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Intronic polymorphism of TSHR gene (rs179247) and its role in the susceptibility to Graves' disease in a sample of Iraqi patients

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Abstract

Thyroid stimulating hormone receptor (TSHR) is thought to be a significant candidate for genetic susceptibility to Graves' disease (GD). The aim of the present study was to determine whether A>G dimorphism at position 15678 of intron1 (rs179247) in the TSHR gene contributes to the severity and clinical manifestations of Graves' disease. We performed genetic and hormonal studies on 60 Graves' patients and 60 apparently healthy controls. We determined the subjects' genotypes for the rs179247 A>G polymorphism of the TSHR gene using TaqMan genotyping kit by RT-PCR. The concentrations of T3, T4, TSH and anti-TSHR in serum were determined. Comparisons of studied variables between genotypes was performed using statistical analysis system (SAS).

The frequency of AG genotype was significantly ($p<0.05$) higher in patients with Graves' disease compared with apparently healthy controls. The GG genotype frequency was significantly ($p<0.01$) higher in patients with Graves' disease than in apparently healthy subjects. Generally, all serum parameters were unaffected by the studied SNP of TSHR in both Graves' disease patients and apparently healthy subjects. The levels of serum T_3 and T_4 hormones, in addition to serum anti-TSHR were significantly ($p<0.01$) higher in Graves' disease patients than in apparently healthy subjects. Serum TSH levels were significantly lower in GD patients than in apparently healthy subjects.

The g.15678 A>G (rs179247 SNP) of TSHR gene is related to the development of Graves' disease in Iraqi patients and more studies are necessary to clarify the role of the TSHR gene in influencing Graves' disease susceptibility.

Key words: TSHR gene, polymorphism, Graves' disease.

INTRODUCTION

Graves' disease (GD) is one of the most common organ-specific endocrine diseases, and also a multifactorial disease with genetic susceptibilities and environmental factors. It affects up to approximately 1% of the general population (1). Many previous studies have identified several gene loci that are associated with the risk to develop Graves' disease which include cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) gene (2), thyroglobulin (TG) gene (3), protein tyrosine phosphatase (PTPN) gene (4), vitamin D receptor (VDR) gene (5), interleukin-1 gene (6) and thyroid stimulating hormone receptor (TSHR) gene (7). TSHR gene is a significant candidate, and failure of tolerance to TSHR is central to the pathogenesis of GD.

Intronic polymorphisms has been entertained because of that intronic DNA may be responsible for regulatory small RNAs as well as providing and influencing different start sites for TSHR mRNA generation (8). Thyroid cell expresses a variety of TSHR mRNA splice variants (9), indicating that SNPs or small RNAs in this intronic DNA may be important in the generation of different receptor forms and their control. A study from Singapore demonstrated an association of a TSHR intron 1 SNP with GD (10). Brand *et al.* (11) found that the SNP rs179247 of TSHR gene showing a strongest association with Graves' disease. Then, the association of TSHR gene SNP rs179247 with the development of Graves' disease was proved in Polish (12), Spanish (13), Chinese (14), Brazilian (15) and Japanese (16) populations. Nevertheless, many studies have investigated the association between TSHR gene polymorphism and the risk of GD, but yielded conflicting results.

To further examine its potential role in influencing the risk of GD, we performed a study on intron1 of TSHR gene (rs179247) to investigate its effects on the incidence of Graves' disease in a sample of Iraqi patients.

MATERIAL AND METHODS

This study was conducted in the institute of genetic engineering and biotechnology for higher studies- university of Baghdad during a period from February to May 2017. The study included 60 patients with Graves' disease. The diagnosis of GD was based on standard clinical criteria including increased serum levels of free T_4 and T_3 and decreased or undetectable TSH concentrations in addition to the increased anti-TSHR levels. We included a control group of very carefully screened 60 healthy individuals recruited from blood donors from our region. None of the control subjects had any history of personal or familiar autoimmune diseases nor presented any abnormality at thyroid palpation. Blood samples were collected from severe Graves' disease patients and apparently healthy subjects in the Endocrinology Centre of Al-Kindi hospital, Baghdad to obtain blood for DNA extraction and serum for hormonal assays. Genomic DNA was extracted from the whole blood which collected in EDTA-containing tubes by using DNA purification kit (WizPrep™ DNA Extraction Kit). The purity and concentrations of DNA samples were estimated by using Nanodrop (2000C apparatus, Thermo Scientific, USA).

All DNA samples were genotyped for rs179247 SNP of TSHR gene by Real-Time PCR TaqMan® SNP Genotyping Assays (C_26928532_10), using a 7500 Real-Time PCR System (Applied Biosystems, CA, USA) and analyzed through the allelic discrimination endpoint analysis mode of the Sequence Detection Software Package (SDS).

Serum T3, T4 and TSH hormones concentrations were determined using VIDAS kits (bioMereux, France). Anti-TSHR levels were determined using ELISA kit.

The statistical analysis was conducted using the SAS statistical software (17). Chi square (X^2) test was used to examine homogeneity between cases and controls. The odds ratio provided the measurement of association strength. ANOVA was used to evaluate the effect of rs179247 genotypes and clinical risk factors including age, sex, smoking status, treatment and duration of disease.

RESULTS AND DISCUSSION

The distribution of genotypes alleles frequency at rs179247 SNP of TSHR gene presented in table 1.

As related with AA genotype, the frequency was in patients with Graves' disease significantly ($p<0.01$) lower than in apparently healthy controls (43.33 *versus* 71.67%, respectively; $X^2=9.903$; *OR*=1.327). The frequency of AG genotype was significantly ($p<0.05$) higher in patients with Graves' disease compared with apparently healthy controls (30 *versus* 20%, respectively; $X^2=4.372$; *OR*=0.763). The GG genotype frequency was significantly ($p<0.01$) higher in patients with Graves' disease than in apparently healthy subjects (26.67 *versus* 8.33%, respectively; $X^2=6.794$; *OR*=1.008). The frequency of the combined AG+GG genotypes was, also, significantly ($p<0.01$)

higher in patients with Graves' disease than in apparently healthy subjects (56.67 *versus* 28.33%, respectively; X^2=9.145; *OR*=1.563).

The frequencies of A and G alleles were 0.82 and 0.18 in apparently healthy subjects and 0.58 and 0.42 in patients with Graves' disease, respectively.

The results in the present study, as related with rs179247 SNP of TSHR, found a G allele-related risk with Graves' disease in Iraqi patients. These results are in agreement with previous studies on populations of Caucasian (11), Polish (12), Spanish (13), Chinese (14), Japanese (18), Brazilian (15) and Italian (19).

Brand *et al.* (11) interrogated a panel of 98 SNPs spanning an 800 kb region of the TSHR gene in a cohort of 768 GD subjects and 768 controls. The SNP rs179247 showing the strongest association with Graves' disease. They proposed that the TSHR intron 1 GD-associated SNPs regulate mRNA spicing, resulting in increased levels of variants encoding a more autoantigenic TSHR A-subunit. They measured the levels of full length TSHR mRNA and of two TSHR truncated transcripts named ST4 and ST5 in thyroid tissues and showed that the disease-risk alleles of 2 intron 1 SNPs (rs179247 and rs12101255) associate with increased ST4 and ST5 and with decreased full length TSHR levels. Also, they suggested that the truncated ST4 and ST5 variants could be translated into TSHR extracellular A-subunit, the main target of TSHR autoantibodies. Ploski *et al.* (12) found an association of SNP rs179247 with orbitopathy as its relationship with the development of Graves' disease was proved in the Polish population. In their study on Spanish population, Colobran *et al.* (13) found that individuals carrying the disease-protective genotype (AA) at the rs179247 site have higher levels of thymic TSHR mRNA than those with the disease-associated genotypes. They showed that individuals homozygous or heterozygous for the GD-associated allele at the rs179247 SNP have significantly lower TSHR mRNA expression levels in the thymus than individuals homozygous for the protective allele (AA). Liu *et al.* (14) found on TSHR gene that the allele G of rs179247 was associated with opthalmopathy in Chinese patients with Graves' disease. The association of the TSHR intron 1 SNPs with GD was validated by the study of Bufalo *et al.* (15) on Brazilian population. Recently, three meta-analysis studies intended to refine the effects of rs179247 SNP on Graves' disease susceptibility concluded that there is a significant association between this SNP in TSHR intron 1 and Graves' disease (16; 20; 21). The meta-analysis of Qian *et al.* (16) revealed a significant association between TSHR rs179247A/G polymorphism with GD in five different populations (Chinese, Japanese, Polish, British and Brazil). Xiong *et al.* (21) showed that the carriers of rs179247 AA had a 34% less risk of developing GD than the controls. Allele G of rs179247 was more frequent in GD patients (42% in GD patients *versus* 18% in healthy subjects in the present study). Allele A of rs179247 was more common in healthy people (82% in healthy subjects *versus* 58% in GD patients in the present study). The risk allele of TSHR SNP rs179247 was associated with a reduced ratio of full length TSHR:ST4 and full length TSHR:ST5. The increase of ST4 and ST5 resulted in a higher production of the soluble "A" subunit of TSHR in the periphery. This led to the production of thyroid auto-antibodies, which are the cause of GD.

Serum levels of T_3, T_4, TSH and anti-TSHR in Graves' disease patients are presented in table 2. All parameters that shown in table 1 were affected by age. Serum levels of T_3 and T_4 hormones in patients with Graves' disease were significantly ($p<0.01$) decreased with advance in age. Serum T_3 and T_4 levels in GD patients at less than 40 years old were equal to two fold compared with those at more than 40 years' old. In contrast, serum TSH hormone levels in patients with GD were significantly ($p<0.01$) increased with advance in age. Serum anti-TSHR levels in GD patients were significantly ($p<0.01$) decreased with advance in age.

Table 1. The frequency of genotypes and alleles at rs179247 SNP in intron 1 of TSHR gene in Iraqi patients with Graves' disease and controls.

Genotypes	Frequency, n (%)		χ^2	*OR*[3]
	Control[1]	GD[2]		
AA	43 (71.67%)	26 (43.33%)	9.903 **	1.327
AG	12 (20 %)	18 (30 %)	4.372 *	0.763
GG	5 (8.33 %)	16 (26.67%)	6.794 **	1.008
AG+GG	17 (28.33%)	34 (56.67%)	9.145 **	1.563
Alleles				
A	0.82	0.58	-	-
G	0.18	0.42	-	-

[1] apparently healthy subjects. [2] Patients with Graves' disease. [3] Odd ratios.
*and ** means significant at 0.05 and 0.01 levels, respectively.

Table 2. Effect of some parameters on serum levels of T_3, T_4, TSH and anti-TSHR in Iraqi patients with Graves' disease. (Means ± SE)

Parameters		T_3	T_4	TSH	Anti-TSHR
Age (year)	<40	8.03 ±0.58 a	250.7 ± 19.6 a	0.29± 0.16 c	18.3 ± 1.5 a
	40-50	3.88 ± 0.63 b	135.2 ± 13.1 b	0.71± 0.20 b	8.1 ± 1.4 b
	>50	3.54 ± 0.95 b	142.9 ± 37.0 b	1.48± 0.45 a	7.7 ± 2.5 b
	P value	**	**	*	**
Sex	Male	4.26 ± 1.13	155.82 ± 34.9	0.73 ± 0.23	9.5 ± 2.5
	Female	5.78 ± 0.51	189.52 ± 14.6	0.67 ± 0.17	12.8 ± 1.3
	P value	NS	NS	NS	NS
Smoking	yes	4.45 ± 0.89	155.12 ± 26.5	0.58 ± 0.18	9.82 ± 1.9
	no	5.85 ± 0.55	192.84 ± 15.7	0.72 ± 0.18	12.94 ± 1.4
	P value	NS	NS	NS	*
Treatment	yes	1.70 ± 0.05	86.99 ± 2.7	1.42 ± 0.25	3.40 ± 0.3
	no	8.56 ± 0.29	261.15 ± 13.6	0.07 ± 0.01	19.23 ± 0.9
	P value	**	**	**	**
Duration of disease (month)	<6	6.66 ± 0.57 a	214.35 ± 18.4 a	0.29 ± 0.10 b	14.76 ± 1.4 a
	6-12	4.62 ± 0.77 a	158.92 ± 20.6 b	0.80 ± 0.22 b	10.46 ± 1.9 a
	>12	1.64 ± 0.11 b	87.25 ± 4.9 c	2.99 ± 0.92 a	1.82 ± 0.1 b
	P value	**	**	**	**

*and ** means significant at 0.05 and 0.01 levels, respectively.

Table 3. Serum T3, T4, TSH and anti-TSHR concentrations as affected by the genotype of rs179247 SNP in intron 1 of TSHR gene in Iraqi patients with Graves' disease and controls.

Parameters	Rs179247 genotypes	Control[1]	Patients[2]	*p* value
Serum T_3	**AA**	1.57 ± 0.06	5.23 ± 0.79	**
	AG	1.74 ± 0.11	6.60 ± 0.75	**
	GG	1.50 ± 0.05	4.62 ± 0.85	**
	***p*- value**	NS	NS	-
Serum T_4	**AA**	95.63 ±16.6	179.80 ± 23.2	**
	AG	90.51± 2.4	203.89 ± 20.4	**
	GG	84.40 ± 4.2	163.87 ± 25.6	**
	***p*- value**	NS	NS	-
Serum TSH	**AA**	2.60 ± 0.18	0.80 ± 0.22	*
	AG	2.58 ± 0.33	0.33 ± 0.19	*
	GG	3.80 ± 0.38	0.89 ± 0.33	**
	***p*- value**	*	NS	-
Serum anti-TSHR	**AA**	0.39 ± 0.03	11.79 ± 1.85	**
	AG	0.36 ± 0.06	13.83 ± 1.76	**
	GG	0.39 ± 0.07	10.69 ± 2.44	**
	***p*- value**	NS	NS	-

[1] apparently healthy subjects. [2] Patients with Graves' disease. [3]
*and ** means significant at 0.05 and 0.01 levels, respectively.

The present work examines the effect of age on the levels T_4, T_3 and TSH (Table 2) which show a decreased level of T_3 and T_4 when compared the levels at less than 40 years old with the age of more than 40 years old.

This pattern of effect is also in agreement with the findings of Muslim and Khalil (22). Similar trends of changes in $T_4$1evels were also found by Muslim and Khalil (22). The effect of age on TSH level was observed to increase with age. This pattern of result is disagreeing with the results obtained in the previous study (23), while some workers showed a higher TSH level with an increase in age (24). Some other researchers also assayed thyroid hormones and TSH and found no changes in TSH level with age (25). This difference may be due to the fact that the subjects in that study were not screened for any kind of illness that may affect the thyroid function tests. All parameters that shown in table 2 were unaffected by sex and smoking status except serum anti-TSHR levels which increased in nonsmokers GD patients. Franklyn *et al.,* (23) and Muslim and Khalil (22) concluded that level of T_4 was higher in females than males. They further concluded that T_3 and TSH levels are not influenced by gender.

All parameters that shown in table 2 that measured for GD patients were responded to treatment. In response to treatment both T_3 and T_4 levels were significantly ($p<0.01$) decreased while serum TSH levels were significantly ($p<0.01$) increased. Serum anti-TSHR levels were significantly ($p<0.01$) decreased in response to treatment. Roger *et al.* (2017) found that TSH receptor antibodies decreased to a greater degree in patients receiving combination therapy. Serum T_3 and T_4 concentrations in GD patients were significantly ($p<0.01$) decreased with increase the duration of GD disease. In contrast, Serum TSH concentrations in GD patients were significantly ($p<0.01$) increased with increase the duration of GD disease. Serum anti-TSHR concentrations in GD patients were significantly ($p<0.01$) decreased with increase the duration of GD disease.

Serum T_3, T_4, TSH and anti-TSHR concentrations as affected by the genotype of rs179247 SNP of TSHR gene in Iraqi patients with Graves' disease and controls are presented in table 3.

Serum T_3, T_4 and anti-TSHR concentrations were unaffected by the studied SNPs of TSHR in both Graves' disease patients and apparently healthy subjects. However, the levels of serum T_3, T_4 and anti-TSHR were significantly ($p<0.01$) higher in Graves' disease patients than in apparently healthy subjects.

In apparently healthy controls, there were a significant increase in serum TSH levels in GG carriers of SNP rs179247 in TSHR compared with AA and AG genotypes. Serum TSH levels were significantly lower in GD patients than in apparently healthy subjects.

The key feature in untreated GD is the significant increase in the serum triiodothyronine (T_3) level, which is caused by the elevated activity of intrathyroidal type 1 deiodinase (26). TSH binding to its receptor leads to coupling Gs and stimulates cAMP production through activation of adenylyl cyclase (27). At higher concentrations of TSH, the TSHR couples Gq/11, resulting in activation of phospholipase C, and stimulates the inositol phosphate (IP) pathway (27). The increase in cAMP leads to phosphorylation of protein kinase A and to activation of transcription factors, such as CREB, resulting in the increase of iodide uptake, thyroid peroxidase, and thyroglobulin synthesis (27). In addition, stimulation of Gq/11 and the phospholipase C-dependent inositol phosphate /diacylglycerol pathway at the higher doses of TSH activates hydrogen peroxide generation and iodination.

Autoantibodies against the thyroid-stimulating hormone (TSH) receptor (TSH receptor antibodies, TSHRAbs) are directly involved in the pathogenesis of Graves 'disease. The TSHRAbs compete with TSH for TSH receptor (TSHR) binding and mimic the TSH action, resulting in hyperthyroidism.

REFERENCES

1- Hollowell, J.G., Staehling, N.W., Flanders, W.D., *et al*. (2002). Serum TSH, T_4 and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J. Clin. Endocrinol. Metab., 87(2): 489-499.

2- Abdul-Hassan, I. A.; Saad, S. A.; Khalid, M. M. (2015). Promoter -318C>T and exon1 +49A>G polymorphisms of CTLA-4 gene confer susceptibility to Graves' disease in Iraqi population. World J. Pharma. Res., 4(3): 141-155.

3- Ban, Y.; Tozaki, T.; Taniyama, M. *et al.* (2012). Multiple SNPs in intron 41 of thyroglobulin gene are associated with autoimmune thyroid disease in the Japanese population. *PLoS One*,7(5): e37501.

4- Chabchoub, G.; Teixiera, E.P.; Maalej, A. *et al*. (2009). The R620W polymorphism of the protein tyrosine phosphatase 22 gene in autoimmune thyroid diseases and rheumatoid arthritis in the Tunisian population. *Ann. Hum. Biol.*, 36(3):342-349.

5- Chen, R.H.; Chang, C.T.; Chen, H.Y. *et al.* (2007). Association between Vitamin-D receptor gene FokI polymorphism and Graves' disease among Taiwanese Chinese. *J. Clin. Lab. Anal.*, 21(3): 173-177.

6- Liu, N.; Li, X.; Liu, C. *et al.* (2010). The association of interleukin-1alpha and interleukin-1beta polymorphisms with the risk of Graves' disease in a case-control study and meta-analysis. *Hum. Immunol.*,71(4): 397-401.

7- Shao, L.; Jiang, H.; Liang, J. *et al.* (2011). Study on the relationship between TSHR gene and thyroid diseases. *Cell Biochem. Biophys.*, 61(2): 377-382.

8- Nakaya, H.I., Amaral, P.P., Louro, R., Lopes, A., Fachel, A.A., Moreira, Y.B., El-Jundi, T.A., da Silva, A.M., Reis, E.M., Verjovski-Almeida, S. (2007). Genome mapping and expression analyses of human intronic noncoding RNAs reveal tissue-specific patterns and enrichment in genes related to regulation of transcription. Genome Biol 8: R43.

9- Graves, P.N.; Davies, T.F. (2000). New insights into the thyroid stimulating hormone receptor. The major antigen of Graves'disease. *Endocrinol. Metab. Clin. N. Am*., 29:267–286.

10- Ho, S.C.; Goh, S.S.; Khoo, D.H. (2003). Association of Graves' disease with intragenic polymorphism of the thyrotropin receptor gene in a cohort of Singapore patients of multi-ethnic origins. *Thyroid*, 13:523–528.

11- Brand, O.J.; Barrett, J.C.; Simmonds, M.J.; Newby, P.R.; McCabe, C.J.; Bruce, C.K. *et al.* (2009). Association of the thyroid stimulating hormone receptor gene (TSHR) with Graves' disease. *Hum. Mol. Genet.*, 18: 1704-1713.

12- Ploski, R.; Brand, O.J.; Jurecka-Lubieniecka, B.; Franaszczyk, M.; Kula, D.; Krajewski, P. *et al.* (2010). Thyroid stimulating hormone receptor (TSHR) intron 1 variant are major risk factors for Graves' disease in three European Caucasian cohorts. *PLoS ONE*.,5: e15512.

13- Colobran, R.; *et al.* (2011). Association of an SNP with intrathymic transcription of TSHR and Graves' disease: A role for defective thymic tolerance. *Hum. Mol. Genet.*, 20(17):3415–3423.

14- Liu, L.; Wu, H.Q.; Wang, Q. *et al.* (2012). Association between thyroid stimulating hormone receptor gene intron polymorphisms and autoimmune thyroid disease in a Chinese Han population. *Endocr. J*., 59(8): 717-723.

15- Bufalo, N.E.; Dos Santos, R.B.; Marcello, M.A. *et al.* (2015). TSHR intronic polymorphisms (rs179247 and rs12885526) and their role in the susceptibility of the Brazilian population to Graves' disease and Graves' ophthalmopathy. *J. Endocrinol. Invest*., 38(5): 555-561.

16- Qian, W.; Xu, K.; Jia, W.; Lan, L.; Zhang, X.; Yang, X.; Cui, D. (2016). Association between TSHR gene polymorphism at the risk of Graves' disease: a meta-analysis. *J. Biomed. Res.*, 30(6): 466-475.

17- SAS, (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

18- Inoue, N.; Watanabe, M.; Hayashi, F. *et al.* (2013). The association between a functional polymorphism in the CD24 gene and the development of autoimmune thyroid diseases. *Tissue Antigens*, 81(3): 161-163.

19- Lombardi, A.; Menconi, F.; Greenberg, D.; Concepcion, E.; Leo, M.; Rocchi, R. *et al.* (2016). Dissecting the genetic susceptibility to Graves' disease in a cohort of patients of Italian origin. *Front Endocrinol*., 7:21.10.3389/fendo.2016.00021.

20- Gong, J.; Jiang, S.J.; Wang, D.K.; Dong, H.; Chen, G.; Fang, K. *et al.* (2016). Association of polymorphisms of rs179247 and rs12101255 in thyroid stimulating hormone receptor intron 1 with an increased risk of Graves' disease: a meta-analysis. *J. Huazhong Univ. Sci. Technolog. Med. Sci*., 36:473–9. doi: 10.1007/ s11596-016-1611-x.

21- Xiong, H.; Wu, M.; Yi, H.; Wang, X.; Wang, Q.; Nadirshina, S. *et al.* (2016). Genetic associations of the thyroid stimulating hormone receptor gene with Graves diseases and Graves ophthalmopathy: a meta-analysis. *Sci. Rep*.,6:30356. doi: 10.1038/srep30356.

22- Muslim, S.; Khalil, Z. (2000). Effect of Age, Sex, Salt, Water and Climate on T3, T4 and TSH in Healthy Individuals, Department of Zoology Peshawar University.

23- Franklyn, J.A.; Ramsden, B.D.; Sheppard, M.C. (1985). The influence of age and sex on tests of thyroid function. *Am. Clin. Biochem.*,22:502–505.

24- Hoogendoorn, H.E.; Hermus, R.A.; de Vegt, F.; Ross, H.A.; Verbeek, A.L.; Kiemeney, L.A.; Swinkels, D.W.; Sweep, F.C.; den Heijer, M. (2006). Thyroid Function and Prevalence of Anti-Thyroperoxidase Antibodies in a Population with Borderline Sufficient Iodine Intake: Influences of Age and Sex. *Clin. Chem.*, 52(1):104-111.

25- Wilson, I.D.; Foster, D.W. (1992). William's Textbook of Endocrinology, 8 th Edition. Philadelphia: WB. Saunders & Co; p.358–380.

26- De Leo, S.; Lee, S. Y., Braverman, L. E. (2016) Hyperthyroidism. *Lancet*, 388 (10047):906–918. doi: 10.1016/ S0140-6736(16)00278-6.

27- Dumont, J.E., Lamy, F., Roger, P., and Maenhaut, C. (1992). Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. *Physiol. Rev.*,72, 667 – 697.