行政院國家科學委員會專題研究計畫 期中進度報告

葛瑞夫茲氏病遺傳研究-連鎖及關聯研究(1/3)
期中進度報告(完整版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-3112-B-002-026-
執 行 期 間 ：95 年 05 月 01 日至 96 年 04 月 30 日
執 行 單 位 ：國立臺灣大學醫學院內科

計 畫 主 持 人：張天鈞
共 同 主 持 人：楊偉勛、范盛娟

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 96 年 03 月 21 日

行政院國家科學委員會補助專題研究計畫 ■期中進度報告

葛瑞夫茲氏病遺傳研究-連鎖及關聯研究(1/3)

計畫類別：▒個別型計畫　　□ 整合型計畫
計畫編號：NSC 95-3112-B-002-026
執行期間：95 年 5 月 1 日 至 96 年 4 月 30 日

計畫主持人：張天鈞
共同主持人：楊偉勛
計畫參與人員：范盛娟 陳沛隆 張倩青 吳宜凌

成果報告類型(依經費核定清單規定繳交)：□精簡報告　▒完整報告

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：國立臺灣大學醫學院內科

中　華　民　國　　96 年　3 月　21 日

中文摘要

葛瑞夫茲氏病是常見的自體免疫疾病，發病原因一般認為是多重因素，也與遺傳有關。我們的連鎖研究涵蓋 122 個葛瑞夫茲氏病家族，共 536 個人。所有家族皆有兩位以上的兄弟姊妹罹患此病，若有可能，父母之檢體也涵蓋在內。若父母之一或兩者之血液檢體不能取得，則要包含一位未罹病之兄弟姊妹之檢體。共計有 270 對罹患葛瑞夫茲氏病之兄弟姊妹(affected sib-pairs)可以做無母數連鎖分析(non-parametric linkage study)。我們從白血球萃取 DNA，並做 short tandem repeat polymorphism(STRP)標記之基因定型。我們選擇涵蓋 HLA 區 13.7 cM 之 8 個 STRP，平均每個標記的密度為 1.9 cM。此外其他四個候選區為 2q33(CTLA4)15.1 cM 之 5 個標記，5q31(cytokine) 18.9 cM 之 7 個標記，7q22(pendrin)10.1 cM 之 5 個標記，14q31(GD-1 和 TSH receptor)61.1cM 之 9 個標記。多點無母數連鎖分析顯示與 HLA 區有相關，尖峰在標記 UniSTS:239159(LOD score 3.46, P = .00003, NPL Z score 4.1, P = .00002)。其他四個候選區並無有意義之關聯。在以族群為基礎的研究，我們已完成 1016 位葛瑞夫茲氏病病人的檢體收集。男性 166 位，女性 850 位 (男比女 = 1:5.1) 。平均年齡為 40.9 ± 12.8 歲 (範圍 9 – 81 歲)。發病平均年齡為 35.9 ± 12.6 歲 (範圍 6 – 81 歲)。甲狀腺腫程度為 2.1 ± 0.8 (範圍 0 – 4). 。有 498 位病人(49.0%) 有眼病變 (Gr. 1: 126, 12.4%; Gr 2: 233, 22.9%; Gr. 3: 139, 13.7%), 51 位病人(5.0%)有脛前粘液水腫 20 位病人(2.0%)有週期性癱瘓 。14 位病人(1.4%)有重症肌無力。8 位病人(0.8%) 有白斑。現所有病人之 DNA 已萃取完成。300 位有眼病變之女性之 DNA 已先送去做全基因掃描。

關鍵詞：葛瑞夫茲氏病，連鎖研究，關聯研究，基因

英文摘要

Graves' disease (GD) is an autoimmune disorder. The etiology of GD is accepted to be multifactorial with genetic effect. Our linkage study included 536 individuals in 122 multiplex families. All the families contained at least two affected siblings. Parents were also enrolled whenever possible; if samples from one or both parents were unavailable, at least one additional unaffected sibling was included. These participants could be analyzed as 270 affected sib-pairs (ASPs) in non-parametric linkage study. Genomic DNA was extracted from peripheral leukocytes. Genotyping of short tandem repeat polymorphism (STRP) markers was performed. Eight STRPs in a 13.7 cM region covering the HLA were chosen, resulting in a 1.9-cM (average) marker density. For the other four candidate regions, the distribution of these 26 markers was 5 markers in a 15.1-cM region on 2q33 (CTLA4), 7 markers in an 18.9-cM region on 5q31 (cytokine), 5 markers in a 10.1-cM region on 7q22 (pendrin), 9 markers in a 61.1-cM region on 14q31 (GD-1 and the TSHR). Multi-point non-parametric linkage analysis yielded evidence of significant linkage to the HLA region, which peaked around the marker UniSTS:239159 (LOD score 3.46, P = .00003; NPL Z score 4.1, P = .00002). For the other four candidate regions, none of the NPL Z scores reached suggestive level of significance. In the association study, we have completed the collection of 1,016 GD individuals. There are 166 men, and 850 women (ratio 1:5.1). The mean age is 40.9 $\pm$ 12.8 years (range 9 – 81 years). The mean age of onset of hyperthyroidism is 35.9 $\pm$ 12.6 years (range 6 – 81 years). The degree of goiter is 2.1 $\pm$ 0.8 (range 0 – 4). There are 498 patients (49.0%) with ophthalmopathy (Gr. 1: 126, 12.4%; Gr 2: 233, 22.9%; Gr. 3: 139, 13.7%), 51 patients (5.0%) with pretibial myxedema, 20 patients (2.0%) with periodic paralysis, 14 patients (1.4%) with myasthenia gravis, 8 patients (0.8%) with vitiligo. The DNA of all patients has been extracted.

Key words: Graves' disease, Linkage study, Association study, Gene

INTRODUCTION

Graves' disease (GD [MIM 275000], http://www.ncbi.nlm.nih.gov/Omim/) is a common autoimmune disorder characterized by hyperthyroidism, diffuse goiter, thyroid-specific auto-antibodies, with or without ophthalmopathy and dermopathy.[1] Its prevalence is estimated to be around 1.0 to 1.6% in the general population.[2] The etiology of GD is generally accepted to be multifactorial[1,3] with strong evidence of a genetic effect, including family clustering,[4] an increased sibling risk (λs) of approximately 8 to 15,[4,5] and a higher concordance rate in monozygotic as compared to dizygotic twins (0.35 vs. 0.03).6 Data from 8,966 Danish twin pairs have suggested that 79% of the predisposition to GD is attributed to genetic factors.[6] The lack of a clear inheritance pattern implies that multiple genes are involved in the pathogenesis of GD.[7] Previous linkage analysis and association study results have implicated many genomic regions including the HLA region, the cytotoxic T-lymphoctye-associated 4 (CTLA4) gene, and the protein tyrosine phosphatase-22, that might harbor susceptibility genes for GD.[8-10]

The HLA region on chromosome 6p21 contains many important immune response genes. A number of population-based genetic association studies supported the association between the HLA region and GD.[11-18] Nevertheless the associated genes/alleles have not been consistent across multiple populations and negative studies were also published.[8, 9, 19] On the other hand, family-based studies have been more controversial. Only one linkage analysis in Caucasians demonstrated a nominal linkage with an NPL score = 1.95.[20] Yet other linkage studies in families from the US, Tunisia, Japan and China have not shown linkage to the HLA region.[21-26] The apparent discrepancy between association studies and linkage analysis, and the discrepancy between different populations, make the HLA region still an intriguing candidate for additional testing.[8] However, it should also be noted that differences between HLA allele and haplotype frequencies have been observed in different ethnic backgrounds.[27-30] It is therefore not uncommon that the disease-associated HLA haplotypes are not the same across populations.[31] Linkage analysis using family samples have suggested more than 20 different loci that might harbor susceptibility genes of GD and/or autoimmune thyroid disease (AITD).[8, 9] On the basis of these reports and their biological relevance, we also investigated four other candidate regions in our first attempt for linkage analysis: the *CTLA4* region on chromosome 2q33;[20] the cytokine gene cluster region on 5q31;[24, 25] the *pendrin* region on 7q22;32 and the *GD-1* and the thyroid stimulating hormone receptor regions on 14q31 in addition to the HLA region.[25] Here we report significant linkage of GD to the HLA region on chromosome 6p21 with a non-parametric LOD score of 3.46 and a NPL Z score of 4.1, but not to the other 6 candidate regions.

MATERIALS AND METHODS

Subjects

Pedigrees were ascertained through a GD proband attending the outpatient clinic of National Taiwan University Hospital or affiliated clinics, Far Eastern Polyclinic. All the individuals enrolled in this study were interviewed and assessed by board-certified endocrinologists. The diagnosis of GD was made based on thepresence of biochemical hyperthyroidism together with either the presence of thyroid eye disease or a diffuse goiter and a significant titer of

auto-antibodies (including anti-microsomal, anti-thyroglobulin or anti-TSH receptor antibody) as previously reported.[19] To enrich phenotypic homogeneity, pedigrees containing any member with possible Hashimoto's thyroiditis (HT [MIM603372]), either according to medical records of HT or self-stated history of symptoms or signs of hypothyroidism without previous thyroidectomy or radioactive iodine treatment, were not included in this study. Ethnic background was recorded according to the information from these individuals. Only subjects whose four grandparents were of Chinese Han origin were included, whereas those with ancestors of possible Taiwanese aboriginal (of Pacific-Polynesian extraction) or other minority Chinese ethnicity were not. This project was approved by the Institutional Review Board of National Taiwan University Hospital. Written informed consent was obtained from each individual.

This study included a total of 536 individuals in 122 multiplex families. All the families contained at least two affected siblings. Parents were also enrolled whenever possible; if samples from one or both parents were unavailable, at least one additional unaffected sibling was included. Among the pedigrees, 73 had two, 32 had three, 9 had four, 3 had five, 4 had six and 1 had eight affected individuals in one family. The pedigrees included a total of 321 affected patients, including 254 females (79.1%) and 67 males (20.9%). Of the 215 unaffected individuals, 113 were females and 102 males. These participants could be analyzed as 270 affected sib-pairs (ASPs) in non-parametric linkage study.

Short tandem repeat polymorphism (STRP) markers selection and genotyping

Genomic DNA was extracted from peripheral leukocytes using the PureGene kit (Gentra Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's protocol. Fluorescence-labeled primers were purchased from Applied Biosystems (Foster City, CA, USA). Genotyping of STRP markers was performed on an ABI PRISM 3100 Genetic Analyzer, with allele calling done by Genotyper Software v 3.7 (Applied Biosystems). Each genotype was independently reviewed by two members. Mendelian inconsistency was checked with PedCheck (version 1.1).[33] Those genotypes with initial Mendelian inconsistency were rechecked, were corrected ifobvious mistake was identified, or set as missing. All the genotypes reported here were compatible with Mendelian inheritance. The overall genotype call rate was 97.5%.

Eight STRPs (D6S1660- D6S1691- D6S276- D6S273- UniSTS:239159-D6S1568- D6S291-D6S1610) in a 13.7 cM region covering the HLA were chosen, resulting in a 1.9-cM (average) marker density. The genetic positions of the markers were determined using the Marshfield (Center for Medical Genetics) genetic maps (http://research.marshfieldclinic.org/genetics/), and the order was verified with the physical map of National Center for Biotechnology Information (NCBI) build 35 (http://www.ncbi.nlm.nih.gov/). One marker (UniSTS:239159) was chosen from UniSTS database in NCBI build 35 without the information in the Marshfield geneticmaps. Its genetic position was approximated based on the physical distances between flanking markers.

The other 26 markers distributed in the other four candidate regions were as followings: five markers (D2S118- D2S2387- UniSTS:48500- D2S155- D2S2242) in a 15.1-cM region on 2q33, seven markers (D5S2017- D5S436- D5S2090- D5S434-D5S2014- D5S410-D5S422) in an 18.9-cM region on 5q31, five markers (D7S2446-D7S501- D7S496- D7S2459-D7S486) in a 10.1-cM region on 7q22, and nine markers (D14S276- D14S274- D14S63-

D14S258- D14S74- D14S1044- D14S280-D14S1054- D14S65) in a 61.1-cM region on 14q31. The necessary sequence information for primer design was based on the database in NCBI. The information of primers is available upon request.

Statistical analysis

Non-parametric linkage analyses were performed to locate the position of the susceptibility genes for GD. The allele frequency of markers was estimated on the basis of founders' genotypes. MERLIN 1.0.1. program was used to carry out two-point and multi-point nonparametric linkage analyses.[34] The non-parametric linkage (NPL) Z score and non-parametric LOD score under an exponential model were calculated.[35, 36] The information content of the genotypes was estimated with use of entropy information.[35] The Sall scoring function was used to capture the information of the allele sharing between all affected individuals in a pedigree.[37] The 1-LOD support interval was based on our multi-point non-parametric LOD scores.

RESULTS

Our familial collection comprised 270 ASPs (all possible pairs) for the Sall scoring function of non-parametric linkage analysis. For the five promising candidate regions, the maximal multi-point NPL Z scores calculated with MERLIN 1.0.1. program were as followings: 0.97 on chromosome 2q33, 0.82 on chromosome 5q31, 4.1 on chromosome 6p21, -0.89 on chromosome 7q22, and -0.34 on chromosome14q31 (Table 1).

Non-parametric analysis demonstrated linkage of GD to the HLA region onchromosome 6p21. Two-point analysis with MERLIN showed the highest non-parametric LOD score of 2.56 (P = 0.0003) at D6S1568 (Table 2). Multi-point non-parametric LOD score peaked around marker UniSTS:239159, but not exactly at the location of the marker (Figure 1), with the highest score of 3.46 (P =0.00003). The score specifically for this marker is 3.44 (Table 2)**.** Multi-point NPL Z score correlated very well with the LOD score (Table 2), peaking at the same UniSTS:239159 marker (maximal NPL Z = 4.1, P = 0.00002). Our 1-LOD support interval was a ~4.5 cM region (44.6 cM – 49.1 cM, sex-average distance) on theMarshfield genetic map (Figure 1). This 1-LOD support interval corresponds to a ~8.9 Mb region (26.9 Mb – 35.8 Mb) on the NCBI build 35 physical map, which contains the whole ~4.0 Mb HLA region (Figure 1).

DISCUSSION

Our results strongly support linkage of GD to the HLA region on chromosome 6p21. Among previous five studies of familial linkage analysis, the only positive signal at the HLA region was reported from the United Kingdom, with a nominal level of significance with an NPL score =1.95.[20] The other linkage studies, using pedigrees from the United States, United Kingdom, Japan, China, and Tunisia did not detect significant effect at the HLA region.[21-26] However, the results from various population-based association studies also offer support for the roles of HLA as a genetic contributor to GD.[7-9]

In this study, we attempted to decrease genetic heterogeneity, one major problem in genetic studies.[38, 39] We enrolled only pedigrees with Chinese-Han ethnic background. We also try to reduce the genetic heterogeneity by excluding families with a history of hypothyroidism or HT, although it could not be possibly complete. There are both advantages and disadvantages of our

decision to focus on GD. This strategy obviously would negatively influence our sample size, and thus decreased our power to detect susceptibility genes with effect on both GD and HT. However, although GD and HT may share some common pathophysiological pathways, these two diseases have substantial differences in terms of clinical manifestations, laboratory abnormality and histological findings.[40] Previous reports have suggested that there might be different sets of susceptibility genes for GD, HT and AITD.[21, 22] In a recent large-scale genome-wide linkage screen with 1,119 AITD relative-pairs, none of the linkages obtained from GD or autoimmune hypothyroidism (AIH) was the same.[26] In addition, the diagnostic definition of HT is more controversial,[8] and the etiology of HT may be even more heterogeneous than GD.[21] In fact, the clinical course is variable in HT, and thyroid function could be normal or abnormal (overt hypothyroidism, subclinical hypothyroidism, and hyperthyroidism).[41] The measurement of anti-thyroglobulin and thyroid peroxidase antibodies can not provide any help in distinguishing various types of AITD.[42]

Our 1-LOD support interval was a ~4.5 cM region (44.6 cM–49.1 cM, sex-average distance) on the Marshfield genetic map, which corresponds to an ~8.9 Mb region (26.9 Mb – 35.8 Mb) on the NCBI build 35 physical map. This interval contains the whole ~4 Mb HLA region.[43] Class II loci, especially the HLA DRB1*03 and the DRB1*03-DQB1*02-DQA1*0501 haplotype, have repeatedly demonstrated to associate with GD in Caucasian populations.[8, 19, 44] However, in studies of populations of Chinese, Thai, Japanese and Korean ancestry, the DRB1*03 and its haplotype did not show association with GD; instead, there were reports for association with class I and other class II loci.[8, 14, 18, 44, 45] One plausible explanation is that different associated haplotypes reported in different populations actually carry some common polymorphisms driving the same autoimmune response. Due to the extended haplotype spectrum of the HLA genes, it has been extremely difficult to identify the primary etiological variants or to "split" the effect of individual loci from the effect of the whole haplotype.[19, 44] Comparison between association studies from populations with different composition of HLA haplotypes may provide very useful clues. Our result provides strong evidence for the involvement of the HLA region in GD in the Chinese-Han population in Taiwan, which advocates for the importance of a larger association study to help clarify the susceptibility HLA loci and/or alleles. Besides, it is still possible that some genes, other than the classical HLA loci, account for our linkage and the previous association signals. The recent linkage-disequilibrium map at the HLA region will facilitate the interpretation of future association studies.[27, 43] In addition, Simmonds et al. recently using various statistical techniques were able to dissect the effect of individual loci from the effects of neighboring variants, all associated with GD because of strong linkage-disequilibrium in this region.[19] All these recent progresses offer the hope for eventual identification of the primary etiological genetic variants for GD in the future. We also tested the linkage of GD to **four other** candidate regions, including the *CTLA4* region on chromosome 2q33,[20] the cytokine gene cluster region on 5q31,[24, 25] the *pendrin* region on 7q22,[32] and the *GD-1* and the *TSHR* regions on 14q31.[25] The average marker density at different regions varied from 1 marker per 2.5 cM to 1 marker per 7.6 cM. None of the NPL Z scores reached suggestive level of significance, although we did see some positive signal at 2q33 (maximal NPL Z score = 0.97) and 5q31 (maximal NPL Z score = 0.82). Our results can not exclude any of the four regions, as the lack of signals could

be due to power limitation because of the underlying gene effects and our modest sample size and marker density. Linkage analysis has had great success in genetic mapping of **monogenic** diseases. Unfortunately, the power for detecting linkage signal of loci with moderate or small effect, the most possible situation in complex traits, is limited.46-48 Risch's calculation demonstrates that it takes more than 3000 affected sib-pairs to have sufficient power of detecting linkage signal of a susceptibility locus with genotype risk ratio of 2, while less than 1000 cases and controls are needed to find association using case control studies.[46] Therefore, further studies are necessary for these regions.

In conclusion, our work provides strong support of linkage of GD to the HLA region. Taken together with the previous linkage report in Caucasians and other association studies, our results suggest that the HLA region harbors one or more susceptibility genes for GD. Further dissection of this region in our population may provide insight into the immunogentics of GD pathogenesis, therefore is warranted.

Acknowledgements

We thank all the GD patients and their families who graciously agreed to participate in the study. We are grateful to Dr. Pei-Jer Chen and Ms. Shu-Fen Lu for equipment and technical support in genotyping. We also thank Drs. David Valle and Dimitrios Avramopoulos for helpful discussion. This work was supported by grants NSC# 91-3112-B-002-003, 92-3112-B-002-009 and 93-3112-B-002-015 from National Science Council, Taiwan. We also apologize to the authors whose work regarding this issue was not included in the references due to word count limitation.

References

1. Weetman, A.P. (2000) Graves' disease. *New England Journal of Medicine* 343,1236-1248.

2. Hollowell, J.G., Staehling, N.W., Flanders, W.D., Hannon, W.H., Gunter, E.W., Spencer, C.A. & Braverman, L.E. (2002) Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *Journal of Clinical Endocrinology & Metabolism* 87,489-499.

3. DeGroot, L.J. & Quintans, J. (1989) The causes of autoimmune thyroid disease. *Endocrine Review* 10,537-562.

4. Brix, T.H., Kyvik, K.O. & Hegedus, L. (1998) What is the evidence of genetic factors in the etiology of Graves' disease? A brief review. *Thyroid* 8,727-734.

5. Vyse, T.J. & Todd, J.A. (1996) Genetic analysis of autoimmune disease. *Cell* 85,311-318.

6. Brix, T.H., Kyvik, K.O., Christensen, K. & Hegedus, L. (2001) Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts. *Journal of Clinical Endocrinology & Metabolism* 86,930-934.

7. Vaidya, B., Kendall-Taylor, P. & Pearce, S.H. (2002) The genetics of autoimmune thyroid disease. *Journal of Clinical Endocrinology & Metabolism*87,5385-5397.

8. Tomer, Y. & Davies, T.F. (2003) Searching for the autoimmune thyroid disease susceptibility genes: from gene mapping to gene function. *Endocrine Review*24,694-717.

9. Ayadi, H., Hadj Kacem, H., Rebai, A. & Farid, N.R. (2004) The genetics of autoimmune

thyroid disease. *Trends in Endocrinology & Metabolism* 15,234-239.

10. Velaga, M.R., Wilson, V., Jennings, C.E., Owen, C.J., Herington, S., Donaldson, P.T., Ball, S.G., James, R.A., Quinton, R., Perros, P. & Pearce, S.H. (2004) The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. *Journal of Clinical Endocrinology & Metabolism* 89,5862-5865.

11. Yanagawa, T., Mangklabruks, A., Chang, Y.B., Okamoto, Y., Fisfalen, M.E., Curran, P.G.. & DeGroot, L.J. (1993) Human histocompatibility leukocyte antigen-DQA1*0501 allele associated with genetic susceptibility to Graves' disease in a Caucasian population. *Journal of Clinical Endocrinology &Metabolism* 76,1569-1574.

12. Badenhoop, K., Walfish, P.G., Rau, H., Fischer, S., Nicolay, A., Bogner, U., Schleusener, H. & Usadel, K.H. (1995) Susceptibility and resistance alleles ofhuman leukocyte antigen (HLA) DQA1 and HLA DQB1 are shared in endocrine autoimmune disease. *Journal of Clinical Endocrinology & Metabolism* 80,2112-2117.

13. Heward, J.M., Allahabadia, A., Daykin, J., Carr-Smith, J., Daly, A., Armitage, M., Dodson, P.M., Sheppard, M.C., Barnett, A.H., Franklyn, J.A. & Gough, S.C. (1998) Linkage disequilibrium between the human leukocyte antigen class II region of the major histocompatibility complex and Graves' disease: replication using a population case control and family-based study. *Journal of Clinical Endocrinology & Metabolism* 83,3394-3397.

14. Huang, S.M., Wu, T.J., Lee, T.D., Yang, E.K., Shaw, C.K. & Yeh, C.C. (2003) The association of HLA -A, -B, and -DRB1 genotypes with Graves' disease in Taiwanese people. *Tissue Antigens* 61,154-158.

15. Yeo, P.P., Chan, S.H., Thai, A.C., Ng, W.Y., Lui, K.F., Wee, G.B., Tan, S.H., Lee, B.W., Wong, H.B. & Cheah, J.S. (1989) HLA Bw46 and DR9 associations in Graves' disease of Chinese patients are age- and sex-related. *Tissue Antigens* 34,179-184.

16. Onuma, H., Ota, M., Sugenoya, A. & Inoko, H. (1994) Association of HLA-DPB1*0501 with early-onset Graves' disease in Japanese. *Human Immunology* 39,195-201.

17. Cho, B.Y., Rhee, B.D., Lee, D.S., Lee, M.S., Kim, G.Y., Lee, H.K., Koh, C.S., Min, H.K. & Lee, M. (1987) HLA and Graves' disease in Koreans. *Tissue Antigens* 30,119-121.

18. Wongsurawat, T., Nakkuntod, J., Charoenwongse, P., Snabboon, T., Sridama, V. & Hirankarn, N. (2006) The association between HLA class II haplotype with Graves' disease in Thai population. *Tissue Antigens* 67,79-83.

19. Simmonds, M.J., Howson, J.M., Heward, J.M., Cordell, H.J., Foxall, H., Carr-Smith, J., Gibson, S.M., Walker, N., Tomer, Y., Franklyn, J.A., Todd, J.A. & Gough, S.C. (2005) Regression mapping of association between the human leukocyte antigen region and Graves disease. *American Journal of Human Genetics* 76,157-163.

20. Vaidya, B., Imrie, H., Perros, P., Young, E.T., Kelly, W.F., Carr, D., Large, D.M., Toft, A.D., McCarthy, M.I., Kendall-Taylor, P. & Pearce, S.H. (1999) The cytotoxic T lymphocyte antigen-4 is a major Graves' disease locus. *Human Molecular Genetics* 8,1195-1199.

21. Tomer, Y., Barbesino, G., Greenberg, D.A., Concepcion, E. & Davies, T.F. (1999) Mapping the major susceptibility loci for familial Graves' and Hashimoto's diseases: evidence for genetic heterogeneity and gene interactions. *Journal of Clinical Endocrinology & Metabolism* 84,4656-4664.

22. 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. *Human Molecular Genetics* 10,1379-1386.

23. Maalej, A., Makni, H., Ayadi, F., Bellassoued, M., Jouida, J., Bouguacha, N., Abid, M. & Ayadi, H. (2001) A full genome screening in a large Tunisian family affected with thyroid autoimmune disorders. *Genes Immunity* 2,71-75.

24. Jin, Y., Teng, W., Ben, S., Xiong, X., Zhang, J., Xu, S., Shugart, Y.Y., Jin, L., Chen, J. & Huang, W. (2003) Genome-wide scan of Graves' disease: evidence for linkage on chromosome 5q31 in Chinese Han pedigrees. *Journal of Clinical Endocrinology & Metabolism* 88,1798-1803.

25. Tomer, Y., Ban, Y., Concepcion, E., Barbesino, G., Villanueva, R., Greenberg, D.A. & Davies, T.F. (2003) Common and unique susceptibility loci in Graves and Hashimoto diseases: results of whole-genome screening in a data set of102 multiplex families. *American Journal of Human Genetics* 73,736-747.

26. Taylor, J.C., Gough, S.C., Hunt, P.J., Brix, T.H., Chatterjee, K., Connell, J.M., Franklyn, J.A., Hegedus, L., Robinson, B.G., Wiersinga, W.M., Wass, J.A., Zabaneh, D., Mackay, I. & Weetman, A.P. (2006) A genome-wide screen in 1119 relative pairs with autoimmune thyroid disease. *Journal of Clinical Endocrinology & Metabolism* 91,646-653.

27. Yang, H.C., Lin, C.H., Hsu, C.L., Hung, S.I., Wu, J.Y., Pan, W.H., Chen, Y.T. & Fann, C.S. (2006) A comparison of major histocompatibility complex SNPs in Han Chinese residing in Taiwan and Caucasians. *Journal of Biomedical Sciences* 13,489-498.

28. Cao, K., Hollenbach, J., Shi, X., Shi, W., Chopek, M. & Fernandez-Vina, M.A. (2001) Analysis of the frequencies of HLA-A, B, and C alleles and haplotypes in the five major ethnic groups of the United States reveals high levels of diversity in these loci and contrasting distribution patterns in these populations. *Human Immunology* 62,1009-1030.

29. Dean, M., Stephens, J.C., Winkler, C., Lomb, D.A., Ramsburg, M., Boaze, R., Stewart, C., Charbonneau, L., Goldman, D. & Albaugh, B.J. (1994) Polymorphic admixture typing in human ethnic populations. *American Journal of Human Genetics* 55,788-808.

30. Cao, K., Moormann, A.M., Lyke, K.E., Masaberg, C., Sumba, O.P., Doumbo, O.K., Koech, D., Lancaster, A., Nelson, M., Meyer, D., Single, R., Hartzman, R.J., Plowe, C.V., Kazura, J., Mann, D.L., Sztein, M.B., Thomson, G. & Fernandez-Vina, M.A. (2004) Differentiation between African populations is evidenced by the diversity of alleles and haplotypes of HLA class I loci. *Tissue Antigens* 63,293-325.

31. Ghodke, Y., Joshi, K., Chopra, A. & Patwardhan, B. (2005) HLA and disease. *European Journal of Epidemiology* 20,475-488.

32. Hadj Kacem, H., Rebai, A., Kaffel, N., Masmoudi, S., Abid, M. & Ayadi, H. (2003) PDS is a new susceptibility gene to autoimmune thyroid diseases: association and linkage study. *Journal of Clinical Endocrinology & Metabolism* 88,2274-2280.

33. O'Connell, J.R. & Weeks, D.E. (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *American Journal of Human Genetics* 63,259-266.

34. Abecasis, G.R., Cherny, S.S., Cookson, W.O. & Cardon, L.R. (2002) Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nature Genetics* 30,97-101.

35. Kruglyak, L., Daly, M.J., Reeve-Daly, M.P. & Lander, E.S. (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. *AmericanJournal of Human Genetics* 58,1347-1363.

36. Kong, A. & Cox, N.J. (1997) Allele-sharing models: LOD scores and accurate linkage tests. *American Journal of Human Genetics* 61,1179-1188.

37. Whittemore, A.S. & Halpern, J. (1994) A class of tests for linkage using affected pedigree members. *Biometrics* 50,118-127.

38. Cardon, L.R. & Bell, J.I. (2001) Association study designs for complex diseases. *Nature Review Genetics* 2,91-99.

39. Glazier, A.M., Nadeau, J.H. & Aitman, T.J. (2002) Finding genes that underlie complex traits. *Science* 298,2345-2349.

40. Armengol, M.P., Juan, M., Lucas-Martin, A., Fernandez-Figueras, M.T., Jaraquemada, D., Gallart, T. & Pujol-Borrell, R. (2001) Thyroid autoimmune disease: demonstration of thyroid antigen-specific B cells and recombination-activating gene expression in chemokine-containing active intrathyroidal germinal centers. *American Journal of Pathology* 159,861-873.

41. Lorini, R., Gastaldi, R., Traggiai, C. & Perucchin, P.P. (2003) Hashimoto's Thyroiditis. *Pediatric Endocrinology Review* 1 Suppl 2,205-211.

42. Weetman, A.P. (2004) Autoimmune thyroid disease. *Autoimmunity* 37,337-340.

43. Miretti, M.M., Walsh, E.C., Ke, X., Delgado, M., Griffiths, M., Hunt, S., Morrison, J., Whittaker, P., Lander, E.S., Cardon, L.R., Bentley, D.R., Rioux, J.D., Beck, S. & Deloukas, P. (2005) A high-resolution linkage-disequilibrium map of the human major histocompatibility complex and first generation of tag single-nucleotide polymorphisms. *American Journal of Human Genetics* 76,634-646.

44. Simmonds, M.J. & Gough, S.C. (2004) Unravelling the genetic complexity of autoimmune thyroid disease: HLA, CTLA-4 and beyond. *Clinical Experiemtal Immunology* 136,1-10.

45. Park, M.H., Park, Y.J., Song, E.Y., Park, H., Kim, T.Y., Park, D.J., Park, K.S. & Cho, B.Y. (2005) Association of HLA-DR and -DQ genes with Graves disease in Koreans. *Human Immunology* 66,741-747.

46. Risch, N.J. (2000) Searching for genetic determinants in the new millennium. *Nature* 405,847-856.

47. Wang, W.Y., Barratt, B.J., Clayton, D.G. & Todd, J.A. (2005) Genome-wide association studies: theoretical and practical concerns. *Nature Review Genetics* 6,109-118.

48. Altmuller, J., Palmer, L.J., Fischer, G., Scherb, H. & Wjst, M. (2001) Genomewide scans of complex human diseases: true linkage is hard to find. *American Journal of Human Genetics* 69,936-950.

Table 1 Maximal Multi-Point NPL Z Scores at the Five Candidate Chromosome Regions

REGION	MARKER[a]	LOCATION[b] (cM)	Maximal NPL Z[c]	*P*=
2q33	D2S2319	210.43	0.97	0.2
5q31	D5S422	164.19	0.82	0.2
6p21	UniSTS:239159[d]	46.34	4.10	0.00002
7q22	D7S2446	113.92	-0.89	0.8
14q31	D14S274	63.25	-0.34	0.6

[a] The marker with the highest NPL Z score within the respective region.
[b] Genetic map locations were determined using sex-average distance on the Marshfield genetic map.
[c] Non-parametric Linkage (NPL) Z score calculated with the MERLIN 1.0.1. program.
[d] UniSTS:239159 is not included in the Marshfield genetic map. Its genetic map location was approximated based on physical distances between flanking markers.

Table 2 The Results of Non-Parametric Linkage Analysis at the HLA Region on 6p21

MARKER	LOCATION		TWO-POINT ANALYSIS			MULTI-POINT ANALYSIS				
	Genetic Map[a] (cM)	Physical Map[b] (Mb)	LOD[c]	*P*	Info. Content	LOD[c]	*P*	NPL Z[d]	*P*	Info. Content
D6S1660	40.14	23.4	0.04	0.3	0.51	1.45	0.005	2.44	0.007	0.86
D6S1691	42.27	24.0	1.36	0.006	0.76	1.69	0.003	2.80	0.003	0.94
D6S276	44.41	24.3	0.35	0.1	0.60	2.32	0.0005	3.31	0.0005	0.95
D6S273	44.96	31.8	0.48	0.07	0.61	2.64	0.0002	3.53	0.0002	0.95
UniSTS:239159[e]	46.34	33.3	1.39	0.006	0.72	**3.44**	**0.00003**	**4.10**	**0.00002**	**0.96**
D6S1568	47.71	34.1	**2.56**	**0.0003**	**0.76**	3.32	0.00005	3.87	0.00005	0.95
D6S291	49.50	36.3	0.94	0.02	0.63	1.30	0.007	2.44	0.007	0.92
D6S1610	53.81	39.4	0.82	0.03	0.67	1.37	0.006	2.41	0.008	0.85

[a] Genetic map locations were determined using sex-average distance on the Marshfield genetic map.
[b] Physical map locations were determined using NCBI human genome map build 35.
[c] Non-parametric LOD score based on the exponential model calculated with the MERLIN 1.0.1. program.
[d] NPL Z score calculated with the MERLIN 1.0.1. program.
[e] The UniSTS:239159 marker is not included in the Marshfield genetic map. Its genetic map location was approximated based on physical distances between flanking markers.

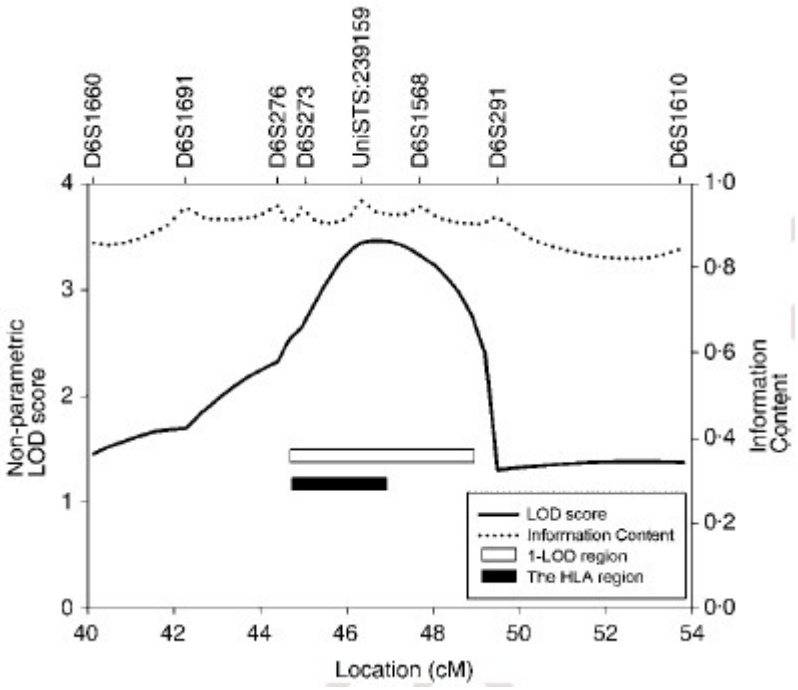

Figure 1 Multi-point non-parametric LOD scores on 6p21-p22 from linkage analysis of 270 ASPs in 122 GD families. The scores were calculated with MERLIN v 1.0.1. Multi-point non-parametric LOD scores (solid line), information content (dotted line), the 1-LOD region (empty bar) and the HLA region (filled bar) are plotted. The X-axis values are distances from the p-telomere, in Kosambi cM.

The above paper will be published in Clinical Endocrinology in 2007

Clinical Endocrinology (2007) 66, 000–000 doi: 10.1111/j.1365-2265.2007.02787.x

ORIGINAL ARTICLE

Linkage of Graves' disease to the human leucocyte antigen region in the Chinese-Han population in Taiwan

Pei-Lung Chen*#, Cathy Shen-Jang Fann§¶, Chien-Ching Chang§, I-Lin Wu*, Wei-Yih Chiu*, Chin-Yu Lin§, Wei-Shiung Yang*†‡§ and Tien-Chun Chang*‡

*Division of Endocrinology and Metabolism, Department of Internal Medicine, National Taiwan University Hospital, †Graduate Institute of Clinical Medicine and ‡Department of Medicine, College of Medicine, National Taiwan University, §Institute of Biomedical Sciences, Academia Sinica, ¶Institute of Public Health, National Yang-Ming University, Taipei, Taiwan, and #Program in Human Genetics, Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD, USA

Association Study

In the population-based association study of 2006-2009, we have completed the collection of 1,016 GD individuals. There are 166 men, and 850 women (men to women =1:5.1). The mean age is 40.9 $\pm$ 12.8 years (range 9 – 81 years). The mean age of onset of hyperthyroidism is 35.9 $\pm$ 12.6 years (range 6 – 81 years). The degree of goiter is 2.1 $\pm$ 0.8 (range 0 – 4). There are 498 patients (49.0%) with ophthalmopathy (Gr. 1: 126, 12.4%; Gr 2: 233, 22.9%; Gr. 3: 139, 13.7%), 51 patients (5.0%) with pretibial myxedema, 20 patients (2.0%) with periodic paralysis, 14 patients (1.4%) with myasthenia gravis, 8 patients (0.8%) with vitiligo. Now the DNA of all patients has been extracted. The DNA of 300 women with ophthalmopathy is sent to perform whole genome scan now. If there are significant findings after comparing with supercontrol of Academia Sinica, these findings will be checked in other 200 GD patients with ophthalmopathy, then other GD patients.