Supplemental Table 1. The list of targeted 23 genes, coding base pair numbers and coverage ratios

Gene	**Transcripts Reference Sequence**	**Base pair numbers of coding regions**	**Percentage of covered (@20x) coding base pairs**		
			Patient 4	**Patient 8**	**Other 46 patients**
DUOX1	NM_175940.2	4656	98.45	97.64	100
DUOX2	NM_014080.4	4647	97.93	96.82	100
DUOXA2	NM_207581.4	963	100	100	100
FOXE1	NM_004473.4	1122	100	100	100
GLIS3	NM_152629.3	2793	100	100	100
GNAS	NM_080425.3	3114	100	100	100
IGSF1	NM_001555.4	4026	100	100	100
IYD	NM_203395.2	982	99.29	100	100
NKX2-1	NM_001079668.3	1206	97.35	96.43	100
NKX2-5	NM_004387.4	975	100	100	100
PAX8	NM_003466.4	1353	100	100	100
SECISBP2	NM_024077.4	2565	99.81	100	100
SLC16A2	NM_006517.4	1620	100	100	100
SLC26A4	NM_000441.2	2343	99.91	100	100
SLC5A5	NM_000453.3	1932	100	100	100
TG	NM_003235.5	8307	100	100	100
THRA	NM_003250.5	1596	100	100	100
THRB	NM_000461.4	1386	100	100	100
TPO	NM_000547.5	2802	100	100	100
TRH	NM_007117.4	729	100	100	100
TRHR	NM_003301.5	1197	100	100	100
TSHB	NM_000549.4	417	100	100	100
TSHR	NM_000369.3	2295	97.56	98.95	100
Total		**53 026**	**99.994**	**99.993**	**100**
Mean coverage			**41**	**55**	**270**

Supplemental Table 2. Clinical characteristics of the patients and detailed information of variants identified from the reclassified cohort

No.	Clinical characteristics									Detailed information and classification of variants									Final status
	Age (day)	Dx.	Sex	Consan-guinity	Family History	TSH mU/l Ref.*	FT4 ng/dl Ref.*	Tg ng/ml Ref.*	Thyroid Morphology (US / Scan)	Gene	Inheri tance	cDNA change	Amino acid change	ExAC/ GnomAD MAF	Zygosity	HGMD Class	ACMG Class	*In silico* prediction	
Permanent Congenital Hypothyroidism																			
#9	2604	C	F	Yes	No	84.6	0.426	<0.04	US: Goiter	*TG*	R	c.961C>T	R321*	0.000008	Hom	Known	LP	Pathogenic	Solved
#25	18	NS	M	No	No	>170	0.12	<0.04	US/Scan: Normal	*TG*	R	c.2744_2748del	P915Qfs*10	NA	Het	Novel	LP	NA**	Ambiguous
#32	**240**	**NS**	**M**	**No**	**Yes**	**9.5**	**0.98**	**1.32**	**Scan: Normal**	***TG***	**R**	**c.3149G>T**	**W1050L**	**0.000592**	**Het**	**Known**	**VUS**	**Pathogenic**	**Ambiguous**
#44	43	NS	F	No	No	>100	0.54	4.4	Scan: Normal	*TG*	R	c.3217+1G>A	-	NA	Het	Novel	P	Pathogenic	Ambiguous
#2	7	NS	F	Yes	Yes	>100	0.007	0.06	US: Goiter	*TG*	R	c.4528+1G>A	-	NA	Hom	Known	P	Pathogenic	Solved
#43	13	NS	M	Yes	No	>100	0.02	<0.04	US: Goiter	*TG*	R	c.4528+1G>A	-	NA	Hom	Known	P	Pathogenic	Solved
#30	**19**	**NS**	**M**	**No**	**No**	**58.3**	**0.887**	**165**	**US: Normal**	***TG***	**R**	**c.638+5G>A**	**P161Ffs5***	**0.000025**	**Het**	**Known**	**VUS**	**Pathogenic**	**Ambiguous**
										DUOX2	**R, D**	**c.1300C>T**	**R434***	**0.000091**	**Het**	**Known**	**P**	**Pathogenic**	
#34	21	NS	M	No	No	>100	<0.40	4.96	Scan: Normal	*TG*	R	c.638+5G>A	P161Ffs5*	0.000025	Het	Known	VUS	Pathogenic	Ambiguous
										SLC26A4	R	c.1031G>A	S344N	0.000008	Het	Novel	VUS	Benign	
#41	22	NS	M	Yes	No	>100	0.23	<0.04	US: Goiter	*TG*	R	c.2103dupA	E702Rfs*6	NA	Hom	Novel	LP	NA**	Solved
										IYD	R, D	c.679G>T	A227S	0.000025	Het	Novel	VUS	Pathogenic	
#27	11	NS	M	No	No	>100	0.023	334.4	US/Scan: Normal	*TPO*	R	c.265C>T	R89*	0.000008	Het	Known	P	Pathogenic	Solved
										TPO	R	c.1477G>A	G493S	0.000074	Het	Known	P	Pathogenic	
#20	6	NS	M	Yes	No	>100	0.237	461.9	US: Goiter	*TPO*	R	c.1618C>T	R540*	0.000012	Hom	Known	P	Pathogenic	Solved
#37	26	NS	F	Yes	No	52.2	1.05	17.3	US: Normal	*TSHR*	R, D	c.326G>A	R109Q	0.000016	Hom	Known	VUS	Pathogenic	Solved
#33	16	NS	M	Yes	Yes	>100	0.6	7.42	Scan: Normal	*TSHR*	R	c.1422C>A	D474E	0.000008	Hom	Known	P	Pathogenic	Solved
#23	4006	C	M	Yes	Yes	>100	0.59	3.67	US: Hypoplasia	*TSHR*	R	c.1422C>A	D474E	0.000008	Hom	Known	P	Pathogenic	Solved
										FOXE1	R	c.689G>T	R230L	0.000024	Het	Novel	VUS	Benign	
										TG	R	c.8006A>G	N2669S	0.000008	Het	Novel	VUS	Pathogenic	
#29	20	NS	M	Yes	No	>100	0.43	3.17	US: Hypoplasia	*TSHR*	R, D	c.1582C>T	R528C	0.000008	Hom	Known	P	Pathogenic	Solved
										DUOX2	R, D	c.2056C>T	Q686*	0.0000039	Het	Known	P	Pathogenic	
#48	137	NS	M	No	No	>100	1.04	4.08	US/Scan: Hypoplasia	*PAX8*	D	c.91C>T	R31C	0.000008	Het	Known	LP	Pathogenic	Solved
#13	27	NS	F	No	No	>100	0.279	17.2	US/Scan:	*PAX8*	D	c.251_265del	A84-V88del	NA	Het	Novel	LP	NA**	Solved

									Hypoplasia										
#5	18	NS	F	No	Yes	34.8	0.92	-	US: Normal	-		-	-	-	-	-	-	-	Unsolved
#8	17	NS	F	No	No	>150	0.254	17.8	US/Scan: Hypoplasia	-		-	-	-	-	-	-	-	Unsolved
#31	12	NS	F	Yes	No	56.3	1.34	-	Scan: Normal	-		-	-	-	-	-	-	-	Unsolved
#35	28	NS	M	No	No	>100	0.57	>300	Scan: Normal	-		-	-	-	-	-	-	-	Unsolved
Persistent Hyperthyrotropinemia																			
#7	**6**	**NS**	**M**	**Yes**	**Yes**	**87.84**	**1.09**	**5.03**	**US: Normal**	***TSHR***	**R, D**	**c.1422C>A**	**D474E**	**0.000008**	**Het**	**Known**	**P**	**Pathogenic**	**Solved**
#39	**3**	**NS**	**M**	**Yes**	**Yes**	**68.2**	**1.25**	**9.48**	**US: Normal**	***TSHR***	**R, D**	**c.1422C>A**	**D474E**	**0.000008**	**Het**	**Known**	**P**	**Pathogenic**	**Solved**
#46	**37**	**NS**	**F**	**Yes**	**No**	**14.96**	**1.1**	**12.3**	**US: Normal**	***TSHR***	**R, D**	**c.1422C>A**	**D474E**	**0.000008**	**Het**	**Known**	**P**	**Pathogenic**	**Solved**
#11	**7**	**NS**	**F**	**No**	**No**	**>100**	**0.46**	**25.4**	**Scan: Normal**	***TSHR***	**R, D**	**c.202C>T**	**P68S**	**0.0000045**	**Het**	**Known**	**VUS**	**Pathogenic**	**Solved**
										DUOXA2	**R, D**	**c.769+2T>A**	**-**	**0.000004**	**Het**	**Novel**	**P**	**Pathogenic**	
Transient Congenital Hypothyroidism																			
#47	20	NS	M	No	No	>100	0.184	>300	US: Normal	*DUOX2*	R, D	c.2056C>T	Q686*	0.0000039	Het	Known	P	Pathogenic	Solved
#4	13	NS	F	No	No	88.35	0.48	>300	Scan: Normal	*DUOX2*	R, D	c.2176C>T	R726W	0.00024	Het	Known	LP	Pathogenic	Solved
#40	16	NS	F	No	No	42.7	0.85	253.1	US: Normal	*DUOX2*	R, D	c.3251G>A	R1084Q	0.000074	Het	Known	LP	Pathogenic	Solved
#19	**66**	**NS**	**M**	**No**	**No**	**18.4**	**1.4**	**106.9**	**US: Normal**	***DUOX2***	**R, D**	**c.4408C>T**	**R1470W**	**0.0000419**	**Het**	**Known**	**VUS**	**Pathogenic**	**Solved**
#21	15	NS	M	Yes	No	>100	0.5	>300	US: Normal	*DUOXA2*	R, D	c.748T>G	F250V	NA	Het	Novel	VUS	Pathogenic	Solved
#3	22	NS	M	Yes	No	>100	0.98	>500	US/Scan: Normal	*SLC26A4*	R	c.718C>T	L240F	NA	Hom	Novel	VUS	Pathogenic	Solved
										SLC5A5	R	c.818G>A	R273H	0.00000795	Het	Novel	VUS	Benign	
#12	52	NS	M	Yes	No	7.33	0.49	28.9	Scan: Normal	*TG*	R	c.6517C>T	R2173*	0.000017	Het	Novel	LP	Pathogenic	Ambiguous
										GNAS	D	c.1744G>A	D582N	0.000009	Het	Novel	VUS	Benign	
#6	17	NS	M	Yes	Yes	>100	0.47	-	US: Normal	*FOXE1*	R	c.689G>T	R230L	0.000024	Het	Novel	VUS	Benign	Ambiguous
#17	**25**	**NS**	**M**	**Yes**	**No**	**86**	**0.72**	**193.8**	**US: Normal**	***GNAS***	**D**	**c.1390G>A**	**D464N**	**NA**	**Het**	**Novel**	**VUS**	**Benign**	**Ambiguous**
#45	**46**	**NS**	**F**	**No**	**No**	**33.07**	**0.86**	**134.5**	**US: Normal**	***GNAS***	**D**	**c.940G>A**	**A314T**	**0.000017**	**Het**	**Novel**	**VUS**	**Benign**	**Ambiguous**
#1	10	NS	M	No	No	67	1.24	151.0	US: Normal	-		-	-	-	-	-	-	-	Unsolved
#10	**15**	**NS**	**F**	**No**	**No**	**100**	**0.52**	**>300**	**US: Normal**	**-**		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Unsolved**
#14	**21**	**NS**	**M**	**No**	**No**	**15.5**	**1.22**	**124.1**	**US: Normal**	**-**		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Unsolved**
#15	16	NS	F	No	No	52.6	0.578	376.1	Scan: Normal	-		-	-	-	-	-	-	-	Unsolved
#16	**17**	**NS**	**M**	**No**	**No**	**33.6**	**1.12**	**-**	**Scan: Normal**	**-**		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Unsolved**
#18	8	NS	M	No	No	68.9	0.8	228	US: Normal	-		-	-	-	-	-	-	-	Unsolved
#22	11	NS	M	No	No	>100	0.654	>300	Scan: Normal	-		-	-	-	-	-	-	-	Unsolved

#24	**16**	**NS**	**F**	**No**	**No**	**182.4**	**0.69**	**>300**	**US: Normal**	-		-	-	-	-	-	-	-	**Unsolved**
#26	9	NS	M	Yes	No	53.88	0.96	340.5	US: Normal	-		-	-	-	-	-	-	-	Unsolved
#28	14	NS	M	No	No	75	1.55	>300	US: Normal	-		-	-	-	-	-	-	-	Unsolved
#36	8	NS	F	No	No	>100	0.52	>300	Scan: Normal	-		-	-	-	-	-	-	-	Unsolved
#38	**7**	**NS**	**F**	**No**	**No**	**55.39**	**1.09**	**172.3**	**US/Scan: Normal**	-		-	-	-	-	-	-	-	**Unsolved**
#42	11	NS	M	Yes	No	42.6	1.23	119.4	US/Scan: Normal	-		-	-	-	-	-	-	-	Unsolved

Bold character indicates the patients reassessed after genetic testing.

* Reference ranges for TSH, FT4 and Tg according to age groups are presented below.

** NA in the column of *in silico* prediction means that there is no data in any of 30 prediction algorithms presented in supplemental method in next page.

ACMG: American College of Medical Genetics, ACMG Class: 1, P: Pathogenic; 2, LP: Likely Pathogenic; 3, VUS: variant of unknown significance;
C: Clinical presentation, D: Autosomal dominant, Dx: Diagnosis, ExAC: Exome Aggregation Consortium, F: Female; FT4: Free thyroxin,
GnomAD: Genome Aggregation Database, Het: Heterozygous, HGMD: Human Genome Mutation Database, Hom: Homozygous, M: Male, MAF: Minor allele frequency,
NA: Not available, NS: Neonatal screening, R: Autosomal recessive, Tg: Thyroglobulin, TSH: Thyroid stimulating hormone, US: Ultrasound, Scan: Scintigraphy

Reference ranges for TSH, FT4 and Tg according to age groups*

Ages	**TSH (µU/ml)**	**FT4** (ng/dl)**	**Tg (ng/ml)**
Newborns	0.70 – 15.2	0.86 – 2.49	25 – 307
6 day–3 month	0.72 – 11.0	0.89 – 2.20	20 – 228
4–12 month	0.73 – 8.35	0.92 – 1.99	18 – 125
1–6 year	0.70 – 5.97	0.96 – 1.77	9.0 – 67
7–11 year	0.60 – 4.84	0.97 – 1.67	5.1 – 43
12–20 year	0.51 – 4.30	0.98 – 1.68	2.6 – 36
Adult	0.27 – 4.20	0.93 – 1.70	1.4 – 78

* Serum TSH, free T4, and thyroglobulin levels were measured by electrochemiluminescence immunoassay using Elecsys® 2010 modular analytics E170. (Roche Diagnostics, GmbH 68298, Mannheim, Germany).

** To convert free T4 values to Système Internationale (SI) units, multiply by 12.87 (to pmol/L).

Supplemental Method

***In silico* analysis:** In this study, we included missense, nonsense, frameshift and splice site variants, which are pathogenic, likely pathogenic and uncertain significance according to ACMG classification, and analyzed their effect on protein structure by *in silico* prediction algorithms. For *in silico* analysis, 30 prediction algorithms were used through the varsome database (https://www.varsome.com/), or the relevant websites of the algorithm programs. A pathogenicity score was calculated dividing the number of algorithms reporting the variant as damaging (pathogenic or functional) by the total number of all algorithms reporting the variant as damaging or neutral (benign or non-functional). In this way, a pathogenicity score was obtained ranging from 0 to 1 (0 = all algorithms are benign-neutral, 1 = all algorithms are pathogenic-damaging). Variants determined as deleterious in most of the prediction algorithms (pathogenicity score >0.5) were considered potentially pathogenic, which means supporting of pathogenicity.

The prediction algorithms used in the calculation of pathogenicity scores of the variants are as follows: SIFT,[1] SIFT4G,[2] Polyphen2-HDIV, Polyphen2-HVAR,[3] LRT,[4] MutationTaster2,[5] MutationAssessor,[6] FATHMM, FATHMM-MKL, FATHMM-XF,[7] MetaSVM, MetaLR,[8] CADD,[9] VEST4,[10] PROVEAN,[11] Eigen, Eigen-PC,[12] M-CAP,[13] REVEL[14] MutPred,[15] MVP,[16] MPC,[17] PrimateAI,[18] DEOGEN2,[19] BayesDel_addAF, BayesDel_noAF,[20] ClinPred,[21] LIST-S2,[22] ALoFT[23] and LINSIGHT.[24]

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