iodine, uptaken from the blood, to thyroid hormones, T3 and T4, released into the circulation. Most of these processes occur at the apical membrane level where the proteins devoted to hormogenesis are located. Defects in these genes have been identified, leading to partial dyshormonogenic defects (PIOD), such as mutations in the pendrin gene [3] or to total organification defects, such as mutations in the TPO gene [4]. This review will focus on a recently discovered “actor” in the hormonogenic process.

2. DUOXs

Although it was well known since 1970 that the availability of hydrogen peroxide was a limiting factor in protein iodination and thyroxine formation, only 30 years after two highly homologous genes, DUOX1 and DUOX2, were cloned [5,6]. Duox1 and Duox2 contain two Ca2+-binding motifs (EF-hand), which could be involved in the direct activation of the H2O2 generator by calcium. Thyroid NADPH oxidase requires the presence of micromolar concentrations of calcium to acquire a functional conformation and to generate H2O2. As expected for this proteins, both DUOXs genes are expressed at the apical membrane of the thyroid cells [7]. They display a lower expression at the airway and tongue epithelia, cerebellum and testis (Duox 1), and at the salivary and rectal glands, gastrointestinal and airway epithelia, uterus, gallbladder and pancreatic islets (Duox2) [8–11].

At the thyroid level, Duox1 and 2 are differently expressed either in normal or in pathological tissues. Indeed, Duox2 is more expressed with respect to Duox1, and it is also more efficient in the production of peroxide [7,12]. Interestingly, the first experiments done to test the activity of Duox2 mutated proteins showed that no peroxide production could be detected in transfected cells lines [12,13]. Following studies demonstrated that transfected cells express only the immature form of the protein. Indeed, in the thyroid, Duox1/Duox2 show two N-glycosylated states: the fully glycosylated mature form (190 kDa) expressed at the plasma membrane, and the high mannose glycosylated immature form (180 kDa) expressed exclusively inside the cell in the endoplasmic reticulum [13]. These data, indicating that only adequately matured and glycosylated Duoxs can generate H2O2 in vitro, suggested the existence of Duox maturation factors.

3. DUOXAs

These maturation factors have been identified in 2007 [14]. They have been called DUOXA (DUOXA1 and DUOXA2) and they also are transmembrane proteins. In vitro experiments demonstrated that DuoxA are required for Duox targeting to the membrane and function in cells. Duox maturation factors form functional heterodimeric complexes with Duox1 and Duox2 (Duox1-DuoxA1 and Duox2-DuoxA2). Complex formation is essential for the translocation of the dimer to particular subcellular locations (from ER to the Golgi and to the membrane) and constitutes the initial form of Duox regulation [15,16]. After the discovery of these complexes, the co-transfection of DUOX2 and DUOXA2 allowed functional studies of DUOX2 mutations in cellular systems, showing that mutated proteins did not target to the membrane, leading to an impairment in peroxide production [17]. Thus, as for DUOX2, the maturation factors were immediately identified as good candidates for CH.

4. DUOX2: genotype-phenotype correlations

To date, around 20 different DUOX2 mutations have been described associated to CH and in one case to a multinodular goiter [18]. The majority of them are located in the extracellular portion of the molecule, devoted to the binding to TPO, or in the first intracellular loop. These variants have been found in a total of 17 patients, owning to seven different series [19–25]. Due to the paucity of patients, clinical data are scarce and the precise phenotype associated with DUOX2 mutations is far to be fully elucidated. Nevertheless, derived from the data available, a patient harbouging DUOX2 mutations is predicted to have at birth a mild-severe CH and a thyroid gland of normal or enlarged volume. At re-evaluation some DUOX2 patients will recover a normal thyroid function (transient CH), whereas other will present a permanent but mild hypothyroidism with a normal or reduced thyroid volume. Almost all patients will have a partial iodide organification defect (PIOD). Unfortunately, the genetic and clinical data available show the lack of genotype-phenotype correlations. In particular, there are no correlations between the class of mutation (truncated, mis-sense, monoallelic, biallelic) and the phenotype in terms either of TSH levels or of discharge rate or of CH duration (i.e. transient or permanent). Indeed, monoallelic mutations can lead to permanent or transient hypothyroidism and there are no differences in the percentage of discharge after perchlorate between patients with mono or biallelic mutations or between transient and permanent cases (Table 1). Moreover, even considering only biallelic mutations, an extreme variability is observed, being the CH transient or permanent and the discharge rate highly variable. In particular, two cases have been described with biallelic stop codon mutations (R434X/R434X and fsX481/fsX638) associated in one with permanent CH [19] and in the other patient to a transient CH [23]. These findings are not consistent with the animal model in which the biallelic mutations invariably lead to a severe hypothyroidism [26], whereas monoallelic mutations are associated with a normal phenotype.

The variability of DUOX2 phenotype is not only interfamilial, but also intrafamilial. Indeed, family members have been described with the same monoallelic mutation but a different phenotype, ranging from euthyroidism with normal discharge rates to CH with radioiodine discharge after perchlorate greater than 50% [19,20,22].
Clinical features of patients with congenital hypothyroidism reported in the literature harbouring mono or biallelic DUOX2 mutations.

<table>
<thead>
<tr>
<th>Monoallelic mutation</th>
<th>CH</th>
<th>Discharge</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Q686X</td>
<td>Transient</td>
<td>66</td>
<td>[19]</td>
</tr>
<tr>
<td>R701X</td>
<td>Transient</td>
<td>41</td>
<td>[19]</td>
</tr>
<tr>
<td>fsX994</td>
<td>Transient</td>
<td>40</td>
<td>[19]</td>
</tr>
<tr>
<td>fsX300</td>
<td>–</td>
<td>–</td>
<td>[22]</td>
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<tr>
<th>Biallelic mutation</th>
<th>CH</th>
<th>Discharge</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>R434X/R434X</td>
<td>Permanent</td>
<td>100</td>
<td>[1]</td>
</tr>
<tr>
<td>R842X/R376W</td>
<td>Permanent</td>
<td>12–28</td>
<td>[20]</td>
</tr>
<tr>
<td>fsX482/splice site</td>
<td>Permanent</td>
<td>60–68</td>
<td>[21]</td>
</tr>
<tr>
<td>fsX994/Q36H</td>
<td>Permanent</td>
<td>46</td>
<td>[21]</td>
</tr>
<tr>
<td>D506N/fsX300</td>
<td>Permanent</td>
<td>–</td>
<td>[22]</td>
</tr>
<tr>
<td>H678R/L1067S</td>
<td>Transient</td>
<td>–</td>
<td>[23]</td>
</tr>
<tr>
<td>K530X/E879K/L1067S</td>
<td>Transient</td>
<td>–</td>
<td>[23]</td>
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<tr>
<td>A649E/R885Q</td>
<td>Transient</td>
<td>–</td>
<td>[23]</td>
</tr>
<tr>
<td>fsX481/fsX638</td>
<td>Transient</td>
<td>–</td>
<td>[23]</td>
</tr>
<tr>
<td>S911L/1052Y</td>
<td>Permanent</td>
<td>13</td>
<td>[24]</td>
</tr>
<tr>
<td>G1518S/del exons 26–33</td>
<td>Transient</td>
<td>–</td>
<td>[25]</td>
</tr>
</tbody>
</table>

5. DUOX2A: genotype-phenotype correlations

The same extreme phenotypic variability seen for DUOX2 has been found also for DUOX2A, even if, to date, only one patient has been published [27] and two additional cases have been reported in a congress communication (Ventura et al., abstract book 10th European Congress of Endocrinology, 2008). In the Chinese patient reported by our group biallelic DUOX2A mutations were associated with a permanent CH with PIOD. Parents and sisters of the proband were all heterozygous carriers of the mutation and had normal thyroid function parameters, including normal neonatal screening results in the proband’s two siblings. Functional studies demonstrated the lack of expression of the mutant at the membrane level and a total defect in peroxide production [27]. Conversely, the other 2 patients, who were briefly described in 2008 had a monoallelic mutation associated with a permanent CH, and a partial reduction in peroxide production.

In summary, the DUOX2/DUOX2A phenotype is characterized by a high intra- and interfamilial variability. According to the data published to date monoallelic DUOX2 mutations seem to be associated with a transient CH, whereas biallelic variants can lead to transient or permanent CH. The perchlorate discharge test reveal in almost all cases a PIOD, being a TIOD described only in one patient with a biallelic stop codon mutation [19]. Biallelic and, possibly, also monoallelic DUOX2A mutations can cause permanent CH with PIOD.

6. The lack of genotype-phenotype correlations: which hypotheses to explain it?

Some hypotheses have been drawn to explain the inter and intrafamilial variability of the DUOX2/A2 phenotype. Among them, the existence of other H2O2 generating systems, the age variability in thyroid hormones requirements, the ethnicity, the intake of iodine (Fig. 1).

6.1. Redundant systems to compensate for DUOX2A deficiency

Recent evidences demonstrated, in cellular systems, that the complex DuoX1/DuoX1 can at least partially replace the function of DuoX2/DuoX2A [28]. Moreover, the possibility to use other peroxide generators emerges from recent mismatch experiments demonstrating the existence of some isoforms of DuoX1, namely DuoX1-1, 1-2 and 1-3. Among these isoforms, the shortest (DuoX1-1) does not reach the membrane and supports DuoX1-dependent H2O2 production only minimally, whereas DuoX1-2 is able to fully target DuoX1 to the cell surface and is the more efficient in the production of peroxide. Finally, the DuoX1-DuoX1-3 complex is moderately active and targets both to the ER and to the membrane [15]. Interestingly, these studies demonstrated that DuoX2 could compensate for DuoX2A inactivation by utilizing DuoX1. Indeed, DuoX1-2 is able to target to the membrane either DuoX1 or DuoX2 and mispaired DuoX2/DuoX1-2 heterodimers are functional, being similar the levels of H2O2 produced by the DuoX2-DuoX1-2 combination and the DuoX2-DuoX2A pair (Fig. 1, panel A). Consistent results have been obtained by another group, confirming that DuoX2 can be functionally rescued by two different DuoX1 variants. In particular, they identified two alternative splicing events of DUOX1 mRNA resulting in four different transcripts, and demonstrated that the α and γ isoforms, that contain the third coding exon, are able to target DuoX2 to the membrane [16].

6.2. Redundant systems to compensate for DUOX2 deficiency

When thyroid hormone levels decrease, TSH concentration increase and triggers, through its G protein-coupled receptor, the intracellular increase of cAMP, diacylglycerol, and Ca2+ concentrations. The latter constitute the primary activator of the dual oxidase function, which is sustained by additional phosphorylations mediated by PKA for DuoX1 and PKC for DuoX2. Thus, it has been proposed that DuoX1 would represent the emergency program in the case of hypothyroidism with PIOD due to mutated and inactive DuoX2 [28]. Nevertheless, based on the fact that DuoX2 transcript is two to five times more abundant than DuoX1, it is likely that DuoX1 cannot fully take over the impairment of DuoX2.

6.3. The age factor

The phenotypic variability of DUOX2 mutated patients includes the transitoriness of CH reported for several cases. It
Fig. 1. Hypotheses formulated to explain the inter- and intrafamilial variability of the DUOX2/A2 phenotype. A. In cellular systems DuoxA1-2 isoform is able to target to the membrane Duox2 and mispaired Duox2/DuoxA1-2 heterodimers are functional [15]. B. The age variability in thyroid hormones requirement varies in the different phases of life. A not fully functional system could not be able to maintain euthyroidism in the first phases of life, but it could be sufficient in the adult life. C. The percentage of DUOX2 mutations carriers with transient congenital hypothyroidism (CH) is variable in different world regions. D. A high iodine intake at birth allows to overcome the DUOX2 impaired function [20].

has been hypothesized that this could be related to the variable requirements for thyroid hormones during life. It is well known that, at birth, the amount of thyroid hormones needed is very high and progressively declines. It is thus possible to speculate that a system not fully functional could not be able to maintain euthyroidism in the first phases of life, but it could be sufficient in the adult life. More insights on this topic will come from the study of the thyroid function in DUOX2 patients with transient CH during those phases of life, such as puberty or pregnancy, when the thyroid hormone requirements increase (Fig. 1, panel B).

6.4. The ethnicity and the iodide intake

The revision of the reported patients indicates that CH due to DUOX2 mutations is transient in most cases in Japan (100% of cases; 22), in the Netherlands (75%; 19) and in Canada (100%; 25), whereas in Argentina [21] and in Italy [20,24] CH is permanent in all the cases published to date (Fig. 1, panel C). This seems to correlate directly with the hypothesis that iodine could be a modifier of the phenotype, since the iodide supply is higher in North America, North Europe and Japan than in other Countries (International Council for the Control of Iodine Deficiency Disorders; http://www.iccidd.org). In this context, we hypothesized that the discrepant phenotype observed in two brothers, harbouring the same biallelic mutations, was tightly related to an overload of iodine that allowed to overcome the hypothyroidism at birth in one of them [20]. Indeed, the child with elevated urinary iodine, due to maternal contamination, developed hypothyroidism at 40 days, whereas the brother with normal urinary iodine was positive at the neonatal screening (Fig. 1, panel D).

7. Conclusions

The peroxide generator system has been described in recent years. In particular, DUOX2 represents the main source of H2O2 for TPO and DUOX2A2 is required for adequate Duox2 targeting and function. Around 20 different DUOX2 mutations have been described distributed all along the gene, while only three allelic variants have been reported to date for DUOX2. The clinical data available allow to speculate that monoallelic DUOX2 mutations are associated with transient CH, whereas biallelic DUOX2 mutations can lead either to transient or to permanent CH with PIOD (rarely TIOD). On the other hand, biallelic, and possibly also monoallelic, DUOX2A2 mutations cause permanent CH with PIOD. Mutations in the peroxide generator system genes are associated with a highly variable intra and inter familial phe-
notype, consistent with the existence of genetic/environmental modulators. Nevertheless, since preliminary data suggest that defects in this system could be extremely more frequent than suspected in patients with mild permanent/transient CH, future precise descriptions of novel cases will certainly give more insights into the knowledge of the genotype/phenotype correlations.

Disclosure of interest

The authors have not supplied their declaration of conflict of interest.

References


