Arteries define the position of the thyroid gland during its developmental relocalisation

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During vertebrate development, the thyroid gland undergoes a unique relocalisation from its site of induction to a distant species-specific position in the cervical mesenchyme. We have analysed thyroid morphogenesis in wild-type and mutant zebrafish and mice, and find that localisation of growing thyroid tissue along the anteroposterior axis in zebrafish is linked to the development of the ventral aorta. In grafting experiments, ectopic vascular cells influence the localisation of thyroid tissue cell non-autonomously, showing that vessels provide guidance cues in zebrafish thyroid morphogenesis. In mouse thyroid development, the midline primordium bifurcates and two lobes relocalise cranially along the bilateral pair of carotid arteries. In hedgehog-deficient mice, thyroid tissue always develops along the ectopically and asymetrically positioned carotid arteries, suggesting that, in mice (as in zebrafish), co-developing major arteries define the position of the thyroid. The similarity between zebrafish and mouse mutant phenotypes further indicates that thyroid relocalisation involves two morphogenetic phases, and that variation in the second phase accounts for species-specific differences in thyroid morphology. Moreover, the involvement of vessels in thyroid relocalisation sheds new light on the interpretation of congenital thyroid defects in humans.

KEY WORDS: Thyroid, Zebrafish, Mouse, Arteries, Vegf, Scl, Hedgehog

INTRODUCTION

The vertebrate body plan is essentially established during early development. However, the final position and shape of organs also depends on later events in morphogenesis. This is particularly evident for endoderm-derived organs, such as the thyroid gland, lung, pancreas and liver. The primordia of these organs bud off from different positions of the primitive gut and then undergo major morphogenetic alterations, such as branching and extension of the lung and lateralisation of the liver primordia. Relocalisation of an organ primordium is particularly evident in thyroid development. Its primordium evaginates from the pharyngeal epithelium after induction, to adopt a distant, species-specific position in the cervical mesenchyme. This relocalisation of the thyroid primordium is often called ‘migration’. We here avoid this term, as active movement of thyroid primordial cells has not been proven, and the molecular mechanisms of thyroid relocalisation are largely elusive. The transcription factor Foxe1 is cell autonomously required for proper relocalisation, as shown in mouse embryos and humans with congenital hypothyroidism (Castanet et al., 2002; Dathan et al., 2003). However, the morphogenetic forces defining the position of organ tissue during the relocalisation process are unknown.

The disruption of morphogenetic events defining position and shape of organs can account for congenital malformations and diseases in humans. The thyroid is particularly prone to morphogenetic variability. Usually present as a bilobed gland in different shapes (Gorbman and Bern, 1962). In zebrafish, the initially small globular thyroid grows massively along the pharyngeal epithelium, the gland eventually adopts its characteristic bilobed shape in front of the trachea. Proper organ function usually depends on normal tissue localisation with respect to adjacent structures during development. Therefore, ectopic localisation of the thyroid gland is often associated with compromised endocrine function, resulting in congenital hypothyroidism (De Felice and Di Lauro, 2004).

Owing to its accessibility for embryonic manipulation, the zebrafish thyroid is an excellent model with which to identify structures required for proper thyroid localisation. As far as investigated, molecules involved in thyroid development are essentially conserved between zebrafish and mammals (Elsalini et al., 2003; Wendl et al., 2002). In zebrafish, rapid development might explain a slightly different timing in thyroid differentiation (Elsalini et al., 2003). More striking differences between species exist with respect to the final position and shape of the gland. Although in all vertebrates investigated the primordium relocalises after evagination from the pharyngeal epithelium, the gland eventually adopts different shapes (Gorbman and Bern, 1962). In zebrafish, the initially small globular thyroid grows massively along the anteroposterior axis in the hypopharyngeal area, expanding into a long strand of tissue (Alt et al., 2006). In mouse and humans, the gland adopts its characteristic bilobed shape in front of the trachea.

In the present study, we investigate how thyroid tissue is positioned during relocalisation. Using zebrafish, we show that ectopic vascular cells are sufficient to mislocate thyroid tissue in a non-cell autonomous manner during growth. Based on this finding and on analysis of zebrafish mutants, we conclude that an adjacent vessel, the ventral aorta, determines thyroid tissue localisation. In the derived circulation of mammals, a vessel homologous to the ventral aorta does not persist. By investigating normal and mutant mouse embryos, we identify the carotid arteries as candidates for vessels that have assumed a comparable role in mammalian thyroid morphogenesis.

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MATERIALS AND METHODS

Animals

Zebrafish work was carried out according to standard procedures and staging in hours post fertilisation (hpf) refers to development at 28.5 to 29°C. The term ‘lara’ is used in the text for fry that have hatched from the chorion and generally refers to an age older than 72 hpf. The mutant zebrafish line kdr<sup>flk1</sup> (Covassini et al., 2006) is allelic to the kdr (flk1) lines described previously (Habeck et al., 2002) and develops heart oedema owing to compromised circulation or other specific defects at stages older than 40 hours, so that determination of mutant phenotypes was possible based on morphology. Homozygous cloche mutant embryos (clo<sup>3798</sup>) (Stainer et al., 1995) were also identified before fixation according to their phenotype at 24 hpf. Phenotypic details described in this study were always evident in all homozygous specimens (more than 10 in each experiment).

For analysis of short digits (Dsh/Dsh) (Niedermaier et al., 2005) and Xf/Xf mutant mouse embryos (Persson et al., 2002) timed matings of mutants were generated. The homozygous phenotypes are distinguishable from wild type owing to severe morphological defects, and in the case of Dsh-Xf matings we determined the genotype by PCR from extra-embryonic membranes (primer information available upon request).

Preparation of specimens

In situ hybridisation on zebrafish was carried out according to standard procedures, using nk2.1a (Rohr and Concha, 2000) as molecular marker for the thyroid primordium. Whole-mount immunohistochemistry with antibodies against thyroid hormone (T4) or thyroglobulin (TG) in whole-mount zebrafish larvae was performed as described elsewhere (Elsalini and Rohr, 2003).

For histological analysis, paraffin embedded mouse embryos were sectioned and stained with Haematoxylin-Eosin according to standard protocols. Three-dimensional images were reconstructed using every fifth section with the program SURFdriver 4.0, available at http://www.surfdriver.com. For immunohistochemistry on mouse sections, we used a Nkx2.1/Ttf-1 specific antibody generated against rat recombinant antigen (DAKO, Carpenteria, USA). Zebrafish embryos were embedded in Durcupan and sectioned. Diameters of the thyroid were measured using Discus software on a DMRA2 compound microscope (Leica).

Embryonic manipulation

Synthetic mRNA was produced using the message machine kit (Ambion) and approximately 25 pg scf mRNA and 25 pg lmo2 mRNA were injected into one cell stage embryos. As a control, we injected synthetic gfp mRNA at same concentrations. As lineage tracer for grafting experiments, we injected biotin-dextran (10,000 MW, 5 mg/ml: Invitrogen) together with synthetic mRNA and detected biotin-labelled donor-derived cells after in situ hybridisation using the ABC kit (Vector Laboratories). Injection and grafting experiments were repeated at least twice. At least 50 embryos were analysed for each mRNA injection experiment.

Morpholino RNA targeted against vegf (vegf-A-3) (Nasevicius et al., 2000), an scl splice morpholino (Patterson et al., 2005), a tnt2 morpholino (Sehnert et al., 2002) and an unspecific control morpholino were purchased (Gene Tools), and dissolved as recommended by the provider. Morpholinos were always injected into one cell stage embryos. At least 50 morphants were analysed for each experiment. Sections were made from five specimens or more in each experiment. All phenotypes described were consistently found in over 70% of morphants, with the rest showing milder defects or wild-type appearance.

RESULTS

The zebrafish thyroid primordium starts to develop at the outflow tract of the heart, which directly leads into the first primordial branch of pharyngeal arteries (Fig. 1A). From about 40 hours post fertilisation (hpf), the globular primordium expands along the anteroposterior (AP) axis into a strand of follicular tissue, thereby always remaining restricted to the midline (Fig. 1B-F). Close examination reveals that thyroid tissue expands in close contact to the growing ventral aorta (Fig. 1E-G). This major artery connects the heart to the branchial arteries that arise pairwise and sequentially, so that by 120 hpf a full set of five pairs of branchial arteries has formed (Isogai et al., 2001). The close association of thyroid and ventral aorta during development prompted us to investigate a possible relationship between both tissues.

Disturbed pharyngeal vessel architecture coincides with thyroid defects in zebrafish mutants

The mechanisms of ventral aorta and branchial artery development are largely unknown, so we first identified zebrafish embryos with disrupted pharyngeal vasculogenesis. The transcription factor Scl (also known as Tal1) is required for haematopoiesis (Gering et al., 1998; Liao et al., 1998), and endothelial (Liao et al., 1998) and dorsal aorta development (Patterson et al., 2005) in zebrafish embryos. Corroboratively, Scl is expressed in haematopoietic and
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endothelial tissue, as well as in a few cells in the central nervous system (Jin et al., 2006). The vascular endothelial growth factor (Vegf) pathway plays crucial roles in vertebrate haematopoiesis and vasculogenesis (Ferrara, 2004). In zebrafish, Kdr (previously known as Flk1) is an endothelial cell specific Vegf receptor required for vasculogenesis (Covassin et al., 2006; Habeck et al., 2002), and vegf and kdr are also expressed in and around the nascent and growing ventral aorta (Fig. 1E,F,H-K). As expected from data regarding trunk vasculogenesis (Patterson et al., 2005), reduction of Scl or Vegf (using scl and vegf morpholinos) (Lawson et al., 2000; Patterson et al., 2005), or absence of Kdr in kdr<sup>−/−</sup> mutant embryos (Habeck et al., 2002), dramatically interferes with pharyngeal vessel formation (Fig. 2A-D). Analysis of the endothelial marker tie1 in the three deficiency backgrounds indicates that here the ventral aorta fails to form properly. Instead, a misshaped domain of tie1 expression surrounds the outflow tract of the heart, often expanding laterally, but not along the AP axis, into one pair of irregular branches. Thus, Scl and the Vegf pathway are required for normal development and expansion of the ventral aorta along the AP axis.

We then analysed whether altered pharyngeal vessel architecture affects thyroid morphology. In the abovementioned three genetic backgrounds with compromised Scl activity or Vegf signalling, the thyroid starts to develop from a normally induced midline primordium at around 26 hpf (normally shaped and sized domain of nk2.1a expression, data not shown). However, during subsequent growth, the thyroid fails to elongate from its initial position at the cardiac outflow tract along the AP axis (Fig. 2E-H). Instead, thyroid tissue is not limited to the midline and often expands laterally in irregular fashion, always adjacent to or embedded into ectopic tie1 expression (Fig. 2I-L). Correspondingly, follicles do not align along the pharyngeal midline during larval growth, and instead form an irregular group around the cardiac outflow tract (Fig. 2M-P). These data strongly suggest that thyroid outflow tract (Fig. 2M-P). These data strongly suggest that thyroid morphogenesis is linked to pharyngeal vessel development.

To test whether compromised blood circulation evident in all of these deficiency backgrounds causes indirectly thyroid abnormalities, we targeted the tnt2 gene by morpholino knock down. tnt2 encodes the thin-filament contractile protein cardiac troponin T, and corresponding morphants lack heart beat and blood circulation (Sehnert et al., 2002). In these morphants, the thyroid is normal at 55 hpf (n=64, data not shown), showing that specific disruption of circulation does not account for morphological defects of the thyroid. Thus, it is rather vessel patterning that appears to be correlated to thyroid morphology. As the ventral aorta is the only vessel adjacent to the thyroid in wild type, it can be assumed that this is the artery that guides follicular growth along the midline. However, the thyroid phenotype in scl morphants could also be due to low level scl expression in thyroid tissue itself. Furthermore, kdr and vegf are broadly expressed around vessels (Fig. 1E,H,K), probably including thyroid cells, and therefore might act in parallel both in vascular cells and in thyroid cells. Thus, we asked whether ectopic endothelial cells are able to change thyroid morphology cell non-autonomously, as this would prove a direct interaction between vessels and thyroid.

**Ectopic endothelial cells can influence thyroid morphology non-cell autonomously**

To develop an assay in which cells are forced to adopt endothelial fate, we used an approach involving Scl and Lmo2, a transcription factor specifically required for haematopoiesis and angiogenesis (Yamada et al., 2000; Yamada et al., 1998). Co-injection of scl and lmo2 mRNA induces ectopic expression of the endothelial marker fn1 throughout the head mesenchyme of zebrafish embryos, indicating that these factors are sufficient to specify endothelial fate.

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**Fig. 2. Mutants with defects in ventral aorta development show correlating thyroid abnormalities.** (A-H) Frontal views, showing vasculature (tie1 expression) or thyroid (nk2.1a expression). Control morpholino injected embryos are indistinguishable from wild-type embryos or kdr<sup>−/−</sup> siblings. (I-L) Sections showing follicular lumen (thyroglobulin immunostaining, brown) in relation to endothelial cells (tie1 expression, blue). (M-P) At 120 hpf, thyroid follicles (T4 immunostaining, brown) fail to align along the ventral aorta. In F,H,J-L the thyroid appears larger owing to the lateral expansion. However, in wild-type embryos the thyroid extends along the AP axis (compare with Fig. 1B). Concomitant with lateral expansion, its AP extent appears to be reduced, so that the size remains similar. This is also reflected by normal follicle numbers at 120 hpf (M-P). TG, thyroglobulin; Thy, thyroid; Vasc, vasculature; va, ventral aorta; 1st, first pair of branchial arteries.
in all head mesenchymal cells (Gering et al., 2003). Similarly, coinjection of these mRNAs induces strong kdr expression in the whole head mesenchyme (Fig. 3A-D). Such injected embryos develop extreme head abnormalities and die around 24 hpf (data not shown), preventing the analysis of thyroid development. Instead, we grafted cells from scl+lmo2 co-injected embryos into wild-type hosts in order to create a mosaic of wild-type and ectopic endothelial cells.

In this transplantation approach, few cells were grafted at late blastula stage from scl+lmo2 mRNA injected donors into wild-type hosts (Fig. 3E). We analysed some embryos at 12-somite stage, when head vasculogenesis is in progress (Gering et al., 2003), and ectopic endothelial cells can easily be distinguished from normal sites of expression. In all embryos investigated, all grafted cells ending up in head mesenchyme express kdr, and, hence, adopt endothelial fate (12 of 12 sectioned embryos; Fig. 3F,G).

For analysis of thyroid development, we fixed other host embryos at 55 hpf. In most of these embryos, the thyroid appears normal, even if grafted cells are found in the pharyngeal region (n=14). However, exclusively in four embryos where grafted cells ended up directly adjacent to the medially positioned thyroid, its tissue projects towards the grafted cells (Fig. 3H). In all four cases, the thyroid marker nk2.1 itself is not expressed in the grafted cells. As a control, we grafted cells from gfp mRNA-injected embryos into wild-type embryos. Out of 28 embryos that received grafted gfp cells, seven had donor cells very close to the thyroid. All of these embryos, as well as all other embryos where gfp or scl+lmo2 donor cells were further away from the thyroid, had a normal gland without projections towards grafted cells (Fig. 3I). To further compare both
sets of grafting experiments, we measured the width and the length (the smallest and the largest diameter) of the thyroid on its median level in sections. In the seven hosts with gfp cells close to the thyroid, the gland generally adopts the normal slightly ovoid shape in cross-sections, with the quotient of both values being around 0.8 (Table 1). In the four hosts with scl+lm2 cells close to the thyroid, the abnormally elongated shapes of the gland result in a quotient in a preset only around 0.4 (Table 1). Statistical analysis of the quotient values of all 11 specimens (U-test according to Mann, Wilcoxon, and Whitney) reveals that the thyroid glands of embryos with scl+lm2 cells are significantly different from embryos with gfp cells ($p=0.02$). We have shown above that grafted cells in the head mesenchyme behave as endothelial cells. Our grafting experiments show that such cells are able to influence thyroid morphology in a non-cell-autonomous manner in zebrafish.

Together, our zebrafish data indicate that vascular cells influence thyroid morphology. Under wild-type conditions, the ventral aorta is the only vessel adjacent to the thyroid in zebrafish embryos/larvae. Based on both mutant analysis and grafting experiments, we conclude that the ventral aorta acts as an instructive structure for positioning thyroid tissue along the AP axis. Three-dimensional analysis of wild-type and mutant mouse embryos suggest an influence of carotid arteries on thyroid morphogenesis

A role of the vascular system in thyroid development has been suspected in mice based on the close spatial relationship between aortic sac and thyroid primordium. However, these studies focused on an hypothetical early role of the aortic sac in induction and evagination of the primordium (Fagman et al., 2005). To test whether an instructive role of blood vessels in subsequent steps of morphogenesis could be conserved among vertebrates, we analysed three-dimensional reconstruction of cervical regions of wild-type and mutant mouse embryos. Thyroid precursor cells firstly occur within the pharyngeal epithelium in direct contact to the aortic sac (Fig. 4A), a transient structure that gives rise to the cardiac outflow tract. Under wild-type conditions, the primordium first relocates along the AP axis in close contact to the developing aortic arch (Fig. 4B). Later, the primordium loses its contact to the aortic arch and bifurcates (Fig. 4C). During bifurcation the thyroid lobes are again close to vascular structures, the bilateral pair of carotid arteries. Subsequently, the lobes elongate following the carotid arteries further cranially (Fig. 4D). We therefore asked whether carotid arteries play a similar role in mammalian thyroid morphology as the ventral aorta does in zebrafish.

Recent work has shown that an abnormal, unilateral single thyroid lobe develops in Ssh-deficient mouse embryos (Fagman et al., 2004). Furthermore, Shh deficiency leads to defective cardiac rotation and atrial dilatation (Tsukui et al., 1999), suggesting further defects in cervical vessel development. Three-dimensional reconstruction of sections reveals that in the absence of Shh expression (in Dsh/Dsh mice) (Niedermaier et al., 2005), the aortic arch fails to cross the midline and both carotid arteries develop asymmetrically on one side of the oesophagus (Fig. 4E-H). Initially, the thyroid primordium evaginates and relocates along the AP axis in the midline of Shh-deficient mice as in wild type (Fig. 4E). However, the primordium then fails to undergo its symmetric lateralisation and bifurcation. Instead, after embryonic day 12.5, a single lobe always relocates on the same side as and in contact with the mislocated carotid arteries (right n=7, left n=1; Fig. 4F-H, Fig. 5D), suggesting that the carotid arteries define the symmetric lateral position of the thyroid gland under wild-type conditions.

Compromised hedgehog signalling affects many aspects of embryonic development, and so it could be that thyroid and vessels are independently affected in Dsh/Dsh mice. However, we did not find detectable expression of Shh or patched (encoding the receptor for Shh in or around carotid arteries or the thyroid primordium at E12.5, when asymmetry of thyroid and carotid arteries starts to be evident in Dsh/Dsh mice (Fig. 4I-K). Although we cannot exclude low levels of expression, it appears unlikely that Shh is directly involved in thyroid relocalisation at this stage. Instead, it is more likely that Shh deficiency causes vascular problems that indirectly affect thyroid morphology.

Furthermore, we investigated thyroid and carotid artery morphology in a mutant background where the Dsh/Dsh phenotype is partially restored to wild-type appearance. Shh and Gli3 are context-dependent antagonists of each other in mouse limb and neural tube development, so that in Shh+/- Gli3+/- double mutants, some aspects of the Shh-/- mutant phenotype are rescued (Ruiz i Altaba et al., 2003; te Welscher et al., 2002). We found that in this double mutant background (Dsh/Dsh Xr/Xr, Xr is a null allele of

### Table 1. Neighbouring scl+lm2-expressing cells change the shape of the wild-type thyroid

<table>
<thead>
<tr>
<th>Grafted specimen (lab code)</th>
<th>Width</th>
<th>Length</th>
<th>Width/Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>gfp-25</td>
<td>12.4</td>
<td>15.6</td>
<td>0.79</td>
</tr>
<tr>
<td>gfp-14</td>
<td>11.7</td>
<td>15.5</td>
<td>0.75</td>
</tr>
<tr>
<td>gfp-4</td>
<td>12.2</td>
<td>16.7</td>
<td>0.73</td>
</tr>
<tr>
<td>gfp-2</td>
<td>10.8</td>
<td>15.7</td>
<td>0.69</td>
</tr>
<tr>
<td>gfp-18</td>
<td>13.8</td>
<td>14.5</td>
<td>0.95</td>
</tr>
<tr>
<td>gfp-3</td>
<td>13.3</td>
<td>16.1</td>
<td>0.83</td>
</tr>
<tr>
<td>gfp-5</td>
<td>13.7</td>
<td>15.4</td>
<td>0.89</td>
</tr>
<tr>
<td>Mean value (embryos that received gfp grafted cells)</td>
<td>12.6</td>
<td>15.6</td>
<td>0.83</td>
</tr>
</tbody>
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| scl+lm2-4                   | 10.7  | 29.6   | 0.36         |
| scl+lm2-6                   | 15.4  | 36.1   | 0.43         |
| scl+lm2-3                   | 10.2  | 31.9   | 0.32         |
| scl+lm2-8                   | 13.5  | 24.4   | 0.55         |
| Mean value (embryos that received scl+lm2 grafted cells) | 12.5  | 30.5   | 0.42         |

The width and the length (in μm) of the thyroid at 55 hpf are given, as measured on the median thyroid level on cross-sections. Data for the four embryos where scl+lm2-overexpressing cells were found close to the thyroid (lab code: scl+lm2-#), and for seven embryos where control gfp-overexpressing cells were found similarly close to the thyroid (lab code: gfp-#).
DISCUSSION

The thyroid gland in vertebrates develops close to vascular structures. In the present study, we analyse zebrafish and mouse embryos with altered vessel architecture and find that defects in pharyngeal/cervical vessels coincide with abnormal thyroid development in both species. The study of zebrafish embryos with ectopic endothelial cells reveals a functional link between vascular and thyroid development.

Our zebrafish data show that ectopic endothelial cells influence thyroid morphology in a non-cell autonomous manner. In mice, vessels are involved in induction of the endoderm-derived pancreas from the primitive gut (Lammert et al., 2001). A role of the cardiovascular system in induction of the mouse thyroid has been suspected (Fagman et al., 2005), and so ectopic induction could probably also cause lateral expansion of thyroid tissue in the zebrafish embryos lacking Scl or with compromised Vegf signalling. However, as reported above, in all deficiency backgrounds analysed, the thyroid starts to develop from a normally induced midline primordium. Therefore, an inductive role of vascular structures can be excluded to be responsible for the thyroid phenotypes observed in our deficiency backgrounds. Furthermore, in cloche, a zebrafish mutant lacking all vessel progenitors in head and anterior trunk region (Liao et al., 1997; Stainier et al., 1995), the thyroid primordium is still induced (data not shown). As the myocard forms in cloche, it is still possible that the heart or some of its precursor cells are involved in thyroid induction, but an inductive role of vessels can be excluded.

Cell labelling experiments suggest that the whole strand of thyroid tissue in zebrafish derives from the small globular primordium that buds off from the pharyngeal epithelium at around 32 hpf (Alt et al., 2006), and that no further cells contribute to the thyroid from outside during its extension along the AP axis. This, and the fact that in our deficiency backgrounds the thyroid primordium is initially normal, suggest that in our grafting experiments the lateral expansion of thyroid tissue adjacent to endothelial cells is generated by misdirected growth. Taken together, our zebrafish data show that endothelial cells are required for proper alignment of thyroid tissue along the AP axis during tissue growth.

The grafting experiments show an instructive influence of endothelial cells on thyroid morphogenesis, but we were not able to discriminate between different endothelial cell types in this experiment. Under wild-type conditions, the ventral aorta is the only vessel directly adjacent to the thyroid in zebrafish, so that it can be assumed that this is the vessel responsible for interactions with the thyroid. Not only in zebrafish (Wendl et al., 2002), but also in other teleosts such as trouts (Raine and Leatherland, 2000), thyroid tissue remains in close association with the ventral aorta throughout later development and adulthood. It will be interesting to find out how such an interaction is mediated. Extracellular matrix or secreted factors are candidates, but the exact nature of the interaction between vessels and thyroid tissue remains to be elucidated.

In both zebrafish and mouse development, two morphogenetic phases are distinguishable in thyroid relocalisation. After induction and evagination, the thyroid primordium adopts a position close to the cardiac outflow tract in zebrafish or to the aortic sac in mice, respectively (Fig. 6). In all deficiency backgrounds investigated, this first phase is not disrupted. Consequently, follicles cluster at a default position around the cardiac outflow tract in the deficient zebrafish larvae, and the primordium initially relocates correctly in the midline of E11.5 Dsh/Dsh mice. In a second phase of relocalisation that is dependent on ventral aorta or carotid artery development, the thyroid then adopts its species-specific position.
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Further cranially. In the complex and derived circulation of mice, the aortic sac is in part homologous to the cardiac outflow tract and probably also to the ventral aorta in zebrafish. Thus, the first phase of thyroid relocation from the pharyngeal epithelium to cardiac structures appears to be evolutionary conserved, whereas the second phase seems to be adapted to the presence of different cranial vessels.

These data suggest that the dependence of thyroid morphogenesis on the development of adjacent arteries is a conserved mechanism that might have evolved to ensure efficient hormone release into circulation. Interestingly, the identity of vessels with instructive influence on thyroid localisation appears to vary considerably in vertebrates. In most vertebrates, two thyroid lobes are positioned cranial to the aortic arch, midway along the carotid arteries, as is the case in mice and humans (Pischinger, 1937). Conversely, in some vertebrates such as crocodiles, turtles, and certain mammals as the echidna (Haynes, 1999; Pischinger, 1937), a single-lobed thyroid is positioned further caudally at the aortic arch, at the base of the branching carotid arteries (Fig. 6). Thus, it appears that the second morphogenetic phase is omitted in these species.

Our data provide a novel starting point to re-investigate and interpret congenital thyroid defects in humans. Cervical vessel architecture is highly variable in humans, in particular with respect to the branching mode of the carotid arteries from the aortic arch that can be classified into few frequent and many rare variants (Nizankowski et al., 1975). Based on our data, it is conceivable that some variants might influence thyroid morphogenesis and account for cases of unilateral thyroid glands or ectopically positioned thyroid tissue in humans. Indeed, in clinical studies, congenital defects of the cardiovascular system have already been associated with congenital thyroid abnormalities (Casanova et al., 2000; Olivieri et al., 2002), and our study is the first to provide a causal link between both structures in development. Thus, the disruption of signalling pathways linking thyroid and blood vessel development represents a novel mechanism that is likely to be relevant for the molecular pathogenesis of congenital thyroid defects in humans.

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