

Sublingual thyroid ectopy: similarities and differences with Kallmann syndrome

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Abstract

Permanent primary congenital hypothyroidism (CH), the commonest cause of preventable intellectual disability, is due to defects in the embryonic development of the thyroid in the vast majority of cases. These defects are collectively called thyroid dysgenesis. The thyroid may be absent (athyreosis) but, more commonly, a sublingual thyroid ectopy without lateral lobes, is the only thyroid tissue present. Such an ectopy presumably results from an arrest in the downward migration of the median anlage. Thyroid ectopy almost always occurs in a sporadic fashion. However, first-degree relatives are affected more often than chance alone would predict. On the other hand, almost all reported monozygotic twin pairs are discordant for thyroid ectopy. Current research is aimed at reconciling these contradictory epidemiological data. We propose a two-hit mechanism associating a germline predisposing factor with another genetic or epigenetic alteration within the ectopic thyroid tissue itself or, as in some forms of Kallmann syndrome, in the structures surrounding the thyroid during embryogenesis. Thyroid ectopy, a model for sporadic congenital malformations in humans, is also associated with congenital heart disease, and molecular mechanisms common to thyroid and heart development are being unraveled.

Introduction

Permanent primary CH is currently estimated to affect about one child in 2500 [1] and is the commonest cause of preventable intellectual disability [2]. Defects in the embryonic development of the thyroid underlie the vast majority of cases. Among these defects, a round, oval, or dumbbell-shaped ectopic sublingual thyroid lacking lateral lobes is the most common (accounting for 50% of the children with CH in our jurisdiction), and in these cases, there is no thyroid in the normal position [3].

The thyroid originates from a midline anlage and from two lateral anlagen, on both sides of the neck. The midline anlage initially differentiates at the back of the tongue, at the level of the foramen cecum, and then dissociates from the pharyngeal floor. This process

occurs between the 5th and the 7th week in the human embryo. The lateral anlagen, originating from the 4th pharyngeal pouches, then fuse with the median anlage. It is generally assumed that an ectopic sublingual thyroid results from arrested downward migration of the median anlage, but ectopy could conceivably also be due to upward growth of the surrounding structures. The observation that the giraffe has a bilobed thyroid just below the tongue [4] suggests that thyroid organogenesis and neck elongation have evolved separately during phylogeny. Histologically, the ectopic thyroids found in humans have a normal follicular architecture, and the CH, which is almost always associated, may be due to a smaller number of cells (because of the absence of lateral lobes) which cannot be fully compensated by increased thyroid-stimulating hormone [5].

From epidemiology to molecular mechanisms of thyroid ectopy

Because of the irreversible effect of a late diagnosis of CH on cognition and behavior, biochemical screening for this condition has been established in all industrialized countries over the past four decades. Screening strategies vary, as do the diagnostic procedures in the newborns with a positive screening result. In our experience, only very few patients with thyroid ectopy are missed [5]. Thus, there is near-complete population-based ascertainment of this congenital malformation, which makes it a unique model to study. Another aspect of thyroid ectopy that makes it unique among congenital malformations is that its prevalence has not been affected by folic acid fortification [1]. Lastly, it does not show significant seasonal variations [6], which makes a role for viral infections during pregnancy unlikely.

Ectopic thyroid is almost always sporadic, but its occurrence in first-degree relatives is much higher than by chance alone. This has led most investigators to propose dominant inheritance of a mutation with variable penetrance [7]. Studies in mice have suggested the possibility of a multigenic mechanism [8] and in one human pedigree, two siblings had a variant in *NKX2.5* inherited from the father and a variant in the *PAX8* promoter inherited from the mother; however, whereas the girl had athyreosis, her brother was normal [9]. Furthermore, the significance of variants in *NKX2.5* in patients with thyroid dysgenesis has been questioned [10]. On the other hand, our compilation of all pairs of monozygotic (MZ) twins reported since screening began revealed that all were discordant for thyroid ectopy [11], with a single exception [12]. Systematic discordance has since been confirmed by others [13,14] and by personal observations. To reconcile the seemingly contradictory data of greater-than-random familial occurrence and MZ twin discordance, we have proposed a two-hit mechanism combining a germline and a somatic alteration [15]. The higher prevalence of thyroid ectopy among Caucasians than among black Africans [16], who are more genetically diverse, is also an argument for predisposing factors in the germline. Lastly, the three-to-one female predominance of thyroid ectopy [17] may suggest that this putative germline 'hit' is more often lethal in male embryos.

Very early post-zygotic events, a mechanism proposed in 1997 [18], could explain systematic discordance for thyroid ectopy between MZ twins. However, they would be at odds with its greater-than-random occurrence in first-degree relatives. Moreover, our recent study of three discordant MZ twin pairs by exome sequencing of blood DNA did not reveal any somatic mutations in the affected

twin [19]. Likewise, copy number variants in blood did not differ between two other pairs of discordant MZ twins [20]. Ideally, the ectopic tissue itself should be analyzed, rather than blood, but there are very few patients in whom ectopic thyroid tissue needs to be surgically removed.

Over the last decade, we have nevertheless been able to obtain ectopic thyroid tissue from a handful of patients. This invaluable collection is one of the main materials used in our research program. It allowed us to exclude somatic mutations in the candidate genes *NKX2.1*, *FOXE1*, and *PAX8* in one case [5]. Next, a transcriptome analysis revealed the presence of calcitonin mRNA in ectopic thyroids [21]. This was confirmed by reverse transcription-polymerase chain reaction and, at the protein level, by immunocytochemistry [22]. These observations challenge the long-held concept that calcitonin-producing cells derive exclusively from the lateral anlagen.

Whether at the genetic or epigenetic level and whether at the germline or the somatic level, *FOXE1* is one of the genes of interest because it is the only one which has been shown to be involved in thyroid migration in knockout models: in 50% of mouse *Foxe1*^{-/-} embryos at embryonic day 9.5, the median thyroid anlage remains connected to the pharyngeal floor, whereas in the other 50%, it has disappeared altogether, as in all *Foxe1*^{-/-} newborn pups [23]. A regulatory pathway analysis also puts *Foxe1* downstream of *Nkx2.1* and *Pax8*, which are involved in initial specification of the thyroid at the foramen cecum, whereas *Foxe1* is involved in its migration [24]. However, the few reported humans with bi-allelic mutations inactivating *FOXE1* have athyreosis and not ectopy, and they also have other malformations not typically seen in patients with thyroid ectopy—such as cleft palate and choanal atresia [25–27]. Intriguingly, one of the mothers, who carried the A65V mutation of *FOXE1* in the heterozygous state, had unilateral choanal atresia [28], suggesting that random tissue-restricted monoallelic expression of the mutated allele may occur—a mechanism that could account for discordance between MZ twins. Indeed, random monoallelic expression is more widespread than previously thought [29] and is likely involved in several developmental disorders [30].

Our own studies of the *FOXE1* gene are focused on its promoter region, in which we have identified a differentially methylated region (DMR). Consistent with the concept that promoter methylation inhibits transcription, this DMR is more methylated in tissues that do not express *FOXE1*, such as leukocytes. On the other hand, we found no significant difference between ectopic and orthotopic thyroid tissue [31]. Studies comparing *FOXE1* promoter

polymorphisms between patients with ectopy and controls and evaluating their impact on thyroid cell migration *in vitro* are under way.

Though generally isolated, thyroid ectopy has been shown to be associated with congenital heart disease, specifically septation defects, in several studies [7,17,32]. Detailed molecular studies in a patient with an association of thyroid ectopy and ventricular septal defect, coupled with functional studies in Zebrafish, recently led us to identify *netrin1* as a possible link between thyroid and heart development [33]. This illustrates that the molecular causes of defective thyroid development should not be sought in thyroid-specific genes only. Indeed, the 'thyroid transcription factors' [34] NKX2.1, FOXE1, and PAX8 have been excluded by linkage analysis in several multiplex families with dysgenetic CH [35]. Among extra-thyroid genes, those involved in the development of the neck vasculature should be considered because embryonic blood vessels direct thyroid morphogenesis [36]. Kallmann syndrome, another endocrine condition resulting from a defect in migration of hormone-producing cells, can be due to mutations in proteins that are extrinsic to the gonadotropin-releasing hormone expressing neurons but that guide them to their final location during embryogenesis [37]. However, Kallmann syndrome typically follows a Mendelian pattern of inheritance [38], whereas thyroid ectopy, as outlined above, generally does not.

Conclusions

Thyroid ectopy, which underlies the majority of cases of CH and is one of the commonest developmental abnormalities of the thyroid, remains one of the enigmas in the pathophysiology of thyroid diseases [39]. To better understand this common congenital malformation and possibly its impact on cognition and behavior, one has to go beyond a strict Mendelian line of thought. Well-characterized patients, access to rare tissues, up-to-date technologies, and relevant animal models are crucial to advance our knowledge of the mechanisms underlying this unique developmental defect.

Abbreviations

CH, congenital hypothyroidism; DMR, differentially methylated region; MZ, monozygotic.

Disclosures





The authors have no conflict of interest to declare.

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