Acta Genet Med Gemellol 30:213-216 (1981)
The Mendel Institute/Alan R. Liss, Inc.

Received 11 March 1981
Final 23 March 1981

A Search for Stratification-Free Association Between Plasma Lipids and HLA Using Dizygotic Twins

S. S. Winata,[1] A. A. Biegel,[2] K. W. Kang,[3] F. Harmath,[3] and J. C. Christian[3]

[1]Department of Histology, Udayana University Faculty of Medicine, Bali, Indonesia; [2]Department of Medicine, Indiana University School of Medicine, Indianapolis; [3]Department of Medical Genetics, Indiana University School of Medicine, Indianapolis

A total of 71 pairs of like-sexed dizygotic twins were studied, comparing within-pair differences for plasma total, free and esterified cholesterol, and triglyceride with the number of HLA haplotypes the twins had in common. If associations are present between HLA and the blood lipids studied, the twins with no haplotypes in common would be expected to have the largest within-pair mean square, those with two in common the smallest, and those with one in common an intermediate value. No significant differences were found comparing within-pair mean squares for the variables studied.

Key words: Plasma lipids, HLA, Coronary heart disease

INTRODUCTION

Mathews in 1975 [9] reported a significant correlation between the frequency of coronary heart disease and histocompatibility antigen B8 as well as haplotype A1-B8, this study was followed by reports by Scott et al [11] and Logan et al [8] who failed to find any relationship between the HLA system and coronary heart disease.

Studies of associations between genetic markers and disease states are difficult due to the problem of obtaining appropriate controls. In addition associations may represent no more than a chance occurrence of a relatively high frequency of the disease in question and the genetic marker in the same population. This chance occurrence, called stratification [7] is of much less potential interest than associations due to multiple gene effects or pleiotropism.

0001-5660/81/3003-0213$01.50

King et al [7] in 1978 proposed the use of fraternal twins to test for associations between genetic markers and quantitative traits. The twins share half of their genes and much of their environmental backgrounds in common, providing the closest possible controls and theoretically the best model for detecting stratification-free associations.

The present study was designed to search for associations between blood lipids that are important coronary heart disease risk factors [5] and the HLA system. If associations are found between coronary heart disease risk factors and HLA, they would provide important new tools for studying the genetics of this common disease. In addition a secondary goal of this study was to start a panel of HLA-typed dizygotic twins to be used in future association studies.

MATERIALS AND METHODS

Seventy-one sets of like-sexed dizygotic twins were studied (29 male and 42 female pairs) ranging in age from 9–31 years. Both members of each twin set were examined on the same day between April 1977 and September 1979.

Twin zygosity was determined by serological typing of blood-markers (ABO, Rh, MNS, Kell, Duffy, Kidd, and P), haptoglobin, HGM, acid phosphatase, and secretor. All twin pairs were discordant for at least one system studied.

Two HLA loci, HLA-A and HLA-B were used to classify the twin pairs, and whenever available the HLA-C locus HLA-W^{4}/6 markers were also included. Venous heparinized blood was drawn, when the blood was obtained for the lipid determination.

The HLA typing was performed by the microcytotoxicity method, with sera obtained from the NIH-NIAID Serum Bank, from other investigators and from local sources. These sera cover the following A, B, and C specificities: HLA-A1,2,3,9,10,11,28; AW24,25,26,30,31,32,33,34,36; B5,7,8,12, 13,14,15,16,17,18,21,22,27,35,37,40,44,45; BW42,49,50, as well as HLA-CW1,2,3,4,6. Also, polyspecific sera determining BW4 and BW6.

In addition to the twins, at least one, (and in many cases, both parents) was also HLA typed for accurate haplotype assignment, clarification of suspected homozygosity and determination of which haplotypes originated from the father and which from the mother. Based on these HLA-genotypes the twins were classified into the following three haplotype groups: (0) twin pairs having no haplotypes in common, (1 male and 9 female pairs); (1) twin pairs having one haplotype in common, (20 male and 19 female pairs), and (2) twin pairs having 2 haplotypes in common (8 male and 14 female pairs).

Blood for lipid determination was drawn after a 12-hour overnight fast and was collected over EDTA (1 mg/ml). Plasma was separated within 2 hours of collection at 4°C (74 g for 30 min).

The lipid variables measured were total cholesterol, free cholesterol, esterified cholesterol, and triglycerides. Cholesterol was determined by the method of Abell et al [1] and thin-layer chromatography was used to separate free cholesterol, esterified cholesterol, and triglycerides from the lipoproteins [6]. Triglycerides were determined by a modified method of Antonis [3].

RESULTS AND DISCUSSION

The primary comparisons were made among the haplotype groups containing twins with 0, 1, and 2 haplotypes in common. This method of analysis to our knowledge has not previously been used in twin studies of associations and is made possible because of the extremely polymorphic HLA system.

A t′ test was used to test for significant differences between the means of the three haplotype groups (Table 1) and the lipids studied [4]. No significant mean differences were found indicating an absence of association between the plasma lipid levels and the haplotype groups.

TABLE 1. Mean Values of Plasma Lipids in Twin Pairs With Zero, One, or Two HLA Haplotypes in Common

	Mean (mg/dl)			
Haplotypes in common	Free cholesterol	Esterified cholesterol	Total cholesterol	Triglycerides
0	40.7	101.7	154.9	83.4
1	43.3	107.4	169.4	87.0
2	42.6	107.6	168.8	101.8

TABLE 2. Among- and Within-Pair Mean Squares for Plasma Lipids in Dizygotic Twin Pairs Divided by the Number of HLA Haplotypes They Have in Common

	Mean (mg/dl)			
Haplotypes in common	Free cholesterol	Esterified cholesterol	Total cholesterol	Triglycerides
a. Within-pairs mean squares				
0	54.3	79.0	230.4	859.0
1	29.8	211.5	606.1	796.7
2	77.6	236.1	828.2	1204.0
F_{max}	2.60	2.99	3.83	1.5112
b. Among-pairs mean squares				
0	122.5*	491.0	1256.4	1842.0
1	258.9*	838.2	2478.6	2796.2
2	81.5*	430.9	1456.5	4386.5

*$P < 0.05$.

The next step was to search for heterogeneity of the variances among the haplotype groups using the F_{max} test [12].

The mean squares within pairs, among pairs and their sums were compared for the three haplotype groups again using the F_{max} test (Table 2). There were no significant differences among the sums of the within- and among-mean squares, indicating that it would be appropriate to compare the within- and among-pair mean squares [4]. If there was a significant association between blood lipids and HLA type, the within-pair mean square for twins with no haplotypes in common would be expected to be the largest, those with one haplotype in common intermediate, and in those twins with two haplotypes in common the smallest. There was no significant difference among the within-pair mean squares for the four lipids studied. A similar though less sensitive test for associations would come from a comparison of the among-pair mean squares with the exception that the expected order of size would be reversed. Of the lipids studied only free cholesterol had a significant difference ($p < 0.05$), comparing the among-pair mean squares. However the difference was due to a relatively large among-pairs mean square for the twins sharing one haplotype in common, a finding that does not fit expectations and because of the number of tests done may well be due to chance. These analyses were repeated following adjustments of the lipid data for body weight and similar results were obtained.

This study provides little evidence for associations between blood lipids and the HLA complex even though dizygotic twins were used to provide closely matched controls. It agrees with the study of Mathews and Tait [10] who could find no association between HLA-B8 and serum cholesterol levels. This study does not rule out the possibility of associations between blood lipids and the HLA complex or closely related markers on the human chromosome 6, but should have detected such associations if they are due to pleiotropic effects of the HLA genes and are common in the population studied. Most of the strong associations between the HLA system and diseases have been with diseases closely related to the immune system. It therefore is not surprising that associations of blood lipids and HLA were not found.

Acknowledgments. This work was supported in part by the Indiana University Diabetes Center grant P60-AM 20542 and MUCIA-AID-Indonesian Higher Education Development Training Project 497-V-042.

REFERENCES

1. Abell LL, Levy BB, Brodie BB, and Kendall RE (1952): A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J Biol Chem 195:357.
2. Amos DB, et al (1970): In P. Terasaki (ed): "Histocompatibility Testing." Copenhagen: Munksgaard, pp 259–275.
3. Antonis A (1960): The colorimetric determination of ester groups in lipid extracts. J Lipid Res 1: 485.
4. Christian JC (1979): Testing twin means and estimating genetic variance. Basic methodology for the analysis of quantitative twin data. Acta Genet Med Gemellol 28:35.
5. Kannel WB, Castelli WP, Gordon T (1979): Cholesterol in the prediction of atherosclerotic disease. Ann Intern Med 90:85.
6. Kang KW, Taylor GE, Greves JH, Staley HL, Christian JC (1971): Genetic variability of human plasma and erythrocyte lipids. Lipids 6:595.
7. King RJ, Feinlieb M, Carrison RJ, Christian JC (1978): Studies of associations between genetic markers and plasma lipids in twins. In "Progress in Clinical and Biological Research," vol 2c. New York: Alan R. Liss, pp 165–170.
8. Logan RL, Oliver MF, McTavish J, Darg C, White AG (1977): Histocompatibility antigens and myocardial infarction. Tissue Antigen 10:361.
9. Mathews JD (1975): Ischaemic heart disease: Possible genetic markers. Lancet 2/681.
10. Mathews JD, Tait BD (1975): HLA-B8 and serum cholesterol. Lancet 2:1215.
11. Scott BB, McGuffin P, Rajah SM, Stoker JB, Losowsky MS (1976): Histocompatibility antigens and myocardial infarction. Tiss Antig 7:187–188.
12. Sokal RR, Rohlf FJ (1969): In "Biometry: The Principles and Practice of Statistics in Biological Research." San Francisco: WR Freeman and Co.

Correspondence: Dr. Joe Christian, Department of Medical Genetics, 1100 W. Michigan, Indianapolis, IN 46223, USA.