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Author: Joustra, Sjoerd

Title: Pituitary disorders and their extra-pituitary implications : observations in patients with nonfunctioning pituitary macroadenoma and the IGSF1 deficiency syndrome

Issue Date: 2016-09-27

CHAPTER 13

General Discussion and Future Perspectives



The studies presented in this thesis aimed to explore detailed pituitary functioning and extrapituitary implications of two pituitary disorders in humans: nonfunctioning pituitary macroadenoma and immunoglobulin superfamily member 1 (IGSF1) deficiency.

PART A: HYPOTHALAMIC REGULATION OF CIRCADIAN RHYTHMICITY IN NONFUNCTIONING PITUITARY MACROADENOMA

Part A focused on the long-term consequences of being treated for a nonfunctioning pituitary macroadenoma (NFMA) on hypothalamic regulation of circadian rhythmicity. In the absence of tumor-related hormone excess, these tumors are generally only discovered when they compress surrounding tissue. Since the sella turcica forms a bony caudal border for the pituitary gland, suprasellar extension of a pituitary macroadenoma is common. In case of suprasellar extension, the first structure that is encountered by the tumor is the optic chiasm, and its compression causes visual field defects (the pathognomic bilateral hemianopsia). The suprachiasmatic nucleus (SCN) of the hypothalamus is situated directly above the optic chiasm. This tiny region of the brain is responsible for coordinating and regulating circadian rhythms in various organs throughout the entire body. Based on (anecdotal) complaints of decreased sleep quality and decreased quality of life in patients that were in long-term remission after surgical treatment for NFMA in our outpatient clinic, we hypothesized that suprasellar tumor growth may irreversibly damage the SCN. The studies presented in this thesis aimed to test this hypothesis by collecting a number of indices of SCN functionality in this specific patient group.

We observed in patients altered sleep architecture with decreased sleep efficiency, altered diurnal rhythmicity of proximal skin temperature, fragmentation of the day-night dichotomy of motor activity, and subjective decreased sleep quality as well as increased daytime somnolence. In addition, we observed heterogeneous patterns of altered rhythmicity of proximal and distal skin temperature, core body temperature, and melatonin concentration. Lastly, patients were subject to an increased risk for the metabolic syndrome.

Certainty of SCN dysfunction

How certain is it that the alterations in metabolic profile, sleep quality, and rhythmicity of the sleep-wake cycle, temperature, and melatonin are caused by dysfunction of the SCN (or its projections), and not by other parts of the central nervous system involved in their regulation?

First, the only structure linking all perturbed indices is the SCN, and it is the first and generally the only brain structure encountered by the tumor. If the alterations are not caused by a defective SCN, but through defects in multiple other parts of the central

nervous system (e.g. thermoregulatory centers, pineal gland, sleep-wake centers), this would require multiple assumptions. William of Ockham's razor taught us the *lex parsimoniae* (law of parsimony), meaning that among competing hypotheses, the one with the fewest assumptions should be selected, as that hypothesis is more easily falsifiable.

Second, similar alterations were observed in several other conditions associated with hypothalamic damage. For instance, in craniopharyngioma patients (which frequently show hypothalamic involvement) we observed lower daytime proximal skin temperature, lower sleep efficiency with less REM sleep, and decreased subjective sleep quality and increased daytime sleepiness. Others reported in craniopharyngioma patients decreased nocturnal melatonin levels (2, 3), as well as sleep disturbances, daytime sleepiness, and low cerebrospinal fluid hypocretin levels known as 'secondary narcolepsy' (4-8). Also, a syndrome of increased risk for cardiovascular disease called 'hypothalamic obesity' (mimicking the metabolic syndrome) is observed in craniopharyngioma, after cranial radiotherapy or trauma, and in genetic disorders that affect the hypothalamus (e.g. Prader-Willi syndrome) (9-13). Furthermore, SCN lesions in primates are associated with fragmented sleep-wake rhythmicity (14), disruptions of temperature rhythmicity have been described in humans with metastatic hypothalamic lesions (15, 16), and specifically decreased daytime proximal skin temperature (as in NFMA) was observed in patients with narcolepsy (17) and several suprasellar tumors that compress the optic chiasm (among which NFMA) (18). Similar observations of supposed 'hypothalamic obesity' and fragmented sleep-wake rhythms have been made in the elderly (19), in whom the aging SCN is characterized by decreased neuronal activity (20). Interestingly, post-mortem evaluation of the SCN in patients treated for suprasellar tumors that compress the optic chiasm also show reduced immunoreactivity of arginine vasopressin (21).

Third, one might argue that hypopituitarism and the intrinsic imperfection of endocrine replacement therapies, as the major comorbidity in patients treated for NFMA and being omnipresent throughout the body, serves as an alternative explanation for the disturbed circadian rhythms. However, hypopituitarism was not associated with alterations in the circadian sleep-wake rhythm (nor was radiotherapy), and patients with Addison's disease dependent of hydrocortisone replacement (but no history of a pituitary tumor) did not show decreased sleep quality, daytime sleepiness, or fragmented sleep-wake rhythms (**Chapter 5**). The latter also signifies that the alterations in NFMA patients are not merely a result of being a patient with a chronic disease. In contrary to hypopituitarism, preoperative visual field defects and suprasellar tumor extension on radiological imaging were independently associated with fragmentation of the sleep-wake rhythm (**Chapter 5**). Also, all observed alterations, including the increased risk for the metabolic syndrome, were observed in NFMA patients both with and without hypopituitarism.

There are, inevitably, limitations to our conclusions. All are based on observational studies, in which one must refrain from the conflation of association with causation. Therefore, we can only prove the presence of altered circadian rhythmicity in our patients, and not the cause for these alterations. We are also hindered by the current absence of a direct *in vivo* read-out of the integrity and functioning of the SCN. For all bodily functions that were analyzed, however, the SCN serves as the major orchestrator responsible for synchronizing their rhythmic nature to the environmental day-night cycle. These measures of diurnal rhythmicity therefore serve as *indirect* read-outs of the SCN. Of note, a dysfunctioning SCN does not equal a damaged SCN, but might also be the result of decreased SCN input caused by a faulty retinohypothalamic tract, or damage to SCN's output projections. Also, we observed great heterogeneity in affected parameters of circadian rhythmicity. From a pathophysiological point of view, it is conceivable that NFMA cause variable damage to the circadian system, regarding their highly variable clinical presentations and unknown suprasellar growth velocity prior to presentation, as well as the great variety of areas within the SCN and its efferent projections. This is illustrated by studies attempting to specifically ablate SCN in rodents (11). Lastly, all research performed in rare diseases is hampered by relatively small study groups, especially when the protocol is intensive. This might have limited our power to detect small alterations or alterations in indices with large variability. One must however bear in mind that absence of evidence is not evidence of absence.

Strengths of our results include that when offered the choice, we have often taken the most conservative approach, as it is wiser to try to falsify than to verify one's hypothesis. For instance, in **Chapters 3** and **4**, we used extensive exclusion criteria to select the healthiest of patients. This resulted in a homogeneous cohort of patients with limited confounding comorbidity, but who might represent the least likely group with hypothalamic damage. Also, we used bed partners of patients as controls in **Chapters 3-5**, to limit the confounding influence of social-economic factors, lifestyle, and sleep/evening behavior, although the patients' sleep disturbances might have affected that of the controls.

Collectively, the abovementioned arguments in favor of altered SCN function culminate in reasonable certainty.

Implications of results

There are several implications now that we have established that patients in long-term follow-up after supposed successful treatment for NFMA suffer from decreased quality of life, daytime sleepiness, objective and subjective decreased sleep quality, as well as a number of dysregulated circadian bodily functions.

First, the results are relevant to patients. The observations add to the known long-term sequelae of treated NFMA and should therefore be part of the information the

patient receives at diagnosis. Anticipating for the occurrence of these symptoms and acknowledgement of their documented severity might strengthen coping behavior and acceptance, or provide the basis for lifestyle advices. Of note, we studied nonfunctioning pituitary macroadenoma for their absence of a confounding hormone excess syndrome, but it is conceivable that the observed long-term consequences may also be observed in patients with large hormone-producing pituitary macroadenomas.

Second, the results are relevant to treating physicians. It provides the physician with better information to educate and support their patients. Also, one day, after we have established whether the altered circadian rhythmicity is caused by tumor compression before surgery, rapid decompression or surgical manipulation of the suprasellar region in the operating room, or even radiotherapy or (treatment for) hypopituitarism, physicians may be able to interfere earlier and prevent complaints before they happen. For instance, removing the tumor before visual field defects are present, or debulking the tumor with stereotactic radiotherapy or medication before surgery, might prevent patients that have been cured from the illness to remain ill from the cure (or from compression damage). But before that happens, physicians might try to reinforce SCN function in patients with long-term disabilities. Enforcing circadian behavior such as regularization of sleep patterns, for example by maintaining a regular schedule for going to bed and waking up, and engaging in stimulating activities and light exposure immediately after waking up can improve daytime sleepiness (22). Also, bright light therapy is known to significantly improve rest-activity cycles in the elderly (23). Lastly, daily timed intake of melatonin has a profound effect on the electrical activity of SCN neurons (24), in addition to its direct soporific effects on the CNS (25). Re-entraining the SCN with melatonin in hypertensive patients allowed the physiological decrease in blood pressure at night to recur (26), as well as induce visceral fat loss and improvement of the metabolic syndrome in rats (27). Melatonin supplementation may therefore be a promising therapy with limited side-effects, and may be prescribed especially in patients with low melatonin values at bedtime. Lastly, the results advocate stringent control and treatment of cardiovascular risk factors, like in patients with prevalent diseases at increased cardiovascular risk.

Third, the results are relevant to the scientific community. Patients treated for NFMA may now serve as a model of circadian dysrhythmicity with highly probable SCN dysfunction, though with relatively limited comorbidity compared to *e.g.* craniopharyngioma patients. For example, our group recently investigated the influence of the SCN on cardiac autonomic control during sleep using patients treated for NFMA and matched controls (28). Also, comparison of these patients with healthy controls may be used to develop better tools to study *in vivo* SCN functioning, either through imaging (*e.g.* with functional MRI or single-photon emission computed tomography) or with advanced analysis of motor activity (*e.g.* detrended fluctuation analysis to calculate scale-invariant correlations (29)). Next, our results accentuate the fragility of the SCN in withstanding

external influences, as well as its incomplete ability to regenerate. Last but not least, the results show that pituitary diseases can affect the functionality of surrounding tissues, and subsequently a vast array of extra-pituitary organs.

Propositions for future research

Animal models of nonfunctioning pituitary adenoma, such as the MENX-affected p27^{Kip1} rat (30), have the potential to teach us about fragility of the SCN. Normal intrasellar pressure is believed to be around 7-15 mmHg (31, 32), but NFMA can exert pressures on surrounding tissue of up to 60 mmHg (33). In animal models, the diurnal variations of vasopressin in cerebrospinal fluid (34) or immunohistological expression of clock genes in the post-mortem SCN (35) can reveal detailed SCN function in relation to the extent of tumor compression. Those results might aid to estimate the effects of compressing tumors in patients.

For now, it is evident that patients treated for NFMA suffer from long-term sequelae for which there are currently no evidence-based treatment strategies. Although the mentioned therapies of strengthening SCN input and supplementing melatonin are safe and rational, I prefer empiricism over rationalism and suggest studying the effects of these two interventions in patients treated for NFMA, preferably blinded to account for *e.g.* the Hawthorne effect. Clinically relevant outcome measures we now know are sensitive include actigraphy, questionnaires, and serum lipids. Evening melatonin values at start of study might inform us whether treatment effects depend on those baseline values.

Lastly, in order to prevent the discovered long-term sequelae of treated NFMA, the cause of the altered diurnal rhythmicity should be studied. One way would be to assess diurnal rhythmicity, *e.g.* with actigraphy, melatonin or temperature measurements, and questionnaires, at different time points between NFMA diagnosis and long-term follow-up to observe when the alterations occur. Examples of relevant time points are at initial diagnosis, preferably even before and after visual field defects occur, and several weeks after surgery. Detailed analysis of radiological tumor shape and integrity of the optic chiasm (*e.g.* through visual-evoked potentials) might teach us relevant predictors of long-term disability.

PART B: OBSERVATIONS IN THE IGSF1 DEFICIENCY SYNDROME

Clinical care for patients with the IGSF1 deficiency syndrome

Since the discovery of this syndrome three years ago, we have identified 69 hemizygous male patients and 56 heterozygous female carriers with IGSF1 mutations originating from 30 unrelated families. The IGSF1 deficiency syndrome therefore already encompasses

more unique mutations and patients than all other known genetic causes of isolated central hypothyroidism combined (*TSHB* (36-39) and *TRHR* (40, 41)). The observations in patients led to the formulation of recommendations for mutational analysis, endocrine work-up, and long-term care. For a schematic overview of these recommendations, we refer to Table 4 of **Chapter 7**.

Genetic screening

We advise genetic evaluation for *IGSF1* mutations in all patients with central hypothyroidism of unknown cause, especially when accompanied by an X-linked inheritance pattern, prolactin or growth hormone (GH) deficiency, disharmonious pubertal development, macroorchidism, or delayed adrenarche. As asymptomatic adult carriers are likely to benefit from treatment with levothyroxine, family members should be evaluated based on the X-linked inheritance pattern. All children of female carriers (and female children of male patients) should be screened at birth with TSH and T₄.

In the Netherlands, mutational analysis can be performed at the Leiden University Medical Center and the Academic Medical Center of Amsterdam. In other countries, diagnostic centers include the University of Cambridge (United Kingdom), the Hokkaido University School of Medicine in Sapporo (Japan), the National Institutes of Health in Bethesda (Maryland, United States of America), and the University of Adelaide (Australia).

As yet no functional assays to test pathogenicity exist, but pathogenicity of variants of unknown clinical significance may be assessed by transfecting heterologous HEK293 cells with expression vectors specific for the *IGSF1* variant, followed by cell surface biotinylation and immunofluorescence to determine plasma membrane expression of the resulting IGSF1 protein, as described in Sun et al. (42). The laboratory of prof. Bernard at the McGill University in Montréal (Canada) in particular has wide experience with these analyses.

Diagnostic care

After a new family with the IGSF1 deficiency syndrome has been found, we advise referral to a center with expertise in (pediatric) pituitary disease (usually a university hospital) for diagnostic work-up. This work-up comprises careful physical evaluation including height, weight, head circumference, pubic hair, testicular volume, and assessment of signs and symptoms of hypothyroidism. Laboratory tests for serum FT₄, TSH, testosterone, prolactin, cortisol, IGF-1, lipids, and glucose should be conducted, as well as dynamic adrenal axis testing in case of low cortisol values, and (primed) GH stimulation tests and hand X-ray in case of growth failure or low serum IGF-1.

Treatment

We advise treatment with levothyroxine of all affected children, commencement of a trial course in all male adults and in female adults with low(-normal) thyroxine concentrations, and re-assessment of FT₄ concentrations in females before and during pregnancy. Hydrocortisone treatment should be started in neonates with impaired cortisol response in a low-dose ACTH test, recombinant human growth hormone (rhGH) in case of an impaired GH response in a (primed) GH stimulation test, and testosterone in case of a delay in pubertal development in males defined as pubic hair stage 1 or prepubertal testosterone at 14.0 years old.

Follow-up

In children, we advise stringent follow-up of growth, supplemented by IGF-1 concentrations, bone age determination, and (primed) GH stimulation tests in case of growth failure. As GH deficiency (GHD) proved transient in most, but not all, in patients treated with rhGH, GH stimulation tests should be repeated after reaching adult height. Also, we advise to monitor pubertal development annually using height, pubic hair staging, testicular volume, and testosterone concentrations (when testes reach ≥ 4 mL). In addition, treating physicians must be aware of problems with attentional control and with gross motor skills, as some patients benefited from early intervention with psychostimulants, psychotherapy, or physical therapy. Pediatric follow-up should take place at centers with expertise in pediatric pituitary disease or in close relation to such centers.

In adults, follow-up should focus on low testosterone values and the effects of levothyroxine treatment, and may take place at regular endocrine centers with good relations to centers with pituitary expertise. In case of stable situation and little comorbidity, follow-up can be left to the patient's primary physician. Patients with IGSF1 deficiency are generally fertile and have normal life expectancy.

At any time, the Expertise Center Genetics of Growth (which includes expertise on the IGSF1 deficiency syndrome) at the Leiden University Medical Center may be consulted for advice.

IGSF1's role in endocrine physiology

Although the research field involved in unraveling IGSF1's role in endocrine physiology is young, current data enable the formulation of several hypotheses.

The TRH receptivity hypothesis

Theoretically, central hypothyroidism may either be caused by deficient hypothalamic TRH production or delivery to the pituitary; impaired responsiveness of thyrotrophs to endocrine (e.g., TRH, thyroid hormone, somatostatin, glucocorticoids, dopamine) or

autocrine (e.g., neuromedin B, leptin) signals; or a reduced ability to produce or secrete bioactive TSH by the thyrotroph (43). There are several arguments in favor of reduced TRH signaling.

Igsf1^{Dex1} mice show decreased pituitary and serum levels of TSH despite normal pituitary *Tshb* mRNA (42). This combination suggests decreased propagation of the TRH signal, as TRH is mainly involved in maturation, glycosylation, and release of TSH (43), and to a much lesser extent *Tshb* transcription. In agreement, this observation was also made in mice deficient of TRH or TRHR (44, 45). Exogenous TRH stimulation in *Igsf1*-deficient mice induces a blunted serum TSH response (42), and similar observations were made in IGSF1 deficient patients (**Chapter 7**), although this can be caused both by decreased TRH signaling or depletion of thyrotrope TSH reserves. In addition, pituitary *Trhr* mRNA levels in *Igsf1*-deficient mice are decreased by more than 50% compared to wildtype mice (42). Conceivably, this may decrease the potential for TRH to propagate its signal. As there is a concomitant increase in hypothalamic *Trh* mRNA in these animals, suggesting (at least partially) intact feedback of thyroid hormone at the hypothalamic level, the decrease in *Trhr* mRNA may be the result of downregulation in response to the increased TRH levels (46, 47). Dr. Beata Bak (McGill University, Montréal, Canada) investigated this hypothesis by comparing *Trhr* mRNA levels in primary pituitary cultures from male wildtype and *Igsf1*-deficient mice in the absence and presence of TRH (48). In the absence of TRH, *Trhr* was decreased by more than 50% in the knockouts, indicating a pituitary-intrinsic effect of *Igsf1* deficiency on *Trhr* expression. TRH treatment induced a downregulation of *Trhr* mRNA levels in wildtype but not *Igsf1*-deficient cultures, probably because the TRH receptor is needed to induce its own downregulation (48).

In IGSF1-deficient patients, we observed a near loss of diurnal variation of TSH secretion (**Chapter 10**). This observation has also been made in patients with suprasellar extension of a pituitary adenoma both with and without hypothyroidism, suggesting that this feature might reflect hypothalamic dysfunction (*i.e.*, decreased TRH signaling) (49, 50). This notion is furthermore supported by similar secretion patterns in a patient with a mutation in *TRHR* (40) or in rats with lesions in the suprachiasmatic nucleus of the hypothalamus (51). As TRH has a stimulatory effect on GH secretion in neonatal rats and an inhibitory component prevailing in adulthood (52), decreased TRH signaling might also explain the partial GH deficiency in 16% of children with IGSF1 deficiency but increased IGF-1 levels in 20% of adults (**Chapter 7**). Decreased TRH signaling might also contribute to the observed hypoprolactinemia in patients, as TRH is known to stimulate prolactin release (53). However, patients with mutations in *TRHR* show normal levels of prolactin (albeit blunted increases in prolactin after exogenous TRH) (40, 41), in contrast to *Trh-r1* knock-out mice (45).

In **Chapter 11**, we observed expression of IGSF1 in the rat islets of Langerhans within the pancreas and the zona glomerulosa of the adrenal gland. TRH is produced by both

of these structures and acts locally as a growth factor (44, 54-57). Although TRH and its receptor are expressed in Leydig cells of the testis and regulate testosterone secretion (58-60), IGSF1 was not expressed in rat Leydig cells but rather in Sertoli cells (which do not express TRH or its receptor (59)) (**Chapter 11**).

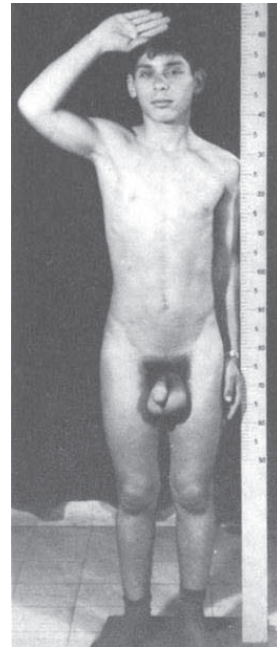
The thyroid hormone signaling hypothesis

The TRH signaling hypothesis cannot explain all alterations seen in patients. For instance, the delayed pubertal testosterone rise despite normal or even advanced testicular enlargement and adult macroorchidism (**Chapter 6**) are not observed in patients with *TRHR* mutations (40, 41). However, these observations may be related to decreased thyroid hormone signaling.

First, there are numerous reports of exactly the same symptoms in untreated prepubertal primary hypothyroidism of various etiology (Figure 1) (1, 61-64), known as the Van Wyk and Grumbach syndrome (65). It has been postulated that in primary hypothyroidism the increased TSH levels stimulate the FSH receptor (64), but as TSH levels are often unmeasurably low in IGSF1 deficiency, our results reject this hypothesis. Rather, it is likely that decreased levels (or signaling) of thyroid hormone are to blame. Thyroid hormone



Patient with untreated primary hypothyroidism at the age of 6.5 years. Note short stature for age, plumpness, and large testicles without pubic hair.



Patient with untreated primary hypothyroidism at the age of 19 years. Note markedly enlarged testicles, volume > 50 ml.

Figure 1. From: Laron et al. (1)

(particularly T_3), shortens the period of immature Sertoli cell proliferation before mitosis ceases and the Sertoli cells differentiate into their non-proliferative adult form (66-69). Hypothyroidism in infancy therefore increases the final number of Sertoli cells (by up to 82-157%), and as the total number of Sertoli cells largely determines testicular size, this results in macroorchidism (66). In addition, T_3 plays a role in postnatal Leydig cell differentiation and steroidogenesis (67), especially around the start of puberty when it increases LH receptors on Leydig cells to aid in the pubertal testosterone rise (70). It is crucial to mention that Van Wyk and Grumbach syndrome is not observed in treated hypothyroidism, and that it is largely reversible after commencing thyroid hormone replacement (1, 71-73). As our observations were generally made in patients with IGSF1 deficiency treated with thyroid hormone since birth, they resemble untreated hypothyroidism, suggesting impaired signaling of (exogenous) thyroid hormone.

A second example is presented by our observation in **Chapter 10** of increased levels of FSH and in some patients prolactin (although others were completely prolactin deficient), while LH secretion was normal. Again, this specific combination has been observed in untreated longstanding primary hypothyroidism (63, 70), possibly due to increased sensitivity of lactotrophs and gonadotrophs to TRH when thyroid hormone levels are low (70). These abnormalities also usually disappear with thyroid hormone replacement (70), which is obviously not the case in IGSF1 deficiency as most were treated during evaluation. Alternatively, the observed relatively high FSH levels (**Chapter 10**) may be caused by slowing of the GnRH pulse generator (74), although IGSF1 is not expressed in GnRH-producing neurons nor in pituitary gonadotrophs (**Chapter 11**) and the pituitary response to GnRH was generally normal (**Chapter 7**). As FSH increases the rate of Sertoli cell proliferation (66), the observed relatively high FSH (although within the reference range) could also contribute to adult macroorchidism.

Third, our observation of mild but robust deficits in attentional control (**Chapter 9**) resemble those in patients and mice with mutations in the thyroid hormone receptor beta, characterized by reduced responsiveness of peripheral and pituitary tissues to thyroid hormone. Several studies confirmed the relation between this defect and especially sustained attention, which was similarly compromised in IGSF1 deficient patients (as was the increased prevalence of attention deficit(-hyperactivity) disorder) (75, 76).

Fourth, we observed expression of IGSF1 in hypothalamic glial cells and the epithelial cells of the choroid plexus, both of which are involved in facilitating feedback of thyroid hormone to the TRH neuron (77).

Limitations

Several limitations prevent definitive conclusions. First, IGSF1 deficiency is a rare disease. Based on the prevalence of central hypothyroidism (1 in 15,000-20,000 (78)), one fourths of which are isolated TSH deficiency, of which the majority may be caused by IGSF1

deficiency, we estimate the prevalence of IGSF1 deficiency to be around 1 in 90,000-100,000. Inherent to studying rare disease is the inability to base conclusions on large sets of patients.

Second, there are limitations to our understanding of where IGSF1 is expressed. The diversity of organs involved in this syndrome prevents a complete analysis of the (endocrine) systems involved, including all cell-types present in *e.g.* the hypothalamus and pituitary gland. The results therefore merely aid in guiding limited time and resources for future research into the right direction. Also, both testicular development and pituitary hormone secretion are influenced by local paracrine crosstalk (52), therefore functioning of cells that do not express IGSF1 can still be influenced by IGSF1 expression on neighboring cells.

Third, our observations in the *Igsf1^{Δex1}*-mice are limited by its incomplete knock-out of a functional IGSF1 protein. The deletion of exon 1 prevents transcription of isoform *Igsf1-1*, but retains isoform *Igsf1-4* (79) which contains the same functional C-terminal domain (but without the N-terminal domain). However, isoform *Igsf1-4* was not readily detected at the protein level in pituitaries of these animals (80), and proved not to be upregulated in *Igsf1^{Δex1}*-mice (48, 79).

Fourth, the presented hypotheses do not allow specification of why expression of the TRH receptor is decreased, *e.g.* by decreased production, transport, depalmitoylation (81), or recycling of the receptor. The same applies to why thyroid hormone signaling would be impaired, *e.g.* by decreased expression or functionality of thyroid hormone membrane transporters, deiodinases, or nuclear receptors (for instance TRα1 in immature Sertoli cells (68, 82)). In addition, thyroid hormone receptivity at the thyrotrope might influence expression of the TRH receptor, and these hypotheses may therefore not be mutually exclusive.

Implications of results

The results of our studies on the recently discovered X-linked IGSF1 deficiency syndrome have several implications.

First and foremost, the results are relevant to patients and their treating physicians, as they led to an understanding of the phenotype of the IGSF1 deficiency syndrome. This will aid in recognizing patients that are longing for a diagnosis, as well as provide them with information on associated signs, symptoms, life expectancy, genetic counseling, possibility to have children, *et cetera*. Also, the recognition of this genetic syndrome offers the opportunity to screen family members for IGSF1 deficiency, with the potential to prevent comorbidity related to untreated hypothyroidism in persons that would have otherwise remained undetected. Next, the results provide the treating physician with previously non-existent recommendations for mutational analysis, endocrine work-up, and long-term care, thereby ultimately improving patient health and wellbeing.

Second, the results are relevant to the scientific community. Rare diseases have the rich potential to teach us about a multitude of common diseases. While classic translational research is used to adapt laboratory results into therapeutic interventions, rare diseases require the opposite path: a keen eye detects the rare patient in clinical practice, the genetic defect is found, and we reproduce this defect in an animal model to study pathophysiology. As rare diseases are generally Mendelian conditions (caused by single-gene defects), in contrast to the often multifactorial etiology of common diseases, they offer a simplified etiology model for studying pathophysiological pathways shared with common diseases. For instance, studying the rare disease familial hypercholesterolemia led to the development of statins, now one of the most widely prescribed drugs in the world (83). Similarly, the single-gene defect knock-out animals serve as a model with similar features to common diseases, as for instance mice with the rare syndrome of generalized resistance to thyroid hormone are used to study attention deficit-hyperactivity disorder and the effects of methylphenidate (76). Studying IGSF1 deficiency and the physiological role of IGSF1 has the potential to elucidate the influence of for instance thyroid hormone transporters or the TRH receptor on the onset and progression of puberty, testicular maturation and function, and attentional control. The results presented in this thesis are one of the first steps in this direction, and may help to focus research resources on relevant tissues, cell types, and pathways.

Last, the results show that genetic pituitary diseases can produce a multitude of extra-pituitary implications that require a multidisciplinary open view.

Future research

In patients, follow-up of the currently phenotyped patient cohort using similar standardized parameters has the potential to teach us about the intra-individual progression of symptoms with age, for instance of adult macroorchidism (no adult growth is consistent with increased number of Sertoli cells, continued growth suggests *e.g.* fibrosis and congestion/edema). Also, a large number of patients did not start treatment with levothyroxine until after phenotyping, and re-analysis of especially the parameters of tissue hypothyroidism will aid in understanding the beneficial effects of treatment and therefore its necessity in patients with few symptoms. Furthermore, obtainment of patient material has been challenging, but physicians may continue to discuss with their patients the opportunities of for instance testicular biopsy (size of seminiferous tubules, transcription of thyroid hormone responsive elements?) during general anesthesia for unrelated surgery, or even post-mortem obtainment of tissue from the pituitary gland (distribution of different cell-types, downregulated TRH receptor?), choroid plexus (integrity of barrier function?), or hypothalamus (upregulated TRH despite treatment, amount of TRH-degrading pyroglutamyl peptidase II from glial cells?). Lastly, in several disorders of altered thyroid hormone signaling, other methods of delivering thyroid

hormone to its nuclear responsive elements have yielded promising results, such as diiodothyropropionic acid (DITPA) in MCT8 deficiency (84) or liothyronine sodium (synthetic triiodothyronine) in generalized resistance to thyroid hormone (85). The latter may not be considered completely experimental, as it has been suggested that perhaps 20% of patients with common causes of hypothyroidism may benefit from T₃/T₄ combination therapy (86). As we learn more about IGSF1's role in endocrine physiology, alternative treatments might benefit outcome in these patients one day.

However, the biggest leaps can be taken by studying IGSF1 knock-out mice, perhaps even in new models lacking isoform *Igsf1*-4. Thyroid hormone or TRH signaling may be studied *in vivo* through altered transcription and translation of responsive genes in different conditions (iatrogenic hypothyroidism, euthyroidism, and hyperthyroidism, or after exogenous TRH). As IGSF1 is expressed in the testes of rat (**Chapter 11**) and human (87, 88) but not in mice (42), studies assessing duration and rate of Sertoli cell proliferation, prepubertal increase in LH receptor expression, blood-testis barrier formation and integrity, and response to exogenous T₄/T₃ may be performed in knock-out rat models. *In vitro*, ligands for IGSF1 may be sought using co-immunoprecipitation or affinity chromatography, and the effects on hormone signaling may be studied in choroid plexus epithelial cells, hypothalamic glial cells, pituitary thyrotropes, or Sertoli cells using primary cell cultures of knock-out and wildtype mice, or knock-down of *Igsf1* expression in *e.g.* thyrotrope-like TaT1 cells using lentiviral vectors expressing anti-*Igsf1* shRNAs. Lastly, given the increased IGF-1 values observed in patients, analysis of 24-hour GH secretion and tissue parameters of GH/IGF-1 action in knock-out mice and patients may reveal novel links between the hypothalamo-pituitary-thyroid axis and somatotroph function.

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