0021-972X/06/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 91(8):3100–3104

doi: 10.1210/jc.2005-2702

Haplotype Analysis Reveals Founder Effects of Thyroglobulin Gene Mutations C1058R and C1977S in Japan

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Context: Thyroglobulin (Tg) mutations were previously believed to be rare, resulting in congenital goitrous hypothyroidism. However, an increasing number of patients with Tg mutations, who are euthyroid to mildly hypothyroid, have been identified in Japan.

Objectives: The purpose of this study was to investigate whether the three frequently found Tg mutations, namely C1058R, C1245R, and C1977S, were caused by a founder effect.

Results: We found 26 different mutations within the Tg gene in 52 patients from 41 families. Thirty-five patients were homozygous for the mutations, whereas the others were compound heterozygous. The occurrence of Tg mutation within the general Japanese population is one in 67,000. Patients with the C1245R mutation were found throughout Japan, whereas those with the C1058R mutation were confined to a small village on a southern island, and those with the C1977S mutation were restricted to a city. The eight patients with the C1058R mutation and the seven patients with the C1977S mutation all showed the same combinations of 18 single-nucleotide polymorphisms in the coding region of the Tg gene, which would appear in one in 810 million and one in 37 billion, respectively, control subjects.

Conclusions: The frequently found mutations, C1058R and C1977S, were caused by founder effects. This result suggests that Tg mutations may provide a genetic basis for the cause of familial euthyroid goiter. **(*J Clin Endocrinol Metab* 91: 3100–3104, 2006)**

MUTATIONS OF THE thyroglobulin (Tg) gene are one of the genetic causes of dyshormonogenesis, which is characterized by the development of goiters with autosomal recessive inheritance (1). Previously, only a few cases of Tg mutations had been reported in subjects displaying severe clinical manifestations, including both impaired physical development and mental retardation (2–8). However, we (9) and others (5) have reported mild cases, where the clinical manifestations include longstanding enlarged goiter from childhood with mild hypothyroidism to euthyroidism.

In this paper, we report on 52 Japanese patients with Tg mutations who were euthyroid to mildly hypothyroid. In addition to the previously reported Tg mutations C1058R, C1245R, C1977S, and G2356R (9, 10), we identified 22 novel mutations. Among them, three mutations, C1058R, C1245R, and C1977S, were frequently identified. In particular, two of the mutations, C1058R and C1977S, were found only in specific regions of Japan. Haplotype analysis confirmed that these two mutations were caused by a founder effect.

First Published Online May 23, 2006

Abbreviations: SNP, Single-nucleotide polymorphism; Tg, thyroglobulin; TPO, thyroid peroxidase.

Subjects and Methods

Subjects and Tg gene analysis

Seventy-six patients from 64 families were screened for mutations within the Tg gene. Twenty patients from 16 families showed high serum TSH at neonatal mass screening and goiters in early childhood. Fifty-six adult patients from 48 families were suspected of having Tg mutations because of clinical symptoms, laboratory tests, and histological findings. This study was approved by the ethical committee of Dokkyo University School of Medicine, and the patients or their parents expressed willingness to enroll in the study by signing informed consents.

Direct sequencing of Tg gene

Genomic DNA was isolated from the peripheral leukocytes using a QIAamp blood kit (QIAGEN, Hilden, Germany). All 48 exons and neighboring introns of the Tg gene were amplified using the Expand High-

Fidelity PCR System (Roche, Mannheim, Germany) and directly sequenced by the Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer, Norwalk, CT) (10).

Haplotype analysis

To study allelic frequencies of single-nucleotide polymorphisms (SNPs) in the coding region of the Tg gene, the nucleotides at 2330 (exon 10), 2443 (exon 10), 2963 (exon 11), 3906 (exon 18), 4493 (exon 21), and 7920 (exon 46) in the Tg cDNA were sequenced in 10 healthy subjects (20 chromosomes) and that at 426 (exon 4) in 50 healthy subjects (100 chromosomes). The nucleotide frequency of the other 11 SNPs was reported previously (9). The likelihood of the haplotypes of the patients with the mutation C1058R, C1245R, and C1977S in a Japanese population was calculated by allelic frequencies of each SNP. First, the likelihood of occurrence of the two nucleotides at a particular SNP position was calculated by multiplying the likelihood of the two nucleotides. Then, the overall likelihood of the haplotype of each patient was calculated by multiplying the likelihood at all of the 18 SNP locations in individual patients.

Results

Tg gene mutations (Table 1)

Direct sequencing of genomic DNA showed mutations within the Tg gene in 52 patients from 41 families. We detected 26 different mutations: 18 missense mutations, four splice mutations, three nonsense mutations, and one single-nucleotide deletion. Thirty-five patients were homozygous for the mutations, whereas 17 patients were compound heterozygous. The mutations are located across the full length of the Tg gene.

Clinical characteristics (Tables 1 and 2)

Among 16 patients born after 1979 when mass screening of hypothyroidism was initiated in Japan, 12 patients were positive at neonatal mass screening. Among the remaining four patients, only one patient born in 2001 with the mutation Q310P/C2135Y was confirmed negative at mass screening by her medical record. The other three patients who were born in 1980 or 1981 had no medical record. Seven patients positive at mass screening were maintained on thyroid hormone replacement therapy. Their serum TSH level was high and free T_4 was low at mass screening (Table 2). Five patients positive at mass screening were either transiently treated with thyroid hormones or not treated at all. Their thyroid function tests showed subclinical hypothyroidism after 5 yr of age.

Patients born before 1979 suffered from longstanding voluminous goiters from childhood. Their thyroid functions were euthyroid to mildly hypothyroid (Table 2). The serum TSH levels were less than 10 mU/liter, except for one patient with the mutation C1245R/Q2638X who had a serum TSH of 51 mU/liter. Thyroid autoantibodies against Tg, thyroid peroxidase (TPO), and TSH receptor were negative in all the patients. Their serum Tg concentrations were low when compared with the size of goiter. Radioactive iodine uptake by the thyroid glands was high. In 12 patients who underwent an operation, abnormal Tg was suspected by typical histological findings of Tg mutations, which include empty-looking colloid in small follicles covered by tall (or cuboidal) follicular cells containing yellow-green granules (11).

Prevalence of Tg mutations

The prevalence of Tg mutation was analyzed in the Kumamoto Prefecture of Japan. The total number of infants who underwent mass screening from 1990–1999 was 199,826.

TABLE 1. Tg mutations

Nucleotide	Amino acid	No. of families	No. of patients	Born after 1979			
				MS(+) on therapy	MS(+) therapy-free	MS(−)	No record
580T→G/580T→G	C175G/C175G	1	1	0	0	0	0
986A→C/IVS30+1G→A	Q310P/Δexon30	1	2	2	0	0	0
986A→C/6461G→A	Q310P/C2135Y	1	1	0	0	1	0
2969G→A/3035C→T	S971I/P993L	1	1	0	0	0	1
IVS10−1G→A/IVS10−1G→A	Δexon11/Δexon11	1	1	0	0	0	0
3229T→C/3229T→C[a]	C1058R/C1058R[a]	5	8	0	0	0	0
3790T→C/3790T→C[a,b]	C1245R/C1245R[a,b]	11	12	0	2	0	1
3790T→C/2131C→T	C1245R/Q692X	1	1	0	1	0	0
3790T→C/4537delG	C1245R/FS1513→1566X	1	2	2	0	0	0
3790T→C/IVS24+1G→C	C1245R/Δexon24	1	1	0	0	0	0
3790T→C/7123G→A[a]	C1245R/G2356R[a]	1	1	0	0	0	0
3790T→C/7969C→T	C1245R/Q2638X	1	1	0	0	0	0
4397G→A/3022C→T	S1447N/R989C	1	1	0	0	0	0
4397G→A/7123G→A	S1447N/G2356R	1	1	0	0	0	1
4820G→T/4820G→T	C1588F/C1588F	2	4	0	0	0	0
4820G→T/4310G→A	C1588F/W1418X	1	1	0	0	0	0
5690G→A/7007G→A	C1878Y/R2317Q	1	1	1	0	0	0
5791A→G/6017G→A	I1912V/C1987Y	1	2	2	0	0	0
5986T→A/5986T→A[a,b]	C1977S/C1977S[a,b]	5	7	0	2	0	0
5986T→A/4820G→A	C1977S/C1588F	1	1	0	0	0	0
6956G→A/6956G→A	G2300D/G2300D	1	1	0	0	0	0
7121G→T/IVS45+2T→A	G2355V/Δexon45	1	1	0	0	0	0
		41	52	7	5	1	3

Nucleotides are numbered denoting the A of the initiator ATG codon as +1. Amino acids are numbered in the mature protein after cleavage of the 19-amino-acid signal peptide. MS(+), Positive at mass screening; MS(−), negative at mass screening.

[a] Patients reported in Ref. 10 were included.

[b] Patients reported in Ref. 9 were included.

TABLE 2. Clinical characteristics of patients with Tg mutations

	Normal range	Born after 1979			Born before 1979
		MS(+) on therapy	MS(+) therapy-free	MS(−)	
TSH (mU/liter)	0.1–4.0	167 ± 145 (n = 7)[a]	6.30 ± 8.75 (n = 5)	4.26 ± 3.76 (n = 4)	4.20 ± 9.22 (n = 29)
Free T_4 (pmol/liter)	8–28	4.68 ± 2.27 (n = 7)[a]	13.10 ± 1.78 (n = 5)	12.55 ± 2.48 (n = 4)	10.94 ± 3.27 (n = 24)
Tg (mg/liter)	1–30	BDL	5.3 ± 2.7 (n = 4)	44.4 ± 41.3 (n = 4)	25.5 ± 34.4 (n = 33)
RAIU (%)	5–20	54.6 ± 19.2 (n = 4)	55.3 ± 18.9 (n = 5)	56.4 ± 3.3 (n = 2)	53.6 ± 16.9 (n = 27)

n, Number of patients whose thyroid function tests were available. BDL, Below detection limit; MS(+), positive at mass screening; MS(−), negative at mass screening; RAIU, radioactive iodine uptake.
[a] $P < 0.01$ *vs.* born before 1979.

Among 155 infants whose serum TSH was more than 10 mU/liter, 111 infants were subsequently evaluated. Twenty-two infants suffered from abnormal thyroid development, six suffered from dyshormonogenesis, 25 suffered from mild persistent hypothyroidism without known causes, and 12 suffered from transient hypothyroidism. Because we identified three infants with Tg mutations, the incidence of Tg mutations is one in 67,000.

Founder effects

C1058R, C1245R, and C1977S mutations were frequently found in this study (Table 1). We found eight cases from five families homozygous for the C1058R mutation, 12 cases from 11 families homozygous for the C1245R mutation, and seven cases from five families homozygous for the C1977S mutation. To distinguish whether these mutations were derived from a common ancestral chromosome or an independently recurrent mutation, we analyzed 18 SNPs within the coding region of the Tg gene (Table 3). Eight patients homozygous for the C1058R mutation and seven patients homozygous for the C1977S mutation showed the same haplotypes, which based on statistical analysis would be expected to occur at a frequency of one in 810 million and one in 37 billion individuals, respectively. Therefore, our results suggest that the homozygous occurrence of these mutations is a result of a founder effect. However, the haplotypes of three patients homozygous for the C1245R mutation were different from the others, suggesting that some of the C1245R alleles were a result of independently recurrent mutations.

Discussion

We have detected 26 different mutations of the Tg gene in 52 patients from 41 families. Because three nucleotides (*i.e.* CAG, after nucleotide 2952) were missing in the original report (12), the numbering of the nucleotides and, hence, the amino acid residues was changed accordingly (9, 13). Moreover, because the signal peptide consisting of 19 amino acids was usually excluded in the description of the Tg protein (1), C1263R and C1995S in our previous report (9) were changed to C1245R and C1977S, respectively, in this report. Among these Tg mutations, 69% (18 of 26) were missense mutations, which in many cases occurred as homozygous and compound heterozygous states.

In our series of patients, the symptoms were mild. Among 12 patients who were positive at neonatal mass screening, five were either transiently treated with thyroid hormones or not treated at all. In adult patients, the only clinical manifestation was longstanding goiter with euthyroidism or mild hypothyroidism. No patients developed mental retardation, even without treatment. Laboratory tests were compatible with Tg gene abnormalities, and the serum Tg concentrations

TABLE 3. Eighteen SNPs in patients homozygous with C1058R, C1977S, and C1245R mutations

Exon	cDNA	Nucleotide	Frequency in general population	C1058R, patients 1–8	C1977S, patients 1–7	C1245R			
						Patients 1–9	Patient 10	Patient 11	Patient 12
e4	426	C;T	99;1	TT	CC	CC	CC	CC	CC
e10	2200	T;G	37;63	GG	TT	TT	*GG*	TT	TT
	2330	C;T	90;10	CC	CC	CC	CC	CC	CC
	2334	T;C	31;69	CC	TT	TT	*CT*	TT	TT
	2443	G;A	85;15	GG	GG	GG	GG	GG	GG
	2488	C;G	98.6;1.4	CC	GG	CC	CC	CC	CC
e11	2963	G;C	85;15	GG	GG	GG	GG	GG	GG
e12	3082	A;G	66;44	GG	AA	AA	*GA*	AA	AA
e18	3906	G;A	90;10	GG	GG	GG	GG	GG	GG
	3935	G;A	31;69	AA	GG	AA	AA	AA	AA
e21	4493	C;T	90;10	CC	CC	CC	CC	CC	CC
	4506	C;T	69;31	TT	CC	TT	*CT*	TT	TT
e29	5512	A;G	50;50	GG	AA	GG	*AG*	*AG*	GG
e33	5995	C;T	79;21	CC	TT	CC	CC	*CT*	*TT*
e43	7408	C;T	81;19	CC	CC	CC	CC	CC	CC
	7501	T;C	91;19	TT	TT	TT	TT	TT	TT
e44	7589	G;A	63;37	GG	GG	GG	GG	GG	GG
e46	7920	C;T	90;10	CC	CC	CC	CC	CC	CC

Changed nucleotides in patients with C1245R are indicated in *italics*.

of the patients were low compared with the size of goiter. No antibodies against Tg and TPO were detected. Radioactive iodine uptake was increased without organification defect under perchlorate discharge test. Histological studies pointed to abnormal protein trafficking from endoplasmic reticulum to Golgi, which is a frequent consequence of Tg gene mutations (14). We have shown that Tg mutations, C1245R and C1977S, which perturb correct folding of Tg protein, caused impaired intracellular transport, leading to accumulation of abnormal Tg in endoplasmic reticulum (9, 15, 16). The mutation C1058R also caused defects in intracellular transport as shown by carbohydrate analysis of Tg protein and increased expression of molecular chaperones in the patients' thyroid (data not shown). Defective intracellular transport of Tg was also confirmed by the *in vitro* expression of mutant proteins in heterologous culture cells (data not shown). Allelic frequency of all the mutations was less than 0.01 (data not shown).

The three mutations, C1058R, C1245R, and C1977S, were frequently found in Japan. None of the mutations have been reported in other countries. In the Japanese population as a whole, the overall incidence of Tg mutations is estimated at one in 67,000 live births. This figure is comparable with the one in 66,000 occurrence of total iodide organification defects, the majority of which are caused by mutations in the TPO gene (17). Patients with the mutations C1058R and C1977S were restricted to specific locations within Japan, whereas those with the C1245R mutation were found all over the country. This observation suggests that the C1058R and C1977S mutations originated from a single person (founder effect). In particular, the small village where the C1058R mutation was found is isolated by steep mountains and sea, inhibiting movement to nearby areas. A public health survey in 1964 reported that 55 people of 379 dwellers (14.5%) in the village suffered from goiter (18). When taking into account that only homozygotes present with symptoms, 47% of the dwellers were deemed heterozygous carriers of the mutation.

To evaluate whether these mutations were inherited from a common ancestral chromosome or independently recurrent mutations in heterogeneous genetic backgrounds, we studied 18 SNPs within the coding region of the Tg gene. We (9) and others (13) previously reported 15 SNPs within the Tg gene. In this report, we identified six more SNPs. The frequencies of the minor alleles of these SNPs were relatively rare, *i.e.* up to 15%. The haplotypes of eight patients with the C1058R mutation and seven patients with the C1977S mutation were identical. When calculated from allelic frequencies of the SNPs in normal controls, the occurrence of the mutations C1058R and C1977S in the Japanese population would be 1.2×10^{-9} and 2.7×10^{-11}, respectively. This confirms the hypothesis that homozygous occurrence of C1058R and C1977S was a result of a founder effect. However, three patients with the C1245R mutation possessed different haplotypes from the other nine patients. In particular, some of the SNPs presented with both major and minor alleles in a single patient. These results suggest that the C1245R mutation is an independently recurrent mutation. However, taking into account that nine patients share a common allele, an alternative explanation is that C1245R is an old mutation with the new SNPs being created independently.

In conclusion, we report 26 different mutations of the Tg gene in 52 patients from 41 families. Among three frequently found mutations (C1058R, C1245R, and C1977S), haplotype analysis confirmed that C1245R and C1977S were caused by a founder effect. Therefore, Tg mutations should be considered as one of the genetic causes of familial euthyroid goiter.

Acknowledgments

We thank N. Ohmika and T. Namatame at Dokkyo University School of Medicine for their technical assistance in genetic analysis.

Received December 12, 2005. Accepted May 17, 2006.

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This study was partially supported by a Grant-in-Aid for Scientific Research (12672248 and 16590462) from the Japan Society for the Promotion of Science (to A.H.).

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