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Molecular genetic basis of thyroid dysgenesis

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Summary

As in other organ malformations, e.g. of the heart and the kidney, congenital defects of the thyroid gland occur mostly sporadic (1 in 3500 newborns). In the few familial cases of organ malformations, genetic defects in transcription factor genes were identified which are inherited mainly dominantly with a variable penetrance. For thyroid malformations, which manifest as the complete absence of the gland (athyrosis) or thyroid ectopy and thyroid hypoplasia, mutations in the transcription factor genes PAX8, NKX2.1 (TITF1), FOXE1 and NKX2.5 were described so far¹. Together these mutations, although investigated in hundreds of patients, were detected in less than 50 of affected children. Therefore, the molecular pathogenesis of organ malformations, including the thyroid, are still unknown. To better understand the pathogenesis of organ malformations, further genes involved in early organ development need to be identified and to be tested as candidate genes. In addition new mechanisms need to be elucidated as potentially involved in the non-classical defects leading to sporadic occurrence. Due to the sporadic occurrence classic mendelian inheritance is an unlikely cause in these conditions. Alternative molecular mechanisms like high rates of spontaneously occurring de-novo somatic mutations and epigenetic defects are possibly involved. Related with this likely non-mendelian inheritance, the variability of the manifestation of organ malformation, even in those cases with already proven mutations within the same transcription factor genes suggest epigenetic modification of the manifestation of organ malformations (fig. 1).

PAX8

Mutations in the PAX8 gene were the first proven to be causally linked to the occurrence of thyroid dysgenesis. Very few single patients with mainly thyroid hypoplasia have been identified since the first report in 1998². However, the phenotype of these heterozygous patients with a thyroid restricted defect without additional other organ malformations represent the typical patients in the large cohorts of children with congenital hypothyroidism and therefore PAX8 still serve as an ideal candidate gene for the majority of patients.

In order to identify “epimutations” in the PAX8 gene we recently established the complete methylation status of the two CpG islands around the transcriptional start site. In peripheral blood leukocytes (PBL) a distinct pattern of hypermethylation in the most 5-prime part of island 1 and an increasingly hypomethylation in island two became evident. Screening for a difference in these methylation patterns in normal controls versus patients with thyroid dysgenesis revealed no significant result. Other regions of the PAX8 gene or in other candidate genes need to be further investigated for “epimutations” as one likely cause of CH.

FOXE1/TTF2

Two cases of recessive FOXE1/TTF2 mutations in patients with thyroid agenesis, cleft palate and mental retardation were described based on the association of these two malformations which resembled the phenotype of the Ttf2 knock

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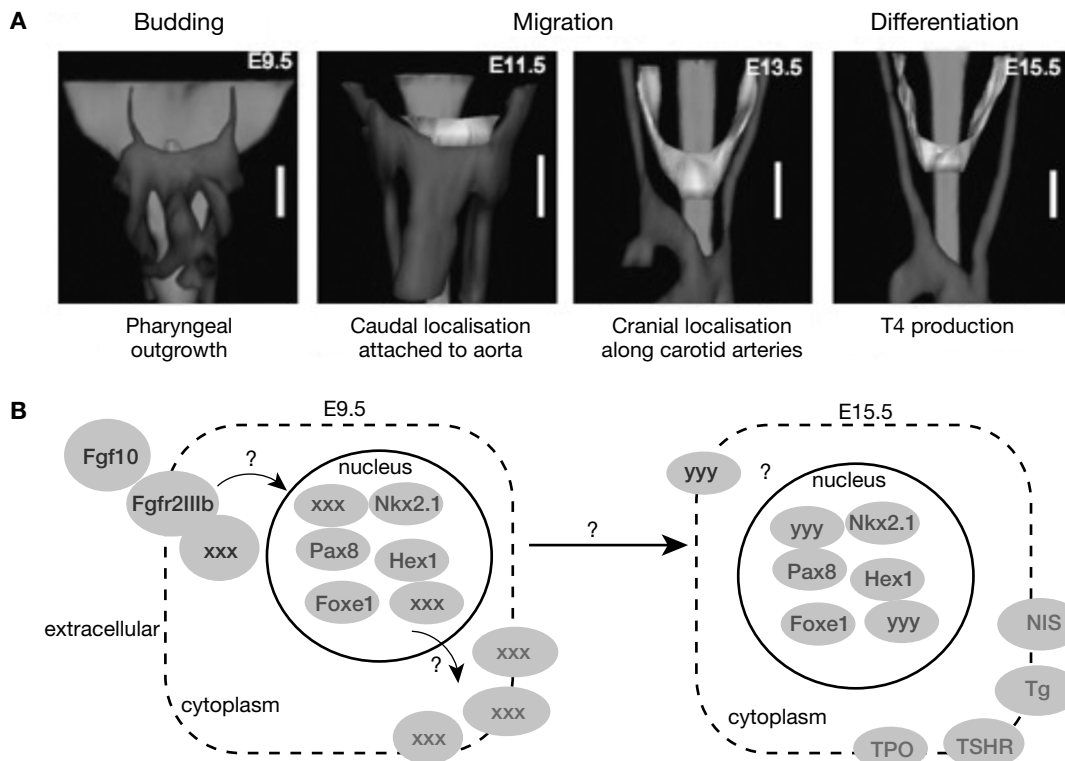


Figure 1 An overview of morphological aspects of thyroid development and the regulatory genetic components known so far. A) The spatial co-development of the thyroid primordium (grey) in relation to the pharynx (green) and the cervical arteries (red) is shown in three dimensional reconstructions of mouse embryonic histological sections at different stages of development (E 9.5: embryonic day 9.5). B) The genes which were identified in mouse knockout studies to be critically linked to thyroid development are shown. The upstream growth factor cascade (blue) is only rudimentary known and so far exists of the Fgf10 and the Fgf2R; the nuclear regulatory network contains four major transcription factors (red); the target genes (green) of this network is so far completely unknown in earlier developmental stages and only for the last step of differentiation the functional relevant target genes were identified which together produce thyroid hormone T4 in the adult gland. Stage specific regulatory transcription factor, co-factor and growth factor genes are unknown.

out mice^{3,4}. So far only single further cases were described with this particular rare syndrome. However, making the diagnosis is clinically useful because the expected mental development of these children is not normal and early intervention programs can be initiated.

NKX2.1/TTF1

The NKX2-1 gene (also named thyroid transcription factor 1, TTF1) is the earliest expressed transcription factor in the thyroid anlage at day E8.5 of mouse development. Targeted deletion revealed absence of the thyroid gland and in addition, due to its role in forebrain and lung development, severe malformations of the basal ganglia and the lung⁵. Due to this broad spectrum of defects in knock out mice, mutation screening was subsequently focused on patients with thyroid dysgenesis and thyroid dysfunction who are affected by additional neurological and pulmonary symptoms. After the first description of heterozygous NKX2-1 gene mutations in 5 of those patients⁶, we now have screened more than 100 patients with different range of neurological and thyroid dysfunction for NKX2-1 gene mutations and identified 25 new mutation carriers. Screening was performed by

direct sequencing as well as by customized oligo array-CGH to detect small deletions in the gene locus which is only 3.5 Kb long. In addition to the deletions and nonsense mutations, we identified few missense and splice mutations which were functionally characterized by either a mini-gene splice assay or by EMSA and subsequent in vivo functional studies in a zebrafish model (Thorwarth et al, manuscript in preparation).

Furthermore a detailed phenotype survey in 30 mutation carriers revealed that this gene defect primarily results in a neurological disease characterized by a choreatic movement defect rather than a thyroid developmental defect. Due to the additional role of the gene in hypothalamus, pituitary and lung development, thyroid dysfunction and chorea in these patients are associated with a variety of other symptoms, thereby defining a disease cluster without a clear genotype – phenotype correlation (fig. 2).

Search for further genetic alterations (CGH-Array in thyroid dysgenesis)

Since thyroid malformations occur sporadically other than classical genetic defects might be relevant for the patho-

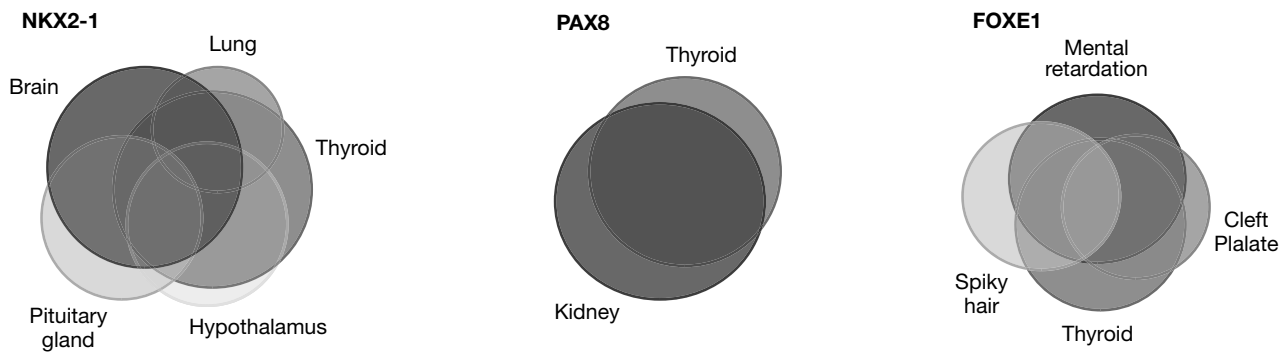


Figure 2 Disease families caused by mutations in interacting thyroid transcriptions factors; Phenotype variations are shown as identified in patients with NKX2-1, PAX8, FOXE1 mutations^{2,4,6}.

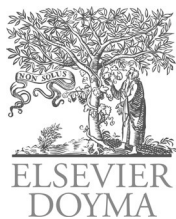
genesis. One possible mechanism could be a high rate of de-novo, dominant mutations as they have been identified in micro-deletion diseases like the CATCH22 syndrome, which shows a comparable incidence rate as thyroid malformations of 1 in 3000 newborns. We therefore performed CGH array in 62 patients with thyroid dysgenesis and found 10-aberrations. No uniform deletion or duplication could be identified in the investigated patients excluding thyroid dysgenesis as a micro-deletion syndrome. However, at least in one deletion, which was found in a patient with athyrosis, a gene could be identified which was annotated in expression data bases to the thyroid gland. So far no information about the gene function and the role in thyroid development is known. Subsequently we performed in situ hybridisation assays and found a specific expression of the gene within the thyroid anlage at day E9.5. The combined data of partial deletion in a patient with thyroid dysgenesis and the expression in the earliest steps of thyroid development suggest a role of the new gene in normal thyroid development.

Together the few patients identified to have mutations leading to CH and thyroid dysgenesis have proven the role of the so far known thyroid transcription factors also for normal human thyroid development. Moreover the elucidation of the FOXE1/TTF2 and TTF1/Nkx2.1 defect were helpful for the understanding of an unfavourable outcome of patients with congenital hypothyroidism despite an early and adequate treatment with thyroxin. The low incidence of these mutations in the large group of patients with congenital hypothyroidism suggests that additional defects in these genes or other so far unidentified genes relevant for normal thyroid development need to be elucidated in the future. In

addition the sporadic occurrence and the discordance in monozygotic twins⁷ argues further for other defects than classical-inherited mutations.

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Usos terapéuticos de IGF-I recombinante humano

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Las acciones de IGF-I están moduladas por una familia de proteínas, IGFBP, la unidad ácido lábil (ALS) y mediadas a través de su unión al receptor tipo 1 de IGF (IGF1R)¹.

Los efectos de rhIGF-I, sola o unida a IGFBP-3, han sido estudiados en un número de estudios clínicos de corta duración, entre ellos estudios de diabetes tipo 1 y tipo 2, en pacientes con quemaduras graves, en osteopenia y en esclerosis amiotrófica lateral o enfermedad de la neurona motora inferior^{2,3}.

Estudios clínicos con rhIGF-I han sido evaluados en pacientes con insensibilidad a la hormona de crecimiento (GHI) y en pacientes con resistencia grave a insulina. Ambos síndromes son poco comunes y ambos están asociados a un amplio rango de fenotipos clínicos y bioquímicos³⁻⁹.

La eficacia y seguridad del tratamiento con rhIGF-I en niños y adolescentes han sido evaluadas principalmente en pacientes con GHI debido a defectos en el gen del receptor de GH o en pacientes con defectos en el gen de GH con anticuerpos neutralizantes después del tratamiento con GH. En ambos grupos de pacientes se ha demostrado que el tratamiento es eficaz en promover el crecimiento lineal, aunque existe variabilidad en la respuesta clínica al tratamiento. Las dosis utilizadas están en el rango de 80 a 120 μ mg por kg, administradas 2 veces al día (inyecciones subcutáneas). La respuesta al tratamiento es buena, especialmente durante los 2 primeros años, disminuyendo en años posteriores. En el tratamiento a largo plazo se han observado ganancias hasta de 1 SDS. Varios factores están asociados a la variabilidad en la respuesta al tratamiento, por ejemplo: edad del inicio del tratamiento, gravedad de la GHI, estado nutricional del paciente y cumplimiento con el tratamiento³.

Las mejores respuestas se observan cuando la terapia se inicia a una edad más temprana y en un niño bien nutrido. Los eventos adversos más frecuentemente asociados a este tratamiento incluyen: hipoglucemia (generalmente en los pacientes más jóvenes), hiperplasia linfoide lipo-hipertrofia, y *pseudotumor cerebri*; cambios en los rasgos faciales, un poco acromegaloides, y un aumento significativo de la masa grasa se han observado con tratamientos a largo plazo⁷.

Actualmente, el uso clínico de rhIGF-I combinada con rhIGFBP-3 (rhIGF-I/rhIGFBP-3; concentraciones equimolares) está limitado a estudios clínicos no relacionados con trastornos del crecimiento.

La eficacia a corto y largo plazo del tratamiento con I-rhIGF-I y rhIGF-I/rhIGFBP-3 ha sido demostrada en pacientes con resistencia a la insulina debido a defectos genéticos en los receptores de insulina o en su vía de señalización intracelular. El beneficio se ha demostrado por cambios significativos en la HbA_{1c} y en una mejoría del crecimiento lineal en algunos pacientes pero, una vez más, asociados a una serie de efectos adversos^{2,3,9}.

Recientemente, hemos iniciado un estudio de colaboración para evaluar la eficacia y seguridad de esta terapia en recién nacidos prematuros. Los bajos niveles IGF-I se han asociado con problemas de crecimiento y la retinopatía del prematuro. Nuestro primer objetivo ha sido estudiar la seguridad y la farmacocinética de la administración intravenosa de rhIGF-I/rhIGFBP-3 a los recién nacidos prematuros. La administración de rhIGF-I/rhIGFBP-3 ha mostrado ser eficaz, aumentando los niveles séricos de IGF-I, y la administración en las condiciones de este estudio fue segura y bien tolerada¹⁰.

En resumen, las aplicaciones clínicas de rhIGF-I, sola o combinada con rhIGFBP-3, representan un avance importante en el desarrollo de medicamentos endocrinos con beneficios clínicos claros en condiciones graves, como son la resistencia a la hormona de crecimiento y síndromes de resistencia a la insulina. Se necesitan estudios clínicos bien diseñados de intervención a corto y largo plazo para evaluar la relación riesgo-beneficio de estos fármacos en otras condiciones clínicas y en pacientes con formas leves de GHI o resistencia a la insulina.

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