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► **To cite this version:**

Rachel Fourneaux, Rachel Reynaud, Gregory Mougel, Sarah Castets, Patricia Bretones, et al.. IGSF1 mutations are the most frequent genetic aetiology of thyrotropin deficiency. *European Journal of Endocrinology*, 2022, 187 (6), pp.787-795. 10.1530/EJE-22-0520 . hal-04035130

HAL Id: hal-04035130

<https://amu.hal.science/hal-04035130>

Submitted on 4 Apr 2023

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IGSF1 MUTATIONS ARE THE FIRST GENETIC ETIOLOGY OF THYROTROPH DEFICIENCY

Journal:	<i>European Journal of Endocrinology</i>
Manuscript ID	Draft
mstype:	Original Research
Date Submitted by the Author:	n/a
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Keywords:	pituitary deficiency, TSH, Growth hormone, Gene transcription, Anterior Pituitary

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1 **IGSF1 MUTATIONS ARE THE FIRST GENETIC ETIOLOGY OF THYROTROPH DEFICIENCY**

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21 **Word count: 3168 words**

22 **Keywords:** pituitary deficiency, TSH, growth hormone, genetics, IGSF1

23 **Short title:** IGSF1 and genetic TSH deficiency

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27

28 **ABSTRACT**

29 Design: Thyroid-stimulating hormone deficiency (TSHD) is a rare disease. It may be isolated, secondary
30 to abnormalities of genes involved in TSH biosynthesis, or associated with other pituitary deficits or
31 abnormalities of genes involved in pituitary ontogenesis. Several genes are involved in thyrotroph
32 development and function.

33 Objective: Our aim was to determine the genetic causes of TSHD, either isolated (ITSHD) or associated
34 with somatotroph deficiency (TSHDGHD), in the cohort of patients from the GENHYPOPIT network.

35 Methods: Next generation sequencing analyses were performed as a panel of genes on a cohort of
36 patients with non-syndromic ITSHD or TSHGHD. The variants were classified according to the ACMG
37 classification reviewed by the NGS-Diag network and correlated with the phenotype. Class 3, 4 and 5
38 single nucleotide variants (SNVs) were checked by Sanger sequencing and copy number variants
39 (CNVs) by MLPA.

40 Results: A total of 64 index cases (22 ITSHD and 42 TSHDGHD) were included in this cohort. A genetic
41 cause was identified in 26.5% of patients, with 36.3% in the ITSHD group (variants in *TSHB* and *IGSF1*)
42 and 21.4% in TSHDGHD (variants in *IGSF1*, *TSHB*, *TRHR*, *GH1*, *POU1F1*, *PROP1*). Among the pathogenic
43 and likely pathogenic variants identified, 42% were in *IGSF1*, including 6 not previously reported.

44 Conclusion: Our results show that *IGSF1* variants represent the first identified etiology of TSH
45 deficiency. Despite a systematic NGS approach and the identification of new variants, most patients
46 remain without a molecular diagnosis. Larger scale studies, such as exome or genome studies, should
47 be considered in the future.

48 INTRODUCTION

49 Congenital central hypothyroidism (CCH) is a rare disorder with an estimated incidence of 1/16000 to
50 1/30000, depending on the neonatal screening strategy (1). It is due to a defect in the hypothalamic–
51 pituitary–thyroid axis, resulting in insufficient stimulation of the thyroid gland by thyroid-stimulating
52 hormone (TSH). CCH can be diagnosed as a single pituitary deficiency (Isolated TSH deficiency, ITSHD)
53 when the defect occurs in genes involved in the TSH biosynthesis pathway, such as thyroid stimulating
54 hormone subunit β (*TSH β*) and the thyrotropin-releasing hormone receptor (*TRHR*). Three additional
55 genes that may be involved have been identified in the last decade: immunoglobulin superfamily
56 member 1 (*IGSF1*), transducin-like protein 1 (*TBL1X*), and insulin receptor substrate 4 (*IRS4*) (2-4). *IGSF1*
57 mutations, first characterized by Sun *et al.* in a complex phenotype that included TSHD in 100% cases,
58 were further associated in larger series with transient partial growth hormone deficiency (GHD) in
59 childhood, increased IGF-1 levels in adulthood, prolactin deficiency, disharmonious puberty with
60 premature testicular growth contrasting with a delayed testosterone rise and late adrenarache, adult
61 macro-orchidism and an increased body mass index (2, 5). CCH can also be diagnosed as part of a
62 multiple pituitary hormone deficiency: in this context, which is the most frequent, constitutional
63 growth hormone deficiency, gonadotroph deficiency or, more rarely, corticotroph deficiency, may be
64 associated: the mechanism is usually due to mutations in genes coding for pituitary transcription
65 factors, as our group and others have shown over the last 20 years (6, 7).

66 The GENHYPOPIT network was launched as a multicentric international adult and paediatric
67 endocrinology network, aimed at defining new mechanisms and aetiologies of constitutional
68 hypopituitarism. With more than 1400 samples included, it is a unique clinical and biochemical
69 database allowing the specific study of a group of patients with a given phenotype (7). Apart from one
70 small Japanese cohort, and with the exception of the study by Joustra *et al.*, which focused only on
71 *IGSF1* variants (8, 9), most of our knowledge concerning genetic etiologies of ITSHD came from case
72 reviews or case reports. The most recent study by Elizabeth *et al.* was also only screening for variants
73 in the extracellular region of *IGSF1* in a large cohort of 94 patients with TSHD and GHD (10). Therefore,

74 using the GENHYPOPIT data, we undertook genetic screening in a large cohort of non-syndromic ITSHD
75 and TSHD associated to GHD (as children with IGSF1 deficiency might present with GHD). Our objective
76 was to determine the prevalence of genetic defects in constitutional TSHD based on a systematic NGS
77 approach, with a special focus on pathogenic and likely pathogenic *IGSF1* variants.

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78 PATIENTS AND METHODS

79 Patients

80 The characteristics of the GENHYPOPIT network have been recently reported (7). Briefly, both pediatric
81 and adult patients, regardless of age or country of origin, were included in the GENHYPOPIT network
82 database when they presented at least one constitutional primary anterior pituitary hormone
83 deficiency. Patients or parents of minor patients gave their written informed consent. The study was
84 approved by the Ethics committee of the University of Aix-Marseille II (France).

85 A declaration was made to the National Commission for Data Protection and Liberties (CNIL-France):
86 1991429v0. Data were anonymously collected in a database declared to health authorities in
87 accordance with local regulations. Investigators were prompted to send an index case DNA sample
88 when other distinguishable etiologies of pituitary deficiency had been ruled out by routine anamnestic,
89 clinical, biological, and neuroradiological workup. A familial case was defined by the presence of at
90 least one other known case of constitutional hypopituitarism among the relatives of the index case.

91 For this specific study, the data of all patients with isolated constitutional thyroid deficiency (ITSHD) or
92 associated with somatotrophic deficiency (TSHD-GHD) who were included in the GENHYPOPIT database
93 between 2008 and 2020 were enrolled. Patients with other pituitary deficiencies (gonadotropic,
94 corticotropic), panhypopituitarism, or syndromic forms (pituitary stalk interruption, midline
95 malformations, ocular malformations etc.) were excluded from the study.

96 Genetic analyses

97 High-throughput sequencing (Next Generation Sequencing, NGS) has been routinely available in
98 clinical laboratories since 2017. First, a retrospective extraction of NGS data from 2017 to 2020 was
99 performed. Variants found in the population of interest, between 2017 and 2020, from the panels of
100 non-syndromic combined pituitary deficiencies (CPHD panel: *GLI2*, *HESX1*, *IGSF1*, *LHX3*, *LHX4*, *POU1F1*,
101 *PROP1*, *SOX3*, *TBX19*, *GH1*) and ITSHD (*TSH β* , *TRHR*, *IGSF1*, *TBL1X*) were therefore retrospectively
102 extracted for analysis. Then, a new panel NGS analysis was performed by selecting patients from the

103 population of interest who did not have a molecular diagnosis between 2008 and 2017. Genetic
104 analyses prior to 2017 were indeed done using Sanger sequencing on phenotype-targeted genes (7).
105 For NGS, exons and 20-nucleotide intronic terminals were amplified by NGS using a QIAseq Targeted
106 DNA Custom Panel library (Qiagen, Courtaboeuf, France) according to manufacturer's
107 recommendations. Sequencing of the samples then took place on a MiseqDX (Illumina) with the Miseq
108 V2 300 cycle Reagent kit (Illumina, San Diego, CA, USA). Alignment and variant calling were performed
109 with the CLC Genomic Workbench software (Qiagen). All variants were annotated using VariantStudio
110 v2.2 software (Illumina), in the GRCh37 human genome with HGVS_c and HGVS_p nomenclatures
111 according to international recommendations. Sanger sequencing was performed to monitor and
112 confirm single nucleotide variation (SNV) class 3, 4 and 5 variants in index cases and to look for their
113 presence in relatives. Using primers specific to the relevant exons, sequencing was performed on an
114 ABI 3500xl DxGenetic Analyzer (Thermo Fisher Scientific, Mass, US). The presence of CNVs (Copy
115 Number Variation) was investigated using the CNV module of the CLC Genomic Workbench software.

116 **Classification of variants**

117 The GnomAD (Genome Aggregation Database) version 2.1.1 was consulted to establish the allelic
118 frequency of variants in the control population and in the most represented subpopulations. Public
119 databases of variants were also consulted (HGMD version, ClinVar and LOVD).

120 For *in silico* analysis, Alamut Visual® software was used to predict the impact of missense variants
121 providing the evolutionary conservation of an amino acid or nucleotide, the location in the protein
122 sequence and the predictions of the biochemical consequence of the amino-acid substitution (Align
123 GVGD, SIFT, Polyphen-2). To study the impact of synonymous, missense and intronic variants on
124 splicing, HSF (Human Splicing Finder) and SPiCE tools were used.

125 A pathogenicity score was established according to the ACMG criteria. This score was used to classify
126 variants as "pathogenic (PV)", "likely pathogenic (LPV)", "uncertain significance (VUS)", "likely benign
127 (LBV)" and "benign (BV)" (11).

128 **Statistical analysis**

129 Statistical analyses were performed with Prism (version 9.1.1, 1994-2021, GraphPad Software, LLC).
130 Univariate comparisons were performed using Student or Welch t-test for quantitative data (a p-value
131 ≤ 0.05 was considered significant).

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132 RESULTS

133 Phenotypic description of the cohort

134 Sixty-four patients in total were evaluated in this cohort study, including 22 ITSHD (34.4%) and 42
135 TSHD-GHD (65.6%). Median age at the time of genetic analysis was 14 years [4.1-19.9]. Sex ratio
136 showed a male predominance (M/F= 2.2). Among all the index cases, 18.7% were familial cases of
137 combined pituitary hormone deficiency and 10.9% were from consanguineous unions. In the 42
138 patients with TSHD-GHD, GHD appeared to be diagnosed earlier than TSHD, with respective median
139 ages of 4 [1.7-10] and 9 years [0.8-14] ($p=0.023$). Total prevalence of likely pathogenic and pathogenic
140 genetic defects was 26.5%, with a significant difference between ITSHD and TSHD-GHD populations
141 (36.3% and 21.4% respectively) ($p=0.038$). Characteristics of the cohort are detailed in Table 1. No CNV
142 was identified in the samples from this cohort.

143

144 Genetic analyses

145 We identified a total of 8 mutations (36.4%) in the ITSHD group, only in genes from the ITSHD panel (6
146 *IGSF1* and 2 *TSHB*). In the second group of patients presenting with TSHD-GHD, we identified 9
147 mutations (21.4%) in 5 genes included in both the ITSHD and CPHD panels (2 *POU1F1*, 3 *PROP1*, 1 *GH1*,
148 1 *TRHR* and 2 *IGSF1*). The classification of these variants and their phenotypic presentation are shown
149 in Table 2A (ITSHD) and 2B (TSHD-GHD).

150 - *IGSF1* pathogenic and likely pathogenic variants

151 Six new truncating pathogenic and likely pathogenic *IGSF1* variants were identified (4 indels inducing
152 frameshift and thus a premature stop codon, and 2 nonsense variants, see Table 2). The seventh likely
153 pathogenic variant was a missense variant, previously described in the literature [5], in three families
154 with a convincing genotype-phenotype correlation.

155 The phenotypes associated to these variants were diverse. Within the ITSHD group, three were
156 diagnosed at birth, two on severe neonatal signs (including one with undetectable thyroid hormone
157 levels) and one on neonatal screening. The last of these was diagnosed later, on delayed psychomotor

158 development associated with overweight. In the group of GHD-TSHD, both were diagnosed on growth
159 failure around puberty (see Table 2). GHD was transient and treated until the age of 13 years for the
160 c.2548C>T (p.Arg850*) variant and until 18 years for the c.3711G>A (p.Trp1237*) variant.

161 Regarding ACMG criteria, only one newly identified *IGSF1* variant (c.2485dup, p.Ala829Glyfs*15) could
162 be classified as pathogenic. Indeed, this variant was observed in 2 different families, including one with
163 7 members (three heterozygous asymptomatic females and four hemizygous symptomatic males)(12).
164 The other five were classified as likely pathogenic since they induced a premature stop codon and were
165 absent from GnomAD (PVS1 + PM2).

166 Of note, another rare *IGSF1* missense variant (c.929A>T, p.Asp310Val) was considered as pathogenic
167 according to most *in silico* predictions. However, it did not fulfil ACMG pathogenic criteria. The
168 modified amino acid was located in the N terminal region of the *IGSF1* protein (no pathogenic variant
169 described in the literature and physiological role different from the C-terminal region), and while rare
170 according to our selection criteria, its GnomAD frequency approached overall ITSHD prevalence. It was
171 thus finally classified as a variant of uncertain significance.

172 - **Other variants of interest identified**

173 *GH1* variant: c.291+4A>C

174 The female patient carrying this variant was born at term and presented with neonatal hypoglycemia,
175 a classical sign of neonatal GHD. We hypothesized that the TSHD was also diagnosed early (before 18
176 months of life, as we received the DNA by this time). She also had dysmorphic features including a
177 bulging forehead, broad nasal root, and late dentition. The c.291+4A>C variant is located in intron 3
178 [10]. It is not listed in the population database (PM2). *In silico*, HSF predicts an alteration of the natural
179 donor site of 14.1% and SPiCE predicts a splicing effect at 99% (PP3). Regarding co-segregation data,
180 the mother and half-sister were both heterozygous for this mutation and displayed IGHD since
181 childhood (PP1). The transmission here is consistent with the autosomal dominant type II familial form
182 of GHD for which several intronic variants have been described in intron 3, in positions +1, +2, +5, +6
183 (13). Moreover a variant was also described in IGHD patients in the same position, but with a different

184 change in nucleotide (c.291+4A>T) (14). It can be therefore considered a mutational hotspot (PM1).
185 Functional studies have shown that these intronic variants in position +1, +2, +5, +6 led to the skipping
186 of exon 3 and encoded an alternative isoform with molecular weight 17.5 kDa (15). In vitro
187 (neuroendocrine cell culture – somatotroph, corticotroph) and in vivo models have established the
188 dominant negative effect of this mutant isoform on the wild-type protein of 22 kDa (15, 16). Thus, we
189 classified this variant as likely pathogenic.

190 TRHR variant: c.317A>G (p.Tyr106Cys)

191 Surprisingly, the initial clinical presentation was an isolated GH deficiency diagnosed on failure to thrive
192 in a 10-year-old boy of Armenian origin. Based on a partial GH response (Glucagon-Betaxolol: basal GH
193 0.2 mUI/L, peak 8.5 mUI/L) he received growth hormone therapy for 2 years, which was effective on
194 his growth curve (-2.5 DS to -1.5 DS, target height 1.65m). TSHD was diagnosed later at the age of 12
195 years. His GHD was transient, as it was reevaluated in adulthood by a provocative test (insulin tolerance
196 test) and response was normal, while TSHD was still present after age 16. Pituitary MRI was normal.
197 Of note, two brothers had short stature without growth failure. The manifestation of TSHD at this age
198 was similar to those reported with other *TRHR* mutations (PP4) (17-20). This missense variant in the
199 first exon of the *TRHR* gene was not listed in the population database (GnomAD V2.1.1) (PM2). *In silico*,
200 the affected nucleotide was moderately conserved (phyloP : 3,43 [-14,1 ; 6,4]) but the amino-acid was
201 highly conserved in up to 18 species. Align GVGD score was high. Prediction software programs were
202 in favor of a pathogenic effect of this variant (PP3). Lastly, the modified amino-acid was located on the
203 third transmembrane domain of the protein adjacent to the other known mutations (positions 49, 242,
204 392, 343) making it a mutational hotspot (PM1) (17-20). We thus classified this variant as *likely*
205 *pathogenic* according to the American College of Medical Genetics (ACMG) criteria (11).

206 TSHB variant: c.373delT (p.Cys125Valfs*15)

207 Mutations in the *TSHB* gene are a well-known cause of TSHD (21-23). This homozygous genotype was
208 encountered in 3 patients. Apart from the 2 cases presenting with a typical neonatal ITSHD, a third
209 phenotype, in an individual born from a consanguineous union, was labelled as GHD-TSHD. TSHD was

210 diagnosed in the neonatal period on typical symptoms of hypothyroidism (see Table 2). Of note,
211 provocative tests on GH were performed in a severe state of hypothyroidism before treatment (TSH
212 0.03 mUI/L; fT4 <0.5 pmol/L; fT3 1.4 pmol/L and IGF-1 29 ng/mL). No precise diagnosis nor follow-up
213 data was available for this subject.

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214 DISCUSSION

215 With a systematic NGS approach in our cohort, the rate of 21.4% and 34.6% identified genetic
216 etiologies of TSHD-GHD and ITSHD, respectively, was higher than in our overall cohort of constitutional
217 hypopituitarism in which only 7.3% had identified genetic etiologies. Our results emphasize the need
218 to focus on a specific group of patients with a homogeneous phenotype when trying to identify new
219 genes involved in constitutional hypopituitarism, an approach which can be easily performed via the
220 GENHYPOPIT network (as an example, ITSHD or combined GHD-TSHD represents 1.8% of our whole
221 cohort of patients). Sugisawa *et al.*, in a smaller cohort of 13 patients, already reported that 46% of
222 these ITSHD patients had identified genetic etiologies (9). This selective approach has proven to be
223 successful, for instance, in the discovery of *IGSF1* pathogenic variants in TSHD (24). Although a large
224 number of patients were presented in other studies (5, 8, 10), the percentage of *IGSF1* mutations in
225 the total ITSHD population was not specified. However, this also means that 63% of our patients (n=14
226 index cases) with constitutional TSHD still have no identified genetic etiology. Obviously,
227 environmental factors, variants in intronic or promoter regions of known genes, or variants in other
228 genes could also be involved. Future studies using a whole genome approach are needed to attempt
229 to identify other genetic causes of TSHD.

230 Pathogenic variants of *IGSF1* should now be considered the first genetic etiology of TSH deficiency
231 (42% of identified genetic etiologies in our cohort). This had been previously suggested but has never
232 been shown in a large cohort of TSHD with a homogeneous phenotype (5, 9). Of note, only *IGSF1* and
233 *TSHB* variants (including the frequently reported c.373del (p.Cys125Valfs*10) variant) (21) were
234 identified in the group of patients with ITSHD. We report here 6 new pathogenic variants of *IGSF1* in
235 the region coding for the C-terminal part of the protein, all inducing either a protein that is truncated
236 in its C-terminal region, or absent, since nonsense mRNA is subject to the nonsense-mediated decay
237 phenomenon. This was also the case for the majority of previously identified variants. We also report
238 slight changes in the clinical presentation of patients with *IGSF1* deficiency. Firstly, we observed
239 transient GH deficiency in 25% of cases, which is higher than the 16% reported in Joustra *et al.* (5).

240 Secondly, we observed a particularly severe case with undetectable levels of thyroid hormones in a
241 child with the *c.2485dup IGSF1* variant, whereas thyroid hormones are usually detectable in patients
242 with *IGSF1* pathogenic variants. This severe phenotype is more frequently observed in children with
243 pathogenic variants of *TSHB* (21). Finally, one of our pediatric patients had bilateral congenital
244 deafness, which has not previously been reported in patients with *IGSF1* deficiency. An association
245 between congenital hypothyroidism and deafness is still a matter of debate; however, as deafness has
246 only been rarely reported in patients with constitutional TSH deficiency (25-27), we hypothesize that
247 this could be due to a direct effect of *IGSF1*.

248 The group of patients with GHD-TSHD shows a high rate of pathogenic and likely pathogenic variants
249 of well-known genes involved in constitutional combined pituitary hormone deficiency, such as
250 *POU1F1* and *PROP1* (7). This was expected based on previously published data, and incomplete follow-
251 up data at an adult age did not provide additional information on other delayed pituitary deficiencies
252 in our patients (7). One likely pathogenic variant of *GH1* was also identified in this group of our cohort:
253 while the phenotype associated with *GH1* dominant mutations usually includes isolated GH deficiency,
254 TSH and ACTH deficiency were also reported (28). Surprisingly, we also identified a new homozygous
255 missense variant of *TRHR* (*c.317A>G*, p.Tyr106Cys). This patient presented with a partial and transient
256 GHD, similar to that reported in 25% of our patients with *IGSF1* pathogenic variants. To our knowledge,
257 this is the first time GHD has been reported in this genetic setting, as the 4 previously reported cases
258 only presented with ITSHD (and growth retardation and overweight during childhood) (17-20). The
259 possibility of an associated mutation in other genes involved in GHD was excluded by further genetic
260 analyses on *GHRHR*, *GHSR* and *RNPC3* genes (data not shown). Our data thus emphasize the need to
261 systematically analyze *IGSF1*, *GH1*, *POU1F1*, *PROP1*, *TSHb* and *TRHR* in patients with non-syndromic
262 TSHD and GHD during childhood, at least in those cases where genetic screening is requested soon
263 after the diagnosis.

264 The main limitation of our study is due to the timing of data collection for each patient. Retrospective
265 analysis of phenotypes showed that we were indeed missing clinical, biochemical and imaging data on

266 a long-term follow-up in several cases. Age at diagnosis may also have been confounded with the age
267 at which the genetic diagnosis was suspected. The adult phenotype of patients with *IGSF1* variants
268 could not be retrieved, likely leading to an incomplete phenotype. Since we had mostly clinical data
269 from childhood, we cannot extrapolate on the rate of macro-orchidism, a sign that was reported in
270 88% of adult patients with *IGSF1* deficiency, nor on prolactin deficiency (60% cases), the biochemical
271 definition of which is difficult to determine at that age and not systematically screened for in routine
272 explorations.

273 To conclude, our study, based on a unique large cohort of patients with constitutional TSH deficiency,
274 shows a high proportion of *IGSF1* variants in this disease, clearly more frequent than *TSHb* and *TRHR*
275 causal variants. Thanks to a homogeneous definition of included phenotypes, we were able to identify
276 a high rate of genetic etiologies, including some atypical etiologies such as the *TRHR* variant. Our study
277 also points to the need to replace the candidate gene approach by a gene panel approach, which could
278 also include genes known to be involved in combined pituitary hormone deficiency. Future work
279 should now focus on as yet unidentified etiologies or mechanisms underlying TSH deficiency in the 14
280 patients of our cohort who, to date, had no genetic diagnosis. One such candidate would be *IRS4* (3),
281 a gene for which putative involvement in central hypothyroidism has been recently reported, after the
282 design of NGS in our study.

283

284 **Declaration of interest:** No conflict of interest that could be perceived as prejudicing the impartiality
285 of the research reported is reported.

286 **Funding:** This work was supported by a grant from the ANR GENOPAT 2008 « MDPHD », from
287 MARMARA Institute (Aix Marseille University) and from ADEREM, Marseille, France.

288 **Acknowledgements:** The authors are indebted to all clinicians (see list of Genhypopit investigators in
289 [7]) who sent clinical information and blood samples for the genetic studies, and especially Dr Bergada
290 and Dr Koren, and to all patients and their families for accepting to take part in this research project.

291 We thank Morgane Pertuit and Sylvie Palazzolo for expert technical support and Bérengère Saliba-
292 Serre for her help in statistical analyses.

293

294 **LEGEND TO TABLES**

295 **Table 1**

296 Characteristics of the whole cohort (index cases). n: number of cases with this information in the
297 database. Values are presented as percentages, median and Interquartile Range (IQR).

298

299 **Table 2**

300 A/Identified variants in HGVS_c and HGVS_p nomenclature and associated phenotypes in patients with
301 isolated TSH deficiency. ACMG classification for SNV (Richards) and ACMG/ClinGen for CNV (Riggs).

302 HMZ : Homozygous - HmZ : Hemizygous - HTZ : Heterozygous.

303 B/Identified variants in HGVS_c and HGVS_p nomenclature and associated phenotypes in patients with
304 combined growth hormone and TSH deficiency. ACMG classification for SNV (Richards) and

305 ACMG/ClinGen for CNV (Riggs). HMZ : Homozygous - HmZ : Hemizygous - HTZ : Heterozygous

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395

	n	Cas index (=64)
ITSHD patients	22	22 (34.4%)
TSHD-GHD patients	42	42 (65.6%)
Sex	64	44M (68.7%) / 20F (31.3%)
Median age at genetic analysis	64	14.2 [4.1-19.9]
Median age at TSHD diagnosis	41	9 [0.8-14]
Median age at GHD diagnosis	25	4 [1.7-10]
Familial cases	49	12 (24.5%)
Consanguineous cases	40	7 (17.5%)
MRI anterior pituitary anomalies	47	21 (44.7%)
Percent with likely pathogenic and pathogenic variants	64	17 (26.5%)

Table 1: Characteristics of the whole cohort (index cases). n: number of cases with available information in the database. M, males; F, females. Age, years. MRI, magnetic resonance imaging. Values are presented in percentages, median and Interquartile Range (IQR)

B - TSHDGH							
Genotype				Phenotype			
A - ITSHD							
Genotype				Phenotype			
Gene	Variant	RS	ACMG criteria	Age at diagnosis	Clinical signs	Pituitary MRI	Others
<i>TSHβ</i>	c.205C>T (p.Gln69*) HMZ	rs121918670	Pathogenic	0.1	Myxedema; constipation	Normal	None
<i>IGSF1</i>	c.2485dupG (p.Ala829Glyfs*15) HmZ		Pathogenic	0	Respiratory Distress Syndrome (RDS); jaundice; hypotonia; breastfeeding difficulties	Normal	None
<i>TSHβ</i>	c.373delT (p.Cys125Valfs*10) HMZ	rs755485552	Pathogenic	0	Jaundice	Normal	None
<i>IGSF1</i>	c.1966G>A (p.Gly656Arg) HmZ		Likely pathogenic	0	Neonatal screening	Unknown	None
<i>IGSF1</i>	c.2485dupG (p.Ala829Glyfs*15) HmZ		Pathogenic	0	Jaundice; hypotonia; breastfeeding difficulties	Unknown	Pneumothorax
<i>IGSF1</i>	c.2909dupA (p.Pro971Alafs*6) HmZ		Likely pathogenic	X	Overweight; delayed psychomotor development at 3 yr	Unknown	None
<i>IGSF1</i>	c.2279_2280delinsC (p.Lys760Thrfs*13) HmZ		Likely pathogenic	4	Neonatal jaundice and hypotonia; overweight and delayed psychomotor development at 3 yr	Normal	None
<i>IGSF1</i>	c.2292delC (p.Ser765Valfs*8) HmZ		Likely pathogenic	0	Neonatal screening	Normal	None

Gene	Variant	ACMG Criteria	RS	Age at TSHD diagnosis	Age at GHD diagnosis	Clinical signs	Anterior Pituitary MRI	Others
<i>IGSF1</i>	c.2548C>T (p.Arg850*) HmZ	Likely pathogenic		14	10	Failure to thrive	Hypoplasia	Bilateral hearing loss; pectus excavatum
<i>IGSF1</i>	c.3711G>A (p.Trp1237*) HmZ	Likely pathogenic		12	13	Failure to thrive	Normal	None
<i>TSHβ</i>	c.373delT (p.Cys125Valfs*15) HMZ	Pathogenic	rs755485552	0	0	Jaundice; hypotonia; constipation; feeding difficulties; constipation	Normal	Atrial septal defect
<i>TRHR</i>	c.317A>G (p.Tyr106Cys) HMZ	Likely pathogenic		10	10	Failure to thrive	Normal	Transient GHD
<i>GHI</i>	c.291+4A>C HTZ	Likely pathogenic		1.6	1.6	Hypoglycemia	Unknown	Dysmorphic feature
<i>POUIF1</i>	c.811C>T (p.Arg271Trp) HTZ	Pathogenic	rs104893755	0.3	X	Jaundice; hypotonia; hypoglycemia	Unknown	Dysmorphic feature
<i>POUIF1</i>	c.404T>G (p.Phe135Cys) HMZ	Pathogenic	rs104893761	0	0	Feeding difficulties; hypotonia	Normal	Dysmorphic feature; bilateral renal hypoplasia; micropenis
<i>PROPI</i>	c.2T>C (p.Met1?) HTZ	Pathogenic		3.8	3.8	Failure to thrive	Normal	Strabism
<i>PROPI</i>	délétion ex 2-3 HTZ	Likely pathogenic		3.8	3.8	Failure to thrive	Normal	Strabism
<i>PROPI</i>	c.301_302delAG (p.Leu102Cysfs*8) HTZ	Pathogenic	rs193922688	9	9	Failure to thrive	Hyperplasia	None
<i>PROPI</i>	c.150del (p. Arg53Aspfs*112) HTZ	Pathogenic	rs587776683	9	9	Failure to thrive	Hyperplasia	None

Table 2

A: Identified variants in HGVS_c and HGVS_p nomenclature and associated phenotypes in patients with isolated TSH deficiency. ACMG classification for SNV (Richards) and ACMG/ClinGen for CNV (Riggs). HMZ : Homozygous - HmZ : Hemizygous - HTZ : Heterozygous.

B: Identified variants in HGVS_c and HGVS_p nomenclature and associated phenotypes in patients with combined growth hormone and TSH deficiency. ACMG classification for SNV (Richards) and ACMG/ClinGen for CNV (Riggs). HMZ : Homozygous - HmZ : Hemizygous - HTZ : Heterozygous