# 行政院國家科學委員會補助專題研究計畫□ 成 果 報 告 ■期中進度報告

## 台灣人的川崎氏症之全基因體關聯性研究（第1年）

計畫類別：■ 個別型計畫　□ 整合型計畫
計畫編號：NSC 98 － 2314 － B － 039 － 004 －MY2
執行期間： 98 年 08 月 01 日至 99 年 07 月 31 日

計畫主持人：蔡輔仁

共同主持人：蔡輔仁、林應如、鄔哲源、張正成。

計畫參與人員：林亭旭、雷玉潔、林欣誼。

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：中國醫藥大學 學士後中醫系
中 華 民 國 99 年 05 月 31 日

## 中文摘要:

川崎氏症是一種未知病因，但可能為基因影響的小兒全身性血管炎。我們初步結果顯示(1) *HLA-E* 基因多形性與川崎氏症病人併發冠狀動脈瘤有相關性;(2) 利用全基因掃瞄相關性研究100位台灣的川崎氏症病人與675位控制組初步鑑定173 個基因多形性與川崎氏症有關聯性。但我們並不能排除之外的相關基因也可能參與川崎氏症的病理機制。在這個計劃中，我們假設川崎氏症是一種複雜的遺傳相關疾病，並且一些未知的基因可能與川崎氏症形成甚至併發冠狀動脈瘤有相關性。為了証明此假說，我們利用一種全基因掃瞄相關性研究尋找有意義的基因，再於再收案病人上重覆研究這些基因，與更詳細研究其基因的功能。利用這些方法研究這些有意義的基因在川崎氏症形成與病理機制的重要性。我們的目標是利用全基因掃瞄相關性研究方法尋找可能影響台灣人川崎氏症形成的遺傳因子。這個資訊可能對於台灣人川崎氏的診斷與治療發展提供可能的貢獻。

## 英文摘要:

Kawasaki disease (KD) is a pediatric systemic vasculitis of unknown etiology for which a genetic influence is supposed. Our preliminary results suggested that (1) a significant association between the *HLA-E* gene polymorphism and the occurrence of CAA in KD patients has been observed; and (2) 173 gene polymorphisms have been initially identified using the genomewide study (which involved 100 Taiwanese KD patients and 675 controls). We cannot exclude additional genes other than *HLA-E* that may be also involved in KD pathogenesis. In this proposal, we hypothesize that KD has a complex mode of inheritance and some unknown genetic loci may be associated with an increased risk of KD as well as the development of CAA. To test this hypothesis, we will use a genomewide association study, replication of candidate gene haplotype tag SNPs in an independent set and fine mapping of candidate genes to investigate the possible molecular mechanism of candidate genes involved in KD susceptibility and pathogenesis. We aim at searching for inherited components that may influence Taiwanese KD susceptibility using a genomewide approach. This information could be useful for the contribution of Taiwanese KD diagnostic and therapeutic development.

**報告內容:**

## 前言與文獻探討:

KD is an acute, self-limited and systemic vasculitis that is one of the leading causes of acquired heart disease in infants and young children (3, 4, 14). This disease is characterized by prolonged fever unresponsive to antibiotics, polymorphous skin rash, erythema of the oral mucosa, lips and tongue, erythema of the palms and soles, bilateral conjunctival injection and cervical lymphadenopathy. The vascular inflammation may cause the development of aneurysms and cardiac complications. Patients with these cardiovascular complications are at increased risk of developing ischemic heart disease, which may lead to myocardial infarction and sudden death (13). Although KD is a mysterious disease of unknown etiology and pathogenesis, it is believed to be caused by infectious agents, host immune dysregulation and genetic susceptibility (12, 16, 17, 28). Several lines of evidence suggest the importance of genetic factors in disease susceptibility and outcome. First, KD is overrepresented in Asian children (2, 3, 8, 10, 24, 27). The annual incidence of KD in Taiwan is estimated to be 66/100,000 in children, the 3rd highest in the world after Japan and Korea (4, 20). Second, the risk of KD in siblings of affected children is 10 times higher than that in the general population (7, 29). In addition, the incidence of KD in children born to parents with a history of KD is twice as high as that in the general population. Although association studies have identified candidate genes that may influence KD susceptibility (19), a systemic genetic approach has not been completely applied to study this disease in Taiwanese population.

Recent high-density genotyping array platforms in human genetic variation studies have made it possible to identify candidate genes across the whole genome for the risk of the disease (5, 22). In addition, statistical methodology based on allele frequencies in case-control studies (association analyses) have more power to identify common alleles that confer a modest risk than do tests based on chromosomal segregation in families (linage analyses) (23). With a large collection of DNA

samples obtained from a population whose clinical characteristics are well defined, cost-effective genotyping and sophisticated statistical analysis, genome-wide association studies are contributing to complex diseases including age-related macular degeneration, myocardial infarction, rheumatoid arthritis and type 2 diabetes (9, 21, 25, 30). Nevertheless, it remains to identify true positive signals in a sea of false positives using a replication study or another genotyping method. In addition, the interactions between genetic factors and environmental factors may contribute to disease predisposition and processes. It is needed to coupling the genotype data and phenotype data to identify genes that predispose to both normal trait variation and disease processes.

In this proposal, genotyping of 250 Taiwanese KD patients will be performed with the use of GeneChip Human Mapping 500K Array Set (Affymetrix), which simultaneously types approximately 500,000 genetic variants. We will also collaborate with National Genotyping Center (NGC) at Academia Sinica. The NGC will provide the information of the control data of Taiwanese population. By statistical analysis and validation of the genomewide association study, the genetic loci with the risk of Taiwanese KD will be identified. The information generated from this study will help to the understanding of the mechanism of Taiwanese KD.

## 研究目的:

Our long-term goal is to investigate possible molecular pathogenesis of identified candidate genes associated with an increased risk of Taiwanese Kawasaki disease (KD) as well as the development of CAA. In this research project, we focus on the identification of candidate genes using a genomewide association study. Based on preliminary data described above, we hypothesized that the genetic contributions may play an important role in a susceptibility to KD. In this proposal, the following aims are designed to test the hypothesis.

***Specific Aim 1:*** *Genomewide association analysis of KD using a case-control study.*

***Specific Aim 2:*** *Replication of candidate gene haplotype tag SNPs in an independent set.*

***Specific Aim 3:*** *Fine mapping of candidate genes.*

## 研究方法:

*Study population*

From 1998 to 2008, individuals fulfilling the diagnostic criteria of KD were identified and will be enrolled into this study from the Department of Pediatrics at China Medical University Hospital in Taichung, Taiwan (6, 15, 18, 31, 32). Every patient would have regular echocardiography examinations at the acute stage, two months after onset, 6 months after onset and once a year thereafter. CAA is identified when either the right coronary artery or the left coronary artery showed a dilated diameter ≧3 mm in children younger than 5 years of age, or ≧4 mm in children older (1). The NGC will provide the information of the control data of Taiwanese population. The control group consisted of 680 healthy subjects randomly selected from the Han Chinese Cell and Genome Bank, in which 3,312 unrelated Han Chinese descendants were recruited based on their geographic distribution across Taiwan (11). The estimated prevalence of KD was less than 1/1000, so it should be assumed no KD cases in the control group. This study will be approved by the Human Studies Committee of China Medical University Hospital. Informed consent will be obtained from patients or their parents. In addition, questionnaires of each patient will be obtained for phenotypic data.

*Genotyping and quality-control filtering*

The genomic DNA was extracted from peripheral blood leukocytes according to standard protocols (Genomic DNA kit; Qiagen). All analyses were performed with the use of the GeneChip Human Mapping 500K Array Set, (Affymetrix), which simultaneously types approximately 500,000 genetic variants. Details on genotype-calling algorithms, quality criteria (at the level of both the individual and the SNP) and validation steps are performed according to the standard protocols available from Affymetrix. The data sets of case and control will be filtered individually on the basis of SNP genotype call rates (>95% completeness), minor allele frequency (>0.01), and the Hardy-Weinberg equilibrium ($P<1x10^{-5}$). Subjects whose percentage of missing genotypes was more than 5%, who had non-Chinese ancestry, who had evidence of relatedness and who had evidence of possible DNA contamination will be omitted.

*Statistical analysis*

The case-control association study will be analyzed using the Chi-square analysis. Allelic association screen was carried out by the Cochran-Armitage Trend test for each SNP (26).

## 結果與討論:

### Genome-Wide Association Study

The final GWA analysis therefore consisted of 156 KD subjects and 376 controls. Of the 500,568 SNPs on the Affymetrix 500 K SNP chip, 149,319 had been excluded from total call rate of <95%, total MAF<0.05% and deviated significantly from HWE in the control group (HWEP < 0.05) (Figure 1). 351,249 SNPs were therefore available for analysis. A total of 78,765 SNPs were significantly associated ($p<0.05$). The quantile-quantile plot between observed and expected allele frequencies showed deviation from expected with $p<10^{-4}$, suggesting the presence of true associations (Figure 2).

Our next step is to try do a follow-up replication study in independent cohorts. Since our initial genomewide association study may identify false positives, a replication study is required to verify these results. In this specific aim 2, another independent Taiwanese KD cohort will be included in this study. Furthermore, the second genotyping of identified candidate gene SNPs will be performed using the probe hybridization method.

Figure 1.

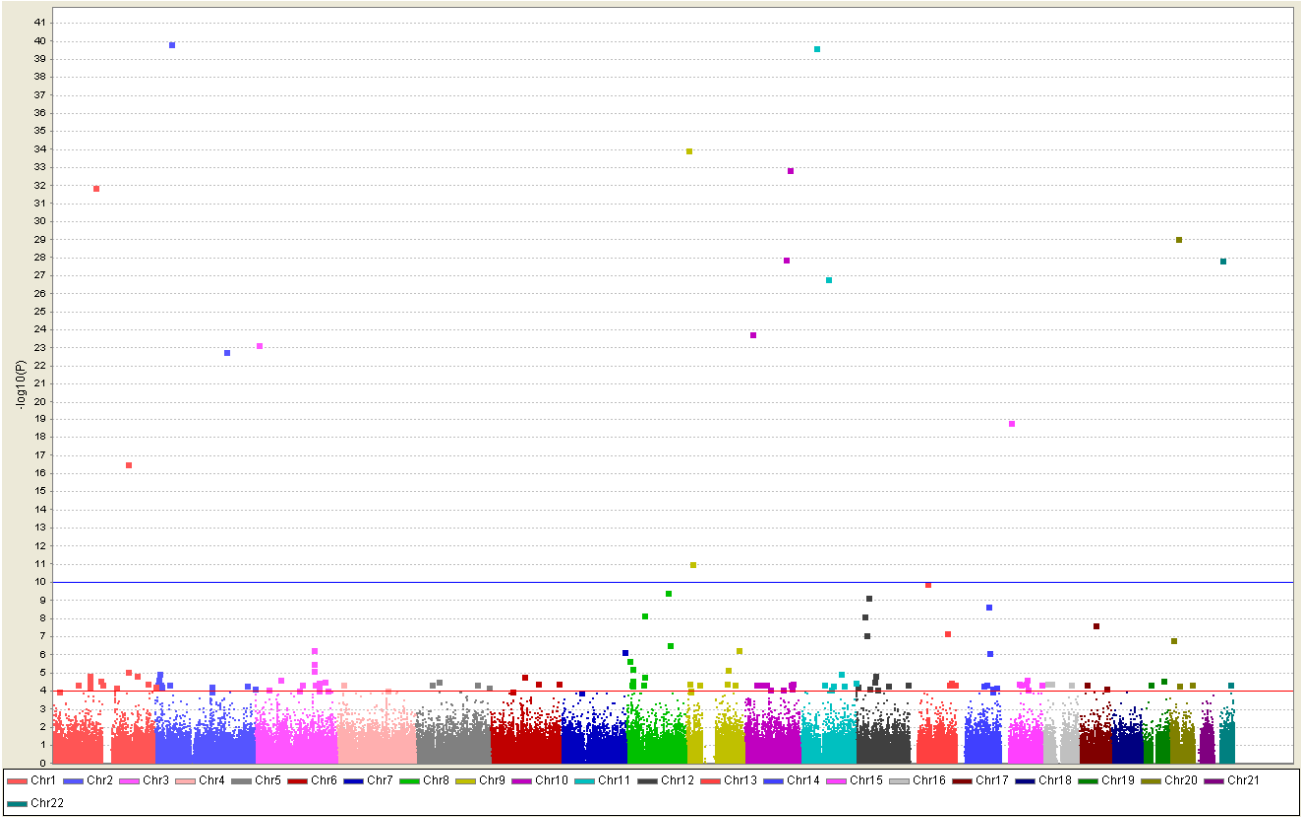
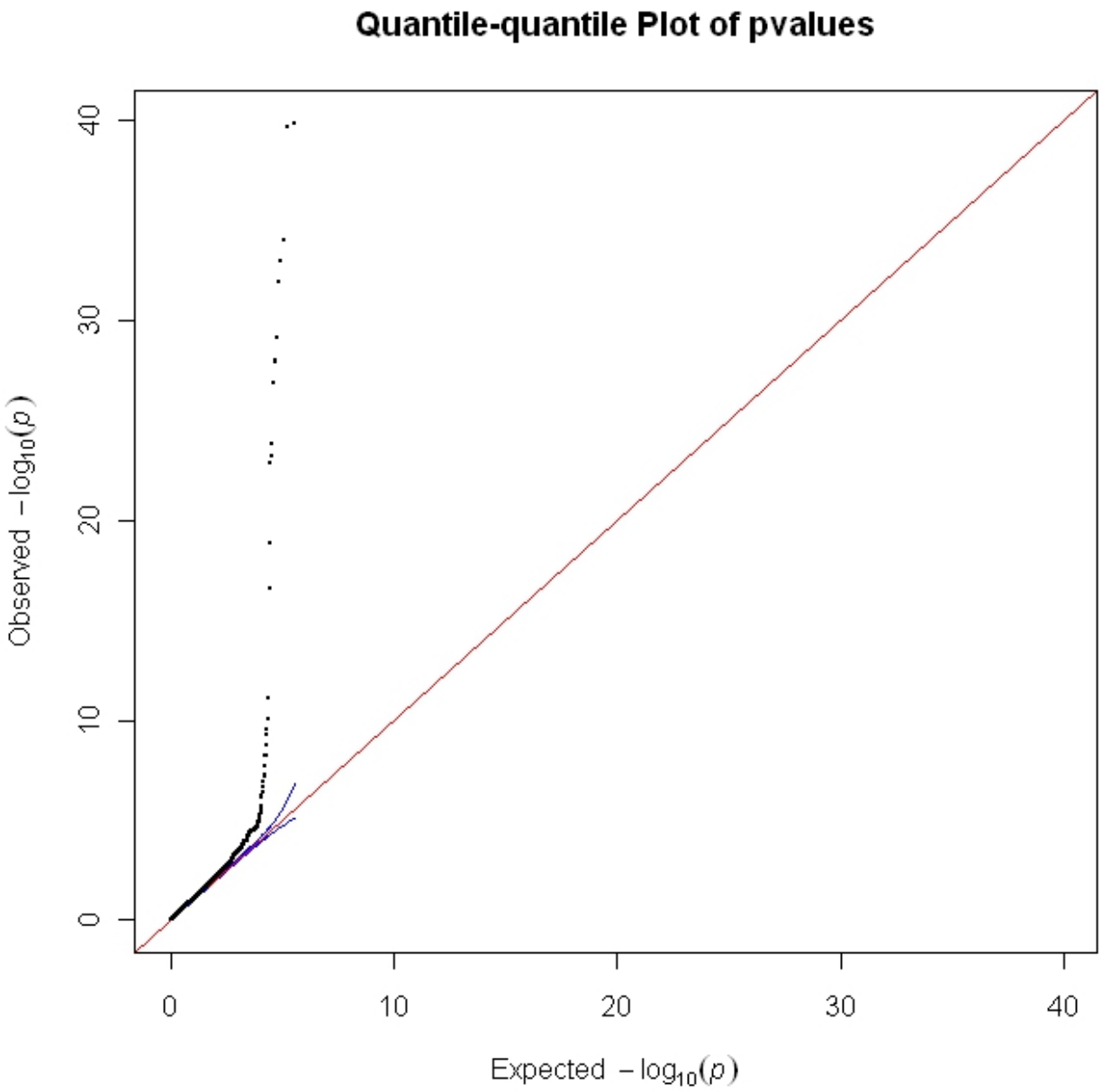


Figure 2.


Quantile-quantile Plot of pvalues
Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$
0
10
20
30
40

## 參考文獻：


1. **Akagi, T., V. Rose, L. N. Benson, A. Newman, and R. M. Freedom.** 1992. Outcome of coronary artery aneurysms after Kawasaki disease. J Pediatr **121:**689-94.
2. **Bronstein, D. E., A. N. Dille, J. P. Austin, C. M. Williams, L. A. Palinkas, and J. C. Burns.** 2000. Relationship of climate, ethnicity and socioeconomic status to Kawasaki disease in San Diego County, 1994 through 1998. Pediatr Infect Dis J **19:**1087-91.
3. **Burns, J. C., and M. P. Glode.** 2004. Kawasaki syndrome. Lancet **364:**533-44.
4. **Chang, L. Y., I. S. Chang, C. Y. Lu, B. L. Chiang, C. Y. Lee, P. J. Chen, J. T. Wang, H. N. Ho, D. S. Chen, and L. M. Huang.** 2004. Epidemiologic features of Kawasaki disease in Taiwan, 1996-2002. Pediatrics **114:**e678-82.
5. **Christensen, K., and J. C. Murray.** 2007. What genome-wide association studies can do for medicine. N Engl J Med **356:**1094-7.
6. **Falcini, F.** 2006. Kawasaki disease. Curr Opin Rheumatol **18:**33-8.
7. **Fujita, Y., Y. Nakamura, K. Sakata, N. Hara, M. Kobayashi, M. Nagai, H. Yanagawa, and T. Kawasaki.** 1989. Kawasaki disease in families. Pediatrics **84:**666-9.
8. **Gardner-Medwin, J. M., P. Dolezalova, C. Cummins, and T. R. Southwood.** 2002. Incidence of Henoch-Schonlein purpura, Kawasaki disease, and rare vasculitides in children of different ethnic origins. Lancet **360:**1197-202.
9. **Hansen, L., and O. Pedersen.** 2005. Genetics of type 2 diabetes mellitus: status and perspectives. Diabetes Obes Metab **7:**122-35.
10. **Holman, R. C., A. T. Curns, E. D. Belay, C. A. Steiner, and L. B. Schonberger.** 2003. Kawasaki syndrome hospitalizations in the United States, 1997 and 2000. Pediatrics **112:**495-501.
11. **Hung, S. I., W. H. Chung, L. B. Liou, C. C. Chu, M. Lin, H. P. Huang, Y. L. Lin, J. L. Lan, L. C. Yang, H. S. Hong, M. J. Chen, P. C. Lai, M. S. Wu, C. Y. Chu, K. H. Wang, C. H. Chen, C. S. Fann, J. Y. Wu, and Y. T. Chen.** 2005. HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. Proc Natl Acad Sci U S A **102:**4134-9.
12. **Kato, H., S. Koike, M. Yamamoto, Y. Ito, and E. Yano.** 1975. Coronary aneurysms in infants and young children with acute febrile mucocutaneous lymph node syndrome. J Pediatr **86:**892-8.
13. **Kato, H., T. Sugimura, T. Akagi, N. Sato, K. Hashino, Y. Maeno, T. Kazue, G. Eto, and R. Yamakawa.** 1996. Long-term consequences of Kawasaki disease. A 10- to 21-year follow-up study of 594 patients. Circulation **94:**1379-85.
14. **Kawasaki, T.** 2001. Kawasaki disease: a new disease? Acta Paediatr Taiwan **42:**8-10.
15. **Kim, S., and F. Dedeoglu.** 2005. Update on pediatric vasculitis. Curr Opin Pediatr **17:**695-702.
16. **Nagashima, M., M. Matsushima, H. Matsuoka, A. Ogawa, and N. Okumura.** 1987.

High-dose gammaglobulin therapy for Kawasaki disease. J Pediatr **110:**710-2.

17. **Newburger, J. W., M. Takahashi, A. S. Beiser, J. C. Burns, J. Bastian, K. J. Chung, S. D. Colan, C. E. Duffy, D. R. Fulton, M. P. Glode, and et al.** 1991. A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. N Engl J Med **324:**1633-9.
18. **Newburger, J. W., M. Takahashi, M. A. Gerber, M. H. Gewitz, L. Y. Tani, J. C. Burns, S. T. Shulman, A. F. Bolger, P. Ferrieri, R. S. Baltimore, W. R. Wilson, L. M. Baddour, M. E. Levison, T. J. Pallasch, D. A. Falace, and K. A. Taubert.** 2004. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. Pediatrics **114:**1708-33.
19. **Onouchi, Y., and A. Hata.** 2008. [Susceptibility genes for Kawasaki disease]. Nippon Rinsho **66:**272-7.
20. **Park, Y. W., J. W. Han, I. S. Park, C. H. Kim, Y. S. Yun, S. H. Cha, J. S. Ma, S. B. Lee, C. H. Kim, H. J. Lee, and Y. C. Tockgo.** 2005. Epidemiologic picture of Kawasaki disease in Korea, 2000-2002. Pediatr Int **47:**382-7.
21. **Plenge, R. M., M. Seielstad, L. Padyukov, A. T. Lee, E. F. Remmers, B. Ding, A. Liew, H. Khalili, A. Chandrasekaran, L. R. Davies, W. Li, A. K. Tan, C. Bonnard, R. T. Ong, A. Thalamuthu, S. Pettersson, C. Liu, C. Tian, W. V. Chen, J. P. Carulli, E. M. Beckman, D. Altshuler, L. Alfredsson, L. A. Criswell, C. I. Amos, M. F. Seldin, D. L. Kastner, L. Klareskog, and P. K. Gregersen.** 2007. TRAF1-C5 as a risk locus for rheumatoid arthritis--a genomewide study. N Engl J Med **357:**1199-209.
22. **Price, A. L., N. J. Patterson, R. M. Plenge, M. E. Weinblatt, N. A. Shadick, and D. Reich.** 2006. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet **38:**904-9.
23. **Risch, N., and K. Merikangas.** 1996. The future of genetic studies of complex human diseases. Science **273:**1516-7.
24. **Royle, J. A., K. Williams, E. Elliott, G. Sholler, T. Nolan, R. Allen, and D. Isaacs.** 1998. Kawasaki disease in Australia, 1993-95. Arch Dis Child **78:**33-9.
25. **Samani, N. J., J. Erdmann, A. S. Hall, C. Hengstenberg, M. Mangino, B. Mayer, R. J. Dixon, T. Meitinger, P. Braund, H. E. Wichmann, J. H. Barrett, I. R. Konig, S. E. Stevens, S. Szymczak, D. A. Tregouet, M. M. Iles, F. Pahlke, H. Pollard, W. Lieb, F. Cambien, M. Fischer, W. Ouwehand, S. Blankenberg, A. J. Balmforth, A. Baessler, S. G. Ball, T. M. Strom, I. Braenne, C. Gieger, P. Deloukas, M. D. Tobin, A. Ziegler, J. R. Thompson, and H. Schunkert.** 2007. Genomewide association analysis of coronary artery disease. N Engl J Med **357:**443-53.
26. **Schaid, D. J., and S. J. Jacobsen.** 1999. Biased tests of association: comparisons of allele frequencies when departing from Hardy-Weinberg proportions. Am J Epidemiol **149:**706-11.

27. **Schiller, B., A. Fasth, G. Bjorkhem, and G. Elinder.** 1995. Kawasaki disease in Sweden: incidence and clinical features. Acta Paediatr **84:**769-74.
28. **Tse, S. M., E. D. Silverman, B. W. McCrindle, and R. S. Yeung.** 2002. Early treatment with intravenous immunoglobulin in patients with Kawasaki disease. J Pediatr **140:**450-5.
29. **Uehara, R., M. Yashiro, Y. Nakamura, and H. Yanagawa.** 2003. Kawasaki disease in parents and children. Acta Paediatr **92:**694-7.
30. **Weeks, D. E., Y. P. Conley, H. J. Tsai, T. S. Mah, S. Schmidt, E. A. Postel, A. Agarwal, J. L. Haines, M. A. Pericak-Vance, P. J. Rosenfeld, T. O. Paul, A. W. Eller, L. S. Morse, J. P. Dailey, R. E. Ferrell, and M. B. Gorin.** 2004. Age-related maculopathy: a genomewide scan with continued evidence of susceptibility loci within the 1q31, 10q26, and 17q25 regions. Am J Hum Genet **75:**174-89.
31. **Wu, S. F., J. S. Chang, C. T. Peng, Y. R. Shi, and F. J. Tsai.** 2004. Polymorphism of angiotensin-1 converting enzyme gene and Kawasaki disease. Pediatr Cardiol **25:**529-33.
32. **Wu, S. F., J. S. Chang, L. Wan, C. H. Tsai, and F. J. Tsai.** 2005. Association of IL-1Ra gene polymorphism, but no association of IL-1beta and IL-4 gene polymorphisms, with Kawasaki disease. J Clin Lab Anal **19:**99-102.

## 計劃成果自評部份:

本計劃研究內容與原計劃相符。我們已利用 Affymetrix SNP chips 方法找出與 Kawasaki disease 相關 SNPs。目前接著希望做 replication study in independent cohorts and 利用不同方法做 genotyping。我們有達成預期目標。其研究成果具學術價值，適合在學術期刊發表。