Takashi Akamizu · Hitomi Hiratani
Satoshi Ikegami · Stephen S. Rich
Donald W. Bowden

Association study of autoimmune thyroid disease at 5q23-q33 in Japanese patients

Received: 22 January 2003 / Accepted: 20 February 2003 / Published online: 9 April 2003
© The Japan Society of Human Genetics and Springer-Verlag 2003

Abstract As part of a genome scan to locate familial Graves' disease (GD) and Hashimoto's thyroiditis (HT) genes, an autoimmune thyroid disease (AITD) susceptibility locus has recently been identified at 5q31-q33 in a Japanese population. We performed an association study using six microsatellite markers located at this locus in a set of 440 unrelated Japanese AITD patients and 218 Japanese controls. We found significant allelic association between AITD and three markers located in 5q23-q33. GD demonstrated significant associations with two of these markers, while HT did not show significant associations with any markers. Further, when patients with GD were stratified according to clinical manifestations, the association was significantly different from the other subgroup of each category. These findings suggest the presence of susceptible genes of AITD, especially distinct subgroups of GD, in or near 5q23-q33.

Keywords Autoimmune thyroid disease · Graves' disease · Susceptibility gene · Microsatellite marker · 5q23-q33 · 5q31-q33

Introduction

The genetic basis of autoimmune thyroid disease (AITD), which includes Graves' disease (GD) and Hashimoto's thyroiditis (HT), is thought to be polygenic (Gough 2000; Brix and Hegedus 1998). Several genetic factors associated with AITD have been tentatively identified by candidate-gene and genome-scanning approaches. They include HLA, cytotoxic T-lymphocyte-associated-4 (*CTLA-4*), and TSHR gene, and other chromosomal regions, such as 14q31 (GD-1), 20q11.2 (GD-2), Xq21.33–22 (GD-3), 13q32 (HT-1), 12q22, 18q21 (IDDM 6), and Xp11 (Farid et al. 1979; Uno et al. 1981; Yanagawa et al. 1995; Donner et al. 1997; Vaidya et al. 1999; Barbesino et al. 1998; Tomer et al. 1999; Vaidya et al. 2000; Sale et al. 1997; Akamizu et al. 2000). Evidence for AITD genes, however, varies among researchers or populations, and no consensus has been obtained.

Recently, Sakai and co-workers mapped susceptibility loci for HT to 8q23-q24 and AITD to 5q31-q33 by multipoint affected sib-pair linkage analysis in a collection of Japanese AITD families (Sakai et al. 2001). First, chromosome 8q23-q24, which encompasses the thyroglobulin gene, yielded suggestive evidence for linkage to HT with an MLS of 3.77 at D8S272. A recent report confirmed that the thyroglobulin gene is linked and associated with AITD (Tomer et al. 2002). Secondly, chromosome 5q31-q33 yielded suggestive evidence for linkage to AITD with an MLS of 3.14 at D5S436. Chromosome 5q31-q33 includes a cytokine gene cluster and other immune-related genes, and has been reported to be associated or linked to allergic diseases such as asthma and atopic dermatitis (Meyers et al. 1994; Postma et al. 1995; Lonjou et al. 2000). Considering that AITD is an autoimmune disease, this is an intriguing region, where susceptible genes may exist.

In this study we attempted to confirm evidence for the presence of susceptibility genes in or near 5q23-q33 by association analysis, using several microsatellite markers,

T. Akamizu (✉) · H. Hiratani · S. Ikegami
Department of Experimental Therapeutics,
Translational Research Center,
Kyoto University Hospital,
54 Shogoin-Kawaharacho,
Sakyo-ku, Kyoto 606-8507, Japan
E-mail: akataka@kuhp.kyoto-u.ac.jp
Tel.: +81-75-7514723
Fax: +81-75-7514731

D.W. Bowden
Department of Biochemistry,
Wake Forest University School of Medicine,
Winston-Salem, North Carolina, 27157, USA

S.S. Rich
Department of Public Health Science,
Wake Forest University School of Medicine,
Winston-Salem, North Carolina, 27157, USA

including D5S436, the linked marker from the genome screen. In addition, we carried out subgroup analysis of Graves' patients based on clinical manifestations in different subgroups and analyzed if the patterns of their associations with these markers were different between subgroups.

Subjects and Methods

Study subjects

Four hundred and forty unrelated Japanese individuals with AITD (337 female, 103 male) were evaluated. In cases where more than one individual from a family was collected, data from only a single individual from the family was included in the genetic analysis. Patients were classed as having either GD ($n=241$), HT ($n=139$), primary hypothyroidism with TSH-binding inhibitory immunoglobulin (TBII) ($n=35$), or hypothyroidism without TBII ($n=25$). These patients were diagnosed and assayed for antibodies as described previously (Sale et al. 1997; Akamizu et al. 2000). As an unaffected control population, we have also collected an expanded set of controls, consisting of 218 unrelated Japanese individuals (89 male, 129 female). Informed consent was obtained from all the subjects used in this study and the present study was approved by the ethical committee of Kyoto University Graduate School of Medicine.

Genotyping loci at 5q23-q33

Loci at 5q15-q33 were genotyped using seven microsatellite markers derived from the ABI PRISM Linkage Mapping Set Version 2 (Table 1). PCR was performed using the GeneAmp System 9600 with standard protocols. The PCR products were analyzed by electrophoresis through a 6% denaturing polyacrylamide gel using an ABI 373 automated sequencer apparatus, and the resulting data were analyzed with Genescan software (V 1.2) (Akamizu et al. 2000).

Association analyses

The frequencies of alleles of loci at 5q23-q33 in the Japanese were calculated from the control populations. Male and female frequencies were compared using the RXC program (George Carmody, Carleton University, Ottawa, Canada), which carries out Monte Carlo simulations (Roff 1989; Waye et al. 1994) to calculate the statistical significance of contingency tables. Because the numbers of male patients were too small, compared with those of females, and patients and controls were not matched for gender, we have presented the data and results for all patients (males + females) throughout the tables. Results from female and male data are noted in the text where their mention seemed relevant. In all cases the allele frequencies in the control male and female allele frequencies were not significantly different, and they were consequently pooled. Rare

alleles, e.g., alleles with frequencies less than 0.014 in the population, were pooled for analysis as noted in Table 2. Association analyses between controls and patients were carried out using a contingency 2x2 table to calculate an odds ratio and χ^2 . For multiple-comparisons procedures, P values from χ^2 analysis were corrected by the method of Bonferroni adjustment (Akamizu et al. 2000; Rosner 1990). P values less than $0.05/N$ (where N is the number of alleles of each marker shown in Table 2) were considered significant. Pooled alleles were considered as one. For example, N was 8 in D5S2115.

Tests for risk associated with specific alleles and interaction between AITD associated alleles

Alleles of markers that showed evidence of significant association with AITD were evaluated for the relative risk which they contributed to disease susceptibility. These evaluations were also carried out using a conventional 2x2 table to calculate an odds ratio and χ^2 .

Clinical manifestations of Graves' patients

Patients with GD were divided into subgroups based upon various clinical manifestations: goiter size, ophthalmopathy, and titer of anti-TSH receptor antibody. Goiter size and titer of anti-TSH receptor antibody were measured at onset or before treatment. The size of goiter was determined by measuring the transverse diameter. The patients were divided into two subgroups, large (≥ 5 cm) ($n=119$) or small (< 5 cm) ($n=78$) goiter groups. Patients with TAO were defined by the presence of proptosis [American Thyroid Association (ATA) class 3] or higher ATA class (diplopia, corneal, and optic-nerve involvement) (Werner 1977). GD patients without and with ophthalmopathy were 111 and 88, respectively. For anti-TSH receptor antibodies, TBII (TSH binding inhibitor immunoglobulin) activity (Rees Smith et al. 1988; Endo et al. 1978) was measured, because TBII activity was most frequently measured at the onset of GD as an anti-TSH receptor antibody. GD patients with potent ($\geq 50\%$) and weak ($< 50\%$) TBII were 64 and 90, respectively. Patients who responded well to anti-thyroid drugs and went into remission were classed as "excellent responders" ($n=32$), while those who needed anti-thyroid drugs (propylthiouracil or methimazol) after two years' treatment to maintain euthyroid function were classed as "poor responders" ($n=119$).

Results

Association studies

Table 1 shows chromosome localization, allele size range and heterozygosity of 5q microsatellite markers in a Japanese population. Six markers are located in 5q23-q33. D5S2011, D5S2090 and D5S2049 were not used in the previous study of Sakai et al. (2001). Table 2 shows

Table 1 Chromosome localization, allele size range, and heterozygosity of 5q microsatellite markers in a Japanese population

Microsatellite marker	Map position		Allele size range (bp)	HET ^a
	Genethon genetic (cM)	Ideogram		
D5S2115	138.6	5q23-q31	152–182	0.787
D5S2011	143.7	5q31	142–160	0.804
D5S436	147.2	5q31-q33	240–258	0.732
D5S2090	149.9	5q31-q33	193–209	0.684
D5S410	156.0	5q33	330–342	0.648
D5S2049	160.4	5q33	87–103	0.560

^aHeterozygosity in Japanese controls of this study

Table 2 Polymorphism allele frequencies in Japanese AITD patients and controls. Frequencies in *bold* are significantly different in patients vs controls at $P < 0.05$ in the method of Bonferroni adjustment; *italics* show that the initial frequencies are $P < 0.05$ but become non-significant after the adjustment; n = total number alleles

Allele	Base pairs	Controls	AITD	GD	HT	<i>Hypothyroidism</i> ^{a,b}	
						TBII(+)	TBII(-)
D5S2115		$n = 326$	$n = 800$	$n = 419$	$n = 266$	$n = 70$	$n = 40$
1	164	0.043	0.035	0.024	0.041	0.057	0.068
2	168	0.049	0.031	0.038	0.030	0.000	0.023
3	170	0.138	0.120	0.131	0.117	0.071	0.114
4	172	0.390	0.490	0.499	<i>0.485</i>	0.514	0.409
5	174	0.150	0.141	0.124	0.173	0.143	0.114
6	176	0.107	0.110	0.126	0.075	0.143	0.114
7	178	0.058	0.020	0.014	<i>0.019</i>	0.043	0.045
Pooled alleles ^a		0.064	0.053	0.043	0.060	0.029	0.114
D5S2011		$n = 368$	$n = 880$	$n = 480$	$n = 282$	$n = 68$	$n = 50$
1	144	0.019	0.003	<i>0.004</i>	0.004	0.000	0.000
2	146	0.215	0.230	0.240	0.220	0.206	0.220
3	148	0.313	<i>0.377</i>	<i>0.400</i>	0.340	0.353	0.400
4	150	0.190	0.175	0.150	0.220	0.221	0.100
5	152	0.071	0.044	0.044	0.043	0.044	0.060
6	154	0.068	0.058	0.060	0.071	0.000	0.040
7	156	0.057	0.044	0.031	0.046	0.132	0.040
8	158	0.052	0.065	0.069	0.053	0.044	0.120
Pooled alleles ^b		0.016	0.003	0.002	0.004	0.000	0.020
D5S436		$n = 342$	$n = 799$	$n = 413$	$n = 266$	$n = 70$	$n = 50$
1	240	0.070	0.091	0.087	0.083	0.157	0.080
2	242	0.447	0.468	0.460	0.508	0.371	0.460
3	244	0.026	0.033	0.034	0.038	0.029	0.000
4	246	0.135	0.135	0.136	0.135	0.114	0.160
5	248	0.120	<i>0.078</i>	<i>0.077</i>	<i>0.068</i>	0.114	0.080
6	250	0.173	0.139	0.143	0.128	0.157	0.140
7	252	0.018	0.025	0.024	0.023	0.000	<i>0.080</i>
Pooled alleles ^c		0.012	0.031	0.039	0.019	0.057	0.000
D5S2090		$n = 376$	$n = 880$	$n = 482$	$n = 278$	$n = 70$	$n = 50$
1	193	0.037	0.063	<i>0.068</i>	0.054	0.100	0.000
2	195	0.508	0.485	0.473	0.471	0.543	0.600
3	197	0.101	0.085	0.093	0.076	0.071	0.080
4	201	0.191	0.173	0.178	0.180	0.157	0.100
5	203	0.021	0.031	0.031	0.014	0.071	0.060
6	205	0.088	0.113	0.108	<i>0.151</i>	0.043	0.040
7	207	0.040	0.039	0.035	0.047	0.014	0.060
Pooled alleles ^d		0.013	0.013	0.012	0.007	0.000	0.060
D5S410		$n = 367$	$n = 816$	$n = 430$	$n = 280$	$n = 62$	$n = 44$
1	330	0.030	0.005	0.002	<i>0.007</i>	0.000	0.023
2	332	0.302	<i>0.237</i>	0.212	<i>0.214</i>	0.403	0.386
3	334	0.493	0.533	0.551	0.557	0.435	0.341
4	336	0.057	0.086	<i>0.098</i>	0.046	<i>0.129</i>	<i>0.159</i>
5	338	0.114	0.137	0.137	0.168	0.032	0.091
Pooled alleles ^e		0.003	0.002	0.000	0.007	0.000	0.000
D5S2049		$n = 338$	$n = 868$	$n = 476$	$n = 284$	$n = 62$	$n = 48$
1	91	0.018	0.025	0.025	0.032	0.000	0.022
2	93	0.163	0.175	0.168	0.169	0.194	0.261
3	95	0.630	0.577	0.574	0.570	0.661	0.543
4	97	0.098	0.100	0.111	0.102	0.016	0.087
5	101	0.077	0.104	0.105	0.106	0.113	0.065
Pooled alleles ^f		0.015	0.018	0.017	0.021	0.016	0.022

^aPooled alleles: 152, 154, 156, 158, 160, 162, 166, 180, 182 bp (control frequencies < 0.015)

^bPooled alleles of 142, 160 bp (control frequencies < 0.012)

^cPooled alleles: 254, 256, 258 bp (control frequencies < 0.013)

^dPooled alleles: 199, 209 bp (control frequencies < 0.012)

^ePooled alleles: 340, 342 bp (control frequencies < 0.012)

^fPooled alleles: 87, 89, 99, 103 bp (control frequencies < 0.012)

the results of the association analysis of polymorphic alleles of these seven markers and AITD. Significant differences between the allele frequencies of cases and controls are reported on the basis of allele-specific tests

of significance, using a χ^2 statistic with one degree of freedom. Table 3 summarizes P values of the χ^2 statistic. Frequencies of alleles of all these markers in the control Japanese males and females were not significantly

Table 3 *P* values of chi square analysis in association study of Japanese AITD patients. *P* values in *bold* are significantly different in patients vs controls at *P* < 0.05 in the method of Bonferroni adjustment

Allele	Base pairs	AITD	GD	HT	Hypothyroidism		Effect
					TBII(+)	TBII(-)	
D5S2115							
Allele 4	172	0.00217	0.00295	0.0198	0.0544	0.803	+
Allele 7	178	7.87×10⁻⁴	9.48×10⁻⁴	0.0154	0.609	0.730	-
D5S2011							
Allele 1	144	0.00479	0.0364	0.0761	0.252	0.325	-
D5S410							
Allele 1	330	3.64×10⁻⁴	0.00140	0.0403	0.167	0.787	-
Allele 2	332	0.0164	0.00330	0.0117	0.1150	0.256	-

different (*P* = 0.12–0.98) and the data were pooled for analysis. We found significant allelic association between AITD and three of six markers. Of three markers, two showed significant allelic association with GD, but D5S2011 did not. For the D5S410 polymorphism, allele 2 (332 bp) was significantly decreased only in GD cases. HT and other hypothyroidisms did not show significant associations with any markers. Although several alleles of these markers showed *P* values less than 0.05 in association analyses (*italic figures* in Table 2), they were considered non-significant after correction by the method of Bonferroni adjustment for multiple-comparisons procedures.

Risks associated with AITD susceptibility alleles

The increased risk for developing AITD in individuals with specific susceptibility alleles of six microsatellite markers was evaluated by calculating the odds ratios (i.e., risk ratios), as shown in Table 4. Allele 4 of D5S2115 showed increased risks of 1.5. Similarly, the reduced risk for developing AITD was evaluated by calculating the odds ratios. The reduced risks were 0.2–0.3.

Clinical manifestations of Graves' disease

To further examine association of GD with 5q markers, patients with GD were categorized into subgroups, based on clinical findings (Table 5). GD without ophthalmopathy and GD of "poor drug responders" were associated with most or all alleles that showed significant association with GD all. Patients with large goiter

(≥5 cm) also showed similar tendency, although they associated with fewer alleles (two alleles). For the TBII activity, GD with weak TBII was associated with allele 4 of D5S410 that also showed significant association with GD of "excellent drug responders".

Discussion

We found significant association between AITD/GD and several microsatellite markers located in 5q23-33. None of these markers were not associated with HT or other hypothyroidism. Although it might be possible that analysis of HT or other hypothyroidism has less power than that of AITD or GD because HT or other hypothyroidism groups are fewer in number, this finding supports the previously published linkage analysis evidence that chromosome 5q31-33 contains AITD/GD susceptibility gene(s), but not HT (Sakai et al. 2001). Although alleles of D5S436 showed a strong linkage with AITD and GD in the previous genome-wide screen, allelic association with them became non-significant after the correction by the method of Bonferroni adjustment. These observations suggest that D5S2115 and/or D5S410 may be more likely locations of AITD and GD susceptibility genes than D5S436 in our population. Considering that genetic distance between D5S2115 and D5S410 is approximately 20 cM, it is unlikely that the two markers are both in linkage disequilibrium with a single variation of the putative susceptibility gene of AITD and GD. Instead, they might suggest that multiple independent susceptibility genes exist within 5q23-33.

Chromosome 5q23-33 contains the human cytokine gene cluster (5q31.1), containing genes for interleukin (IL)-3, IL-4, IL-5, IL-9, IL-13, granulocyte/macrophage

Table 4 Risks associated with AITD susceptibility alleles

Locus		Cases	Controls	Odds ratio	95% confidence interval	<i>P</i> value
D5S2115	Allele 4' 172 bp	392	127	1.5	1.2–2.0	0.00217
	NOT 'allele 4' 172 bp	408	199			
	Allele 7' 178 bp	16	19	0.3	0.2–0.6	7.87×10 ⁻⁴
D5S2011	NOT 'allele 7' 178 bp	784	307			
	Allele 1' 144 bp	3	7	0.2	0.0–0.7	0.00479
D5S410	NOT 'allele 1' 144 bp	877	361			
	Allele 1' 330 bp	4	11	0.2	0.1–0.5	3.64×10 ⁻⁴
	NOT 'allele 1' 330 bp	812	356			

markers spanning this region; (2) to find single-nucleotide polymorphism of candidate genes located in this region, which are not only associated or linked, but also functionally related to AITD.

Acknowledgements This work was supported by grants-in-aid from the Ministry of Education, Science, Culture, Sports and Technology of Japan (nos. 12204065 and 13204046) and the Ministry of Health, Labor and Welfare, Japan to T.A.. We are indebted to many doctors, especially Drs. Keiichi Kamijo and Kishiko Nakamura, for collecting blood samples of AITD patients and normal subjects, respectively, and also to the patients and unaffected individuals who participated in this study. We thank Miss Maki Kouchi for excellent secretarial assistance.

References

- Akamizu T, Sale M, Rich SS, Hiratani H, Noh JY, Kanamoto N, Saijo M, Miyamoto Y, Saito Y, Nakao K, Bowden DW (2000) Association of autoimmune thyroid disease with microsatellite markers for the thyrotropin receptor gene and CTLA-4 in Japanese patients. *Thyroid* 10:851–858
- Barbesino G, Tomer Y, Concepcion ES, Davies TF, Greenberg DA (1998) Linkage analysis of candidate genes in autoimmune thyroid disease. II. Selected gender-related genes and the X-chromosome. International Consortium for the Genetics of Autoimmune Thyroid Disease. *J Clin Endocrinol Metab* 83:3290–3295
- Brix TH, Kyvik KO, Hegedus L (1998) What is the evidence of genetic factors in the etiology of Graves' disease? A brief review. *Thyroid* 8:727–734
- Donner H, Rau H, Walfish PG, Braun J, Siegmund T, Finke R, Herwig J, Usadel KH, Badenhop K (1997) CTLA4 Alanine-17 confers genetic susceptibility to Graves' disease and to type 1 diabetes mellitus. *J Clin Endocrinol Metab* 82:143–146
- Endo K, Kasagi K, Konishi J, Ikekubo K, Okuno T, Takeda Y, Mori T, Torizuka K (1978) Detection and properties of TSH-binding inhibitor immunoglobulins in patients with Graves' disease and Hashimoto's thyroiditis. *J Clin Endocrinol Metab* 46:734–739
- Farid NR, Sampson L, Noel EP, Barnard JM, Mandeville R, Larsen B, Marshall WH, Carter ND (1979) A study of human leukocyte D locus related antigens in Graves' disease. *J Clin Invest* 63:108–113
- Gough SC (2000) The genetics of Graves' disease. *Endocrinol Metab Clin North Am* 29:255–266
- Heuer M, Aust G, Ode-Hakim S, Scherbaum WA (1996) Different cytokine mRNA profiles in Graves' disease, Hashimoto's thyroiditis, and nonautoimmune thyroid disorders determined by quantitative reverse transcriptase polymerase chain reaction (RT-PCR). *Thyroid* 6:97–106
- Hunt PJ, Marshall SE, Weetman AP, Bell JI, Wass JA, Welsh KI (2000) Cytokine gene polymorphisms in autoimmune thyroid disease. *J Clin Endocrinol Metab* 85:1984–1988
- Laurberg P, Buchholtz Hansen PE, Iversen E, Eskjaer Jensen S, Weeke J (1986) Goitre size and outcome of medical treatment of Graves' disease. *Acta Endocrinol (Copenh)* 111:39–43
- Le Beau MM, Espinosa R 3rd, Neuman WL, Stock W, Roulston D, Larson RA, Keinänen M, Westbrook CA (1993) Cytogenetic and molecular delineation of the smallest commonly deleted region of chromosome 5 in malignant myeloid diseases. *Proc Natl Acad Sci USA* 90:5484–5488
- Lonjou C, Barnes K, Chen H, Cookson WO, Deichmann KA, Hall IP, Holloway JW, Laitinen T, Palmer LJ, Wjst M, Morton NE (2000) A first trial of retrospective collaboration for positional cloning in complex inheritance: assay of the cytokine region on chromosome 5 by the consortium on asthma genetics (COAG). *Proc Natl Acad Sci USA* 97:10942–10947
- Mansur AH, Bishop DT, Markham AF, Britton J, Morrison JF (1998) Association study of asthma and atopy traits and chromosome 5q cytokine cluster markers. *Clin Exp Allergy* 28:141–150
- Meyers DA, Postma DS, Panhuysen CI, Xu J, Amelung PJ, Levitt RC, Bleecker ER (1994) Evidence for a locus regulating total serum IgE levels mapping to chromosome 5. *Genomics* 23:464–470
- Michelangeli V, Poon C, Taft J, Newnham H, Topliss D, Colman P (1998) The prognostic value of thyrotropin receptor antibody measurement in the early stages of treatment of Graves' disease with antithyroid drugs. *Thyroid* 8:119–124
- Mirow AL, Kristbjarnarson H, Egeland JA, Shilling P, Helgason T, Gillin JC, Hirsch S, Kelsoe JR (1994) A linkage study of distal chromosome 5q and bipolar disorder. *Biol Psychiatry* 36:223–229
- Postma DS, Bleecker ER, Amelung PJ, Holroyd KJ, Xu J, Panhuysen CI, Meyers DA, Levitt RC (1995) Genetic susceptibility to asthma-bronchial hyperresponsiveness coinherited with a major gene for atopy. *N Engl J Med* 333:894–900
- Rees Smith B, McLachlan SM, Furmaniak J (1988) Autoantibodies to the thyrotropin receptor. *Endocr Rev* 9:106–121
- Rioux JD, Stone VA, Daly MJ, Cargill M, Green T, Nguyen H, Nutman T, Zimmerman PA, Tucker MA, Hudson T, Goldstein AM, Lander E, Lin AY (1998) Familial eosinophilia maps to the cytokine gene cluster on human chromosomal region 5q31-q33. *Am J Hum Genet* 63:1086–1094
- Roff DA, Bentzen P (1989) The statistical analysis of mitochondrial DNA polymorphisms: c2 and the problem of small samples. *Mol Biol Evol* 6:539–545
- Rosner B (1990) Fundamentals of Biostatistics. PWS-Kent, Boston, pp 303–305
- Sakai K, Shirasawa S, Ishikawa N, Ito K, Tamai H, Kuma K, Akamizu T, Tanimura M, Furugaki K, Yamamoto K, Sasazuki T (2001) Identification of susceptibility loci for autoimmune thyroid disease to 5q31-q33 and Hashimoto's thyroiditis to 8q23-q24 by multipoint affected sib-pair linkage analysis in Japanese. *Hum Mol Genet* 10:1379–1386
- Sale MM, Akamizu T, Howard TD, Yokota T, Nakao K, Mori T, Iwasaki H, Rich SS, Jennings-Gee JE, Yamada M, Bowden DW. (1997) Association of autoimmune thyroid disease with a microsatellite marker for the thyrotropin receptor gene and CTLA-4 in a Japanese population. *Proc Assoc Amer Physician* 109:453–461
- Tomer Y, Barbesino G, Greenberg DA, Concepcion E, Davies TF (1999) Mapping the major susceptibility loci for familial Graves' and Hashimoto's diseases: evidence for genetic heterogeneity and gene interactions. *J Clin Endocrinol Metab* 84:4656–4664
- Tomer Y, Greenberg DA, Concepcion E, Ban Y, Davies TF (2002) Thyroglobulin is a thyroid specific gene for the familial autoimmune thyroid diseases. *J Clin Endocrinol Metab* 87:404–407
- Uno H, Sasazuki T, Tamai H, Matsumoto H (1981) Two major genes, linked to HLA and Gm, control susceptibility to Graves' disease. *Nature* 292:768–770
- Vaidya B, Imrie H, Perros P, Young ET, Kelly WF, Carr D, Large DM, Toft AD, McCarthy MI, Kendall-Taylor P, Pearce SH (1999) The cytotoxic T lymphocyte antigen-4 is a major Graves' disease locus. *Hum Mol Genet* 8:1195–1199
- Vaidya B, Imrie H, Perros P, Young ET, Kelly WF, Carr D, Large DM, Toft AD, Kendall-Taylor P, Pearce SH (2000) Evidence for a new Graves disease susceptibility locus at chromosome 18q21. *Am J Hum Genet* 66:1710–1714
- van Leeuwen BH, Martinson ME, Webb GC, Young IG (1989) Molecular organization of the cytokine gene cluster, involving the human IL-3, IL-4, IL-5, and GM-CSF genes, on human chromosome 5. *Blood* 73:1142–1148
- Waye JS, Eng B, Ni HY, Blajchman MA, Carmody G (1994) Trinucleotide repeat polymorphism within the human antithrombin gene (AT3): allele frequency data for three population groups. *Mol Cell Probes* 8:149–154

- Werner SC (1977) Modification of the classification of the eye changes of Graves' disease: recommendations of the Ad Hoc Committee of the American Thyroid Association. *J Clin Endocrinol Metab* 44:203–204
- Wills-Karp M. (2000) The gene encoding interleukin-13: a susceptibility locus for asthma and related traits. *Respir Res* 1:19–23
- Yamada T, Sato A, Komiya I, Nishimori T, Ito Y, Terao A, Eto S, Tanaka Y (2000) An elevation of serum immunoglobulin E provides a new aspect of hyperthyroid Graves' disease. *J Clin Endocrinol Metab* 85:2775–2778
- Yanagawa T, Hidaka Y, Guimaraes V, Soliman M, DeGroot LJ (1995) CTLA-4 gene polymorphism associated with Graves' disease in a Caucasian population. *J Clin Endocrinol Metab* 80:41–45
- Young ET, Steel NR, Taylor JJ, Stephenson AM, Stratton A, Holcombe M, Kendall-Taylor P (1988) Prediction of remission after antithyroid drug treatment in Graves' disease. *Q J Med* 66:175–189