

High Frequency of Skewed X-Chromosome Inactivation in Females with Autoimmune Thyroid Disease: A Possible Explanation for the Female Predisposition to Thyroid Autoimmunity

Thomas Heiberg Brix, Gun Peggy S. Knudsen, Marianne Kristiansen, Kirsten Ohm Kyvik, Karen Helene Ørstavik, and Laszlo Hegedüs

Department of Endocrinology (T.H.B., L.H.), Odense University Hospital, 5000 Odense C, Denmark; Department of Medical Genetics (G.P.S.K., M.K., K.H.Ø.), Rikshospitalet, Faculty Division, University of Oslo, 0027 Oslo, Norway; The Danish Twin Registry (K.O.K.), University of Southern Denmark, 5230 Odense M, Denmark; and Department of Medical Genetics (K.H.Ø.), Rikshospitalet University Hospital, 0027 Oslo, Norway

Context: Autoimmune thyroid diseases (AITD) comprise Graves' disease (GD) and Hashimoto's thyroiditis (HT). They are characterized by loss of immunological self-tolerance and female preponderance. Theoretically, X chromosome inactivation (XCI) and resultant tissue chimerism could offer an explanation for the female predisposition to AITD.

Aim: Our aim was to examine whether skewed XCI is associated with AITD.

Designs: We first conducted a classical case-control study of twin individuals with and without AITD, and then a case-control study of twin pairs discordant for AITD.

Participants: Participants included 32 female twins with AITD and a control group of 96 healthy female twin individuals.

Methods: XCI analysis was performed by enzymatic predigestion of DNA with a methylation-sensitive enzyme followed by PCR of the

polymorphic CAG repeat of the androgen receptor gene. The XCI pattern was classified as skewed when 80% or more of the cells preferentially inactivated the same X chromosome.

Main Outcome Measures: We assessed the prevalence of skewed XCI.

Results: The frequency of skewed XCI in female twins with AITD, GD, and HT was 34, 37, and 31%, respectively, which was higher than the prevalence in the corresponding control populations, 11% ($P = 0.003$), 14% ($P = 0.045$), and 8% ($P = 0.057$), respectively. Similar results were found in twin pairs discordant for AITD. Overall, skewed XCI was associated with an increased risk of developing AITD, with an odds ratio of 9.0 (95% confidence interval, 1.64–49.4) ($P = 0.022$).

Conclusion: These observations suggest a possible role of XCI in the etiology of AITD and may in part explain the female preponderance of AITD. (*J Clin Endocrinol Metab* 90: 5949–5953, 2005)

AUTOIMMUNE THYROID DISEASES (AITD) are a group of disorders characterized by loss of immunological self-tolerance (1). AITD can roughly be divided into Graves' disease (GD) and Hashimoto's thyroiditis (HT). In both phenotypes, lymphocytic infiltration of the thyroid gland with accompanying evidence of both humoral and cellular immune system activation is seen (1). In GD, the autoimmune process results in the production of thyroid-stimulating antibodies that activate the TSH receptor and leads to hyperthyroidism (2), whereas in HT the immune response is destructive, leading to thyroid cell death and hypothyroidism (3).

Together, clinically overt AITD affect 1–2% of the population, with a 5- to 10-fold excess in women (4). This phenomenon of female predisposition to thyroid autoimmunity

is often ascribed to hormonal differences, because in a number of experimental disease models, estrogens exacerbate disease and androgens can inhibit disease activity (5, 6). However, studies in man have failed to demonstrate a clear-cut influence of sex hormones on disease susceptibility to AITD. Moreover, the observed gender differences in AITD and other autoimmune disorders extend far beyond the hormonal differences (7). With this in mind, it is reasonable to consider alternative explanations for the increased prevalence of AITD in females.

A unifying feature of AITD seems to be the loss of immunological tolerance to self-antigens. A potential mechanism through which lack of exposure to X-linked self-antigens could occur in women is a skewing of X-chromosome inactivation (XCI) (8–10). In female mammalian cells, one of the two X chromosomes is inactivated in early embryonic life (11). Thus, females are mosaics for two cell lines, cells with the paternal or cells with the maternal X chromosome as the active X. Females frequently exhibit a random 50:50 ratio of the two cell lines (12). A skewed XCI is a deviation from this ratio and is arbitrarily defined, for instance, as a pattern where 80% or more of the cells inactivate the same X chro-

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Abbreviations: AITD, Autoimmune thyroid disease; DZ, dizygotic; GD, Graves' disease; HT, Hashimoto's thyroiditis; MZ, monozygotic; XCI, X-chromosome inactivation.

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mosome (12, 13). It follows that this can yield a situation in which self-antigens on one X chromosome may fail to be expressed at sufficiently high levels in the thymus, or in other peripheral sites that are involved in tolerance induction, but may yet be expressed with a high frequency in other peripheral tissues and blood cells. Theoretically, some females may be predisposed to express X-linked antigens in the periphery to which they have been insufficiently tolerized (9). Indeed, a skewed XCI in blood cells of women with the autoimmune connective tissue disease scleroderma has very recently been described (14). We speculate whether an analogous mechanism occurs in AITD.

Subjects and Methods

Subjects

Twin individuals with AITD as well as the control twins were identified through the Danish Twin Register. The ascertainment of twins with clinically overt AITD and their healthy twin siblings has previously been described and evaluated in detail (15–17). In brief, twins with clinically overt AITD and their co-twins were identified by means of questionnaires mailed in 1996/1997 to a representative nationwide sample of 6628 same-sex twin pairs born between 1953 and 1976. Based on a careful review of information from questionnaires, hospital files, outpatient clinics, and general practitioners, with special emphasis on blood tests and clinical criteria, the participants were classified as having or having had GD, nodular toxic goiter, HT, nontoxic goiter, or no thyroid disease. Definition of the various phenotypes is given in detail in previous publications based on this twin cohort (15, 16, 18). In all, 69 female twin individuals with AITD were identified (mean age at diagnosis, 27.9 yr; range, 19–40 yr). DNA samples were available from 40 of these individuals, and 32 (19 GD and 13 HT) subjects were heterozygous for the analyzed polymorphism in exon 1 of the androgen receptor gene and hence suitable for X-chromosome analysis. Because of death (two subjects), emigration (two subjects), loss of blood sample or insufficient amount of blood (10 subjects), and unwillingness to give a blood sample (15 subjects), DNA was not available in the remaining 29 subjects with AITD. To increase power, three control twin individuals, who were healthy, biochemically euthyroid, and matched for age (within 5 yr) and zygosity were identified for each case with AITD.

Informed consent was obtained from all the participants, and the study was approved by all the Regional Scientific Ethical Committees in Denmark.

Case-control study with external controls

In the first part of the study, we compared twin individuals with AITD (cases) with matched unrelated control twin individuals (external controls). The external case-control comparison is based on the 32 AITD cases and 96 external control subjects.

Case-control study using the co-twin as a control

The second part of the study was a within-pair comparison of 26 twin pairs discordant for AITD. In this approach, the discordant twin pairs are considered as matched pairs. This method has an additional advantage over other matched-pair designs in that twin pairs are also genetically matched [monozygotic (MZ) twins share 100% of their segregating genes, whereas dizygotic (DZ) twins share 50%, on average]. Moreover, the twins in a pair are of the same age and usually share their early life exposures, such as *in utero* environment, home location, nutrition, in-house toxicant exposures, and socioeconomic background.

XCI analysis and zygosity determination

DNA was extracted from peripheral blood cells. The XCI phenotype was determined by PCR analysis of a polymorphic (CAG)_n repeat in the first exon of the androgen receptor gene (19). After digestion of the DNA with the methylation-sensitive enzyme *HpaII*, a PCR product is obtained from the inactive X chromosome only. The PCR products were separated

on an ABI 3100 automated sequencer and analyzed by GeneScan software (Applied Biosystems, Oslo, Norway) (Fig. 1). Each sample was analyzed in duplicate and blinded as to the clinical phenotype and the result in the co-twin.

The XCI pattern was recorded as the relative amount of the PCR product of the smallest allele to the amount of PCR product from both alleles. Thus, XCI is a number between 0 and 100, where 50 reflects a random XCI pattern, and 0 and 100 indicate a completely skewed XCI. The XCI pattern was classified as skewed and extremely skewed, respectively, when 80% and 90% or more of the cells inactivated the same X chromosome.

Zygosity was established by analysis of nine highly polymorphic restriction fragment length polymorphisms and microsatellite markers scattered widely throughout the genome with a PE Applied Biosystems (Foster City, CA) AmpFISTER Profiles Plus Kit.

Statistical methods

In comparisons between AITD cases and external unrelated controls, group frequencies were analyzed with the χ^2 test or Fisher's exact test. Within-pair comparisons between AITD cases and their healthy co-twins were done using 2×2 contingency tables for paired observations and tested using the McNemar's test. With this approach, the odds ratio is given by the ratio of pairs in which the exposure differs, that is number of pairs in which the twin with AITD has a skewed XCI and the co-twin has a random XCI divided by the number of pairs in which the twin with AITD has a random XCI and the co-twin has a skewed XCI.

All tests applied were two tailed, and $P \leq 0.05$ was considered significant. All analyses were carried out using version 7 of the STATA statistical package.

Results

Case-control study with external controls

The prevalence of skewed XCI ($\geq 80\%$ skewing) in AITD and control subjects is summarized in Table 1. Overall, AITD cases had a significantly higher prevalence of skewed XCI than the external controls (34 *vs.* 11%; $P = 0.003$). Subdividing according to the clinical phenotype of the affected twin (GD and HT) yielded essentially similar results. The frequency of extremely skewed XCI ($\geq 90\%$ skewing) in cases with AITD, GD, and HT was 16% (5 of 32), 16% (3 of 19), and 15% (2 of 13), respectively, which was much higher than the prevalence in the corresponding control populations, 1% (1 of 96;

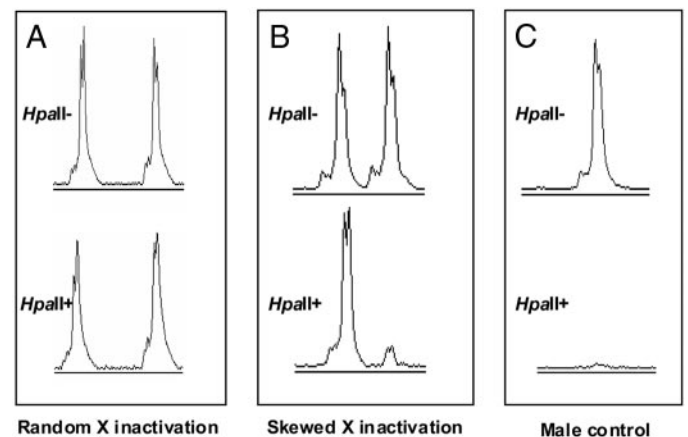


FIG. 1. *HpaII* indicates no predigestion and *HpaII*+ indicates predigestion with *HpaII*. A, Random XCI pattern; B, skewed XCI pattern; C, male control. A PCR product is obtained only from the inactive X chromosome. Note lack of a PCR product after *HpaII* digestion in the male control.

TABLE 1. XCI pattern in relation to phenotype

Phenotype	Random XCI [n (%)]	Skewed XCI ^a [n (%)]	<i>P</i> value
AITD cases (n = 32)	21 (66)	11 (34)	0.003
Control subjects (n = 96)	85 (89)	11 (11)	
GD (n = 19)	12 (63)	7 (37)	0.045 ^b
Control subjects (n = 57)	49 (86)	8 (14)	
HT (n = 13)	9 (69)	4 (31)	0.057 ^b
Control subjects (n = 39)	36 (92)	3 (8)	

^a At least 80% of the cells preferentially used the same X chromosome.

^b Fisher's test.

P = 0.004), 0% (0 of 57; *P* = 0.01), and 3% (1 of 39; *P* = 0.15), respectively.

Case-control study using the co-twin as control

The prevalence of skewed XCI in twins with AITD was 42% (11 of 26), which was significantly higher than the prevalence in the corresponding healthy co-twin population, 12% (3 of 26; *P* = 0.03). Overall, skewed XCI ($\geq 80\%$ skewing) was associated with an increased risk of developing AITD, with an odds ratio of 9.0 (95% confidence interval, 1.64–49.4) and *P* = 0.022 (Table 2). When the twin pairs were stratified according to the phenotype of the affected twin individual (17 pairs with GD and nine pairs with HT) or zygosity (six MZ pairs and 20 DZ pairs), the association did not reach statistical significance. However, even in the small number of pairs discordant for GD and HT, there was a trend toward an increased risk associated with skewed XCI (Table 2).

Discussion

We have demonstrated a significantly higher prevalence of skewed XCI in blood cells of females with AITD compared with a matched control group. Moreover, this frequency was much higher in female twins with AITD than in their healthy co-twins, indicating a possible role of XCI in the etiology of AITD and in the female preponderance of AITD.

We also tested the specificity of the relationship between XCI and AITD by stratifying the AITD cases into GD and HT. In the case-control study with external controls, skewed XCI was associated with both GD and HT, although the association with the latter was not statistically significant (*P* = 0.057). In within-pair comparisons between GD and HT cases and their healthy co-twins, no significant differences in skewed XCI were found. In this approach, however, a strong association will lead to relatively few discordant pairs (on which the odds ratio is calculated), and hence, the comparison will be of low power because of overmatching. The presence of overmatching may well explain why no statis-

tically significant associations were found among twin pairs discordant for GD or HT. Although the effect estimates were imprecise, the prevalence of skewed XCI seems to be much more apparent in twins with GD or HT than in their healthy co-twins. However, additional studies with larger sample sizes are needed to determine the relationship between XCI and GD and HT.

Another feature of our results is that they can explain the relatively low concordance rates (30–50%) for AITD in MZ twin pairs (15, 16) because the pattern of XCI differs if the twinning event is early (20). MZ twins may be monozygotic, where the two embryos have a common placenta and chorion, or dizygotic, where each embryo has its own placenta and chorion. Approximately one third of MZ twins are dizygotic and result from a twinning event that occurs about 0–4 d after conception, whereas the remaining two thirds are monozygotic and seem to be the result of an event that occurs more than 4 d after fertilization (21). A highly similar XCI pattern has been reported for monozygotic but not for dizygotic twin pairs (20). It is therefore possible that monozygotic twin pairs undergo splitting after X chromosome inactivation has taken place, whereas dizygotic pairs split before or around the time of the XCI process. In our study, as in most twin studies, information on the anatomy of chorion and placenta was not available, making it impossible for us to further explore a possible link between chorionic anatomy and concordance for AITD.

If a skewed XCI pattern is a significant factor in AITD, then one would expect this to occur also in other autoimmune disorders. Indeed, supporting data have recently been published for scleroderma (14). However, examination of XCI pattern in female patients with other autoimmune diseases such as systemic lupus erythematosus, insulin-dependent diabetes mellitus, and rheumatoid arthritis did not reveal skewed XCI patterns (10, 20, 22). Conversely, a higher than expected prevalence of autoimmune disease has been described in X-chromosome aneuploidies such as Turner's syndrome (23) and Klinefelter's syndrome (24).

Whether our results, obtained in twins, can be generalized to the background population depends on whether twins can be considered to be no different from non-twin individuals with respect to the XCI process and to the etiology of AITD. XCI occurs at the late blastocyst stage (approximately 5 d after fertilization), which is about the same time as the MZ twinning process. A relationship between the MZ twinning process and XCI has therefore been suggested (25), and a higher frequency of skewed XCI has been described in MZ compared with DZ twins (26). However, in a number of other studies, the degree of skewing in the MZ twins was no

TABLE 2. Number of pairs according to the XCI pattern in the probands and the corresponding healthy co-twins, stratified by phenotype

Phenotype	XCI status of the proband and healthy co-twin				Odds ratio (95% confidence interval) ^a
	Random and random	Random and skewed	Skewed and random	Skewed and skewed	
AITD	14	1	9	2	9 (1.64–49.4) ^b
GD	9	1	6	1	6 (0.94–38.5) ^c
HT	5	0	3	1	

^a Given as a test-based confidence interval.

^b McNemar's test; *P* = 0.022.

^c McNemar's test; *P* = 0.125.

different from that observed in non-twin females (27, 28) or in DZ twins (12). In fact, we found that young (18–54 yr) MZ twins had a slightly lower frequency of skewed XCI than young DZ twins (12). Thus, there is no evidence of any major differences between twins and non-twin individuals with respect to the XCI process. Moreover, the great majority of the twin pairs in the present study were DZ, which further minimizes this potential confounder.

Environmental factors of importance for developing AITD could have considerable impact during fetal life, when the immune system is immature and tolerance to various antigens is induced. If low birth weight *per se*, as recently suggested (29), is associated with AITD, one would expect a higher disease prevalence among twins than in the non-twin background population, because the birth weight of twins on average is 1000 g less than that of singletons. We can find no support of this, neither among twin pairs discordant for overt AITD nor among pairs discordant for thyroid autoantibodies (30, 31). Moreover, the prevalence of thyroid autoantibodies (31) and clinically overt AITD (15, 16) are not different from that reported in the Danish population (32, 33), indicating that there are no major differences between twins and non-twin individuals with respect to the occurrence of AITD.

A skewed XCI pattern may occur by chance, because of genetic factors influencing the XCI process or because of a selection process (34). In humans, the XCI phenotype has been linked to loci on the X chromosome, suggesting an X-linked inheritance of the XCI phenotype (35). It has also been suggested that genes on the X chromosome might show linkage with AITD (36). Thus, it is possible that the observed association between skewed XCI and AITD is not causal but just an epiphenomenon related to the inheritance of X-linked susceptibility genes. It is also important to point out that the XCI patterns vary between tissues because the event occurs at different times in different tissues (13). We have examined XCI in blood cells and it is possible that this does not accurately reflect the XCI patterns of cells in the thyroid gland. Thyroid tissue was, however, not available.

Accepting that a skewed XCI pattern is highly associated with AITD in females, it does not, however, lead to AITD in all. Thus, a skewed XCI is neither necessary nor sufficient for the development of AITD, indicating that environmental factors, such as iodine intake (37), smoking habits (38), stress (39), certain infections (40), or other agents, may trigger events leading to the development of AITD. In addition, the coinheritance of genetic susceptibility factors, such as functional variants of regulator genes of the immune system (36), may exacerbate the effects of skewed XCI and thereby contribute to the development of AITD.

In conclusion, our findings suggest a possible role of skewed XCI in the etiology of AITD and may in part explain the female preponderance of AITD.

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Address all correspondence and requests for reprints to: Thomas Heiberg Brix, M.D., Ph.D., Department of Endocrinology, Odense University Hospital, Sønder Boulevard 29, 5000 Odense C, Denmark. E-mail: thomas.brix@ouh.fyns-amt.dk.

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References

1. Weetman AP 2001 Determinants of autoimmune thyroid disease. *Nat Immunol* 2:769–770
2. McIver B, Morris JC 1998 The pathogenesis of Graves' disease. *Endocrinol Metab Clin North Am* 27:73–89
3. Weetman AP 1997 New aspects of thyroid immunity. *Horm Res* 48(Suppl 4):51–54
4. Wang C, Crapo LM 1997 The epidemiology of thyroid disease and implications for screening. *Endocrinol Metab Clin N Am* 26:189–218
5. Estienne V, Duthoit C, Reichert M, Praetor A, Carayon P, Hunziker W, Ruf J 2002 Androgen-dependent expression of FcγRIIB2 by thyrocytes from patients with autoimmune Graves' disease: a possible molecular clue for sex dependence of autoimmune disease. *FASEB J* 16:1087–1092
6. Cutolo M, Sulli A, Capellino S, Villaggio B, Montagna P, Seriolo B, Straub RH 2004 Sex hormones influence on the immune system: basic and clinical aspects in autoimmunity. *Lupus* 13:635–638
7. Whitacre CC 2001 Sex differences in autoimmune disease. *Nat Immunol* 2:777–780
8. Gregersen PK 1993 Discordance for autoimmunity in monozygotic twins. *Arthritis Rheum* 36:1185–1192
9. Stewart JJ 1999 Theory and treatment of the X-inactivation chimera in female-prevalent autoimmune disease. *Arch Immunol Ther Exp* 47:355–359
10. Chitnis S, Monteiro J, Glass D, Apatoff B, Salmon J, Concannon P, Gregersen PK 2000 The role of X-chromosome inactivation in female predisposition to autoimmunity. *Arthritis Res* 2:399–406
11. Lyon MF 1961 Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature* 190:372–373
12. Kristiansen M, Knudsen GPS, Bathum L, Naumova AK, Sorensen TI, Brix TH, Svendsen AJ, Christensen K, Kyvik KO, Orstavik KH 2005 Twin study of genetic and aging effects on X chromosome inactivation. *Eur J Hum Genet* 13:599–606
13. Sharp A, Robinson D, Jacobs P 2000 Age- and tissue-specific variation of X chromosome inactivation ratios in normal women. *Hum Genet* 107:343–349
14. Ozbalkan Z, Bagislar S, Kiraz S, Akyerli CB, Ozer HT, Yavuz S, Birlik AM, Calguneri M, Ozcelik T 2005 Skewed X chromosome inactivation in blood cells of women with scleroderma. *Arthritis Rheum* 52:1564–1570
15. Brix TH, Kyvik KO, Hegedüs L 2000 A population-based study of chronic autoimmune hypothyroidism in Danish twins. *J Clin Endocrinol Metab* 85: 536–539
16. Brix TH, Kyvik KO, Christensen K, Hegedüs L 2001 Evidence for a major role of heredity in Graves' disease: a population based study of two Danish twin cohorts. *J Clin Endocrinol Metab* 86:930–934
17. Brix TH, Kyvik KO, Hegedüs L 2001 Validity of self-reported hyperthyroidism and hypothyroidism: comparison of self-reported questionnaire data with medical record review. *Thyroid* 11:769–773
18. Brix TH, Kyvik KO, Hegedüs L 1999 Major role of genes in the etiology of simple goiter in females: a population-based twin study. *J Clin Endocrinol Metab* 84:3071–3075
19. Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW 1992 Methylation of *HpaII* and *HhaI* sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. *Am J Hum Genet* 51:1229–1239
20. Trejo V, Derom C, Vlietinck R, Ollier W, Silman A, Ebers G, Derom R, Gregersen PK 1994 X chromosome inactivation patterns correlate with fetal-placental anatomy in monozygotic twin pairs: implications for immune relatedness and concordance for autoimmunity. *Mol Med* 1:62–70
21. Chitnis S, Derom C, Vlietinck R, Derom R, Monteiro J, Gregersen PK 1999 X chromosome-inactivation patterns confirm the late timing of monoamniotic-MZ twinning. *Am J Hum Genet* 65:570–571
22. Huang Q, Parfitt A, Grennan DM, Manolios N 1997 X-chromosome inactivation in monozygotic twins with systemic lupus erythematosus. *Autoimmunity* 26:85–93
23. Chiovato L, Larizza D, Bendinelli G, Tonacchera M, Marino M, Mammoli C, Lorini R, Severi F, Pinchera A 1996 Autoimmune hypothyroidism and hyperthyroidism in patients with Turner's syndrome. *Eur J Endocrinol* 134: 568–575
24. Oktenli C, Yesilova Z, Kocak IH, Musabak U, Ozata M, Inal A, Gul D, Sanisoglu Y 2002 Study of autoimmunity in Klinefelter's syndrome and idiopathic hypogonadotropic hypogonadism. *J Clin Immunol* 22:137–143
25. Nance WE 1990 Do twin Lyons have larger spots? *Am J Hum Genet* 46:646–648
26. Goodship J, Carter J, Burn J 1996 X-inactivation patterns in monozygotic and dizygotic female twins. *Am J Med Genet* 61:205–208
27. Watkiss E, Webb T, Rysiecki G, Girdler N, Hewett E, Bunday S 1994 X inactivation patterns in female monozygotic twins and their families. *J Med Genet* 31:754–757

28. **Monteiro J, Derom C, Vlietinck R, Kohn N, Lesser M, Gregersen PK** 1998 Commitment to X inactivation precedes the twinning event in monozygotic MZ twins. *Am J Hum Genet* 63:339–346
29. **Phillips DI, Osmond C, Baird J, Huckle A, Rees-Smith B** 2002 Is birthweight associated with thyroid autoimmunity? A study in twins. *Thyroid* 12:377–380
30. **Brix TH, Kyvik KO, Hegedüs L** 2000 Low birth weight is not associated with clinically overt thyroid disease: a population based twin case-control study. *Clin Endocrinol (Oxf)* 53:171–176
31. **Brix TH, Hansen PS, Kyvik KO, Hegedüs L** 2004 Aggregation of thyroid autoantibodies in first-degree relatives of patients with autoimmune thyroid disease is mainly due to genes: a twin study. *Clin Endocrinol (Oxf)* 60:329–334
32. **Pedersen IB, Knudsen N, Jørgensen T, Perrild H, Ovesen L, Laurberg P** 2003 Thyroid peroxidase and thyroglobulin autoantibodies in a large survey of populations with mild and moderate iodine deficiency. *Clin Endocrinol (Oxf)* 58:36–42
33. **Laurberg P, Pedersen KM, Vestergaard H, Sigurdsson G** 1991 High incidence of multinodular toxic goitre in the elderly population in a low iodine intake area vs. high incidence of Graves' disease in the young in a high iodine intake area: comparative surveys of thyrotoxicosis epidemiology in East-Jutland Denmark and Iceland. *J Int Med* 229:415–420
34. **Puck JM, Willard HF** 1998 X inactivation in females with X-linked disease. *N Engl J Med* 338:325–328
35. **Naumova AK, Olien L, Bird LM, Smith M, Verner AE, Leppert M, Morgan K, Sapienza C** 1998 Genetic mapping of X-linked loci involved in skewing of X chromosome inactivation in the human. *Eur J Hum Genet* 6:552–562
36. **Tomer Y, Davies TF** 2003 Searching for the autoimmune thyroid disease susceptibility genes: from gene mapping to gene function. *Endocr Rev* 24:694–717
37. **Laurberg P, Bulow Pedersen I, Knudsen N, Ovesen L, Andersen S** 2001 Environmental iodine intake affects the type of nonmalignant thyroid disease. *Thyroid* 11:457–469
38. **Brix TH, Hansen PS, Kyvik KO, Hegedüs L** 2000 Cigarette smoking and risk of clinically overt thyroid disease: a population based twin case-control study. *Arch Intern Med* 160:661–666
39. **Winsa B, Adami HO, Bergstrom R, Gamstedt A, Dahlberg PA, Adamson U, Jansson R, Karlsson A, Bergström R** 1991 Stressful life events and Graves' disease. *Lancet* 338:1475–1479
40. **Wenzel BF, Peters A, Zubashev I** 1996 Bacterial virulence antigens and the pathogenesis of autoimmune thyroid diseases (AITD). *Exp Clin Endocrinol Diabetes* 104(Suppl 4):75–78

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