

The National Heart Institute Twin Study

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During the past two decades prospective epidemiologic studies have established the association between several risk factors and coronary heart disease. Elevations of serum cholesterol and blood pressure, obesity, lack of physical activity and cigarette smoking are among the factors which have been related to an increased risk of heart disease. We are now entering a period in which vigorous efforts will be made to modify these factors in order to prevent the occurrence of heart disease. An important part of this effort will be to determine to what extent these factors are genetically controlled and to what extent they can be modified by manipulation of the individual environment. Recognizing this need, the Field Epidemiological Research Section, National Heart Institute, NIH, has recently embarked on a study of cardiovascular disease risk factors in a large sample of adult male twins. The purpose of the present report is to describe the protocol and methods to be used and the importance of this study to current research in cardiovascular disease.

Twin studies are generally regarded as potentially among the most powerful methods available for studying genetic factors in human disease. Although genetic factors have long been suspected of having a major role in cardiovascular disease, little research has been done in this area among adult twins. Anthropometric studies early established that weight was to a considerable extent determined by genetic factors. Stocks (1930), one of the first investigators to study blood pressure in twins, found that blood pressure from adolescence on is governed by hereditary factors. Subsequent studies on blood pressure in twins have been conflicting. Hines et al (1957) and Lundman (1966) agreed that there is a strong genetic factor for systolic and diastolic pressure. Takkunen (1964) was only able to find genetic factors of importance with regard to systolic pressure and of no importance in determining diastolic pressure. Mathers et al (1961), Osborne et al (1963) and Downie et al (1969) have quite recently reported that blood pressure is only weakly, if at all, influenced by genetic effects as determined by studies of twins.

An important biochemical variable, related to risk of coronary heart disease, is the serum cholesterol level. Most twin studies (Osborne and Adlesberg, 1958; Osborne et al, 1959; Gedda and Poggi, 1960; McDonough et al, 1962; Meyer, 1962; Jensen et al, 1965; Pikkariainen et al, 1966; Blankenhorn et al, 1967; Kulonen, 1967)

done during the past decade report a genetic influence on the level of total serum cholesterol. Two recent studies by Lundman (1966) and Rifkind et al (1968) have failed to confirm these findings and have raised questions about the earlier study designs, twin samples and methods of analysis. During the past few years Fredrickson et al (1967) at the National Heart Institute have suggested that it is more meaningful to consider the genetic factors influencing the levels of lipoprotein fractions than that of total serum cholesterol. They have documented five types of electrophoretic patterns occurring among hyperlipidemic patients. These recent developments in the lipoprotein field suggest that it is important to do twin studies utilizing these newer methodologies.

Another risk factor which has come under considerable scrutiny is cigarette smoking. Although there is still considerable controversy about the relationship of cigarette smoking to the risk of various forms of coronary heart disease, there is little doubt that some effect is demonstrable in most epidemiologic studies. Whether or not this effect might be due to constitutional differences in the smokers is a moot point. Fisher (1958), Friberg et al (1959), and Cederlöf et al (1966, 1967*a*, 1967*b*) have reported on the smoking characteristics of twins; MZ twins tend to be more concordant than DZ twins. Fisher (1958) has shown that MZ twins raised apart show the same degree of concordance as MZ twins raised together. Cederlöf, using MZ twins discordant in smoking habits, has failed to find an appreciable effect of cigarette smoking upon the occurrence of angina pectoris, thus raising a serious question as to the role of cigarette smoking as a direct etiologic factor in angina pectoris.

Another approach to the investigation of cardiovascular disease in twins has been the study of ECG patterns. Early reports (Wise et al, 1939, and others cited by Takkunen, 1964) showed a greater concordance in MZ than DZ twins. Recent reports (Mathers et al, 1961; Takkunen, 1964), using quantitative methods, have been conflicting. More definitive studies in this difficult area are needed.

In order to further clarify the relative importance of genetic factors in determining these and other risk factors, the National Heart Institute is planning to examine 300 pairs of white male twins at the facilities of the Framingham Heart Study (Framingham, Mass.). Access to these twins has been obtained through the cooperation of the NAS-NRC Twin Panel which has assembled a roster of all twins born between 1917 and 1927 who have served in the US Armed Forces. The structure of the Panel has been described by Jablon et al (1967). These twins are currently in their 40's and early 50's — an age at which the important risk factors have become relatively stable and the effect of environmental influences as well as genetic influences have been established. They thus constitute a good group to study the effects of heredity and environment upon these risk factors. Moreover, they are also at the age when heart disease is rapidly becoming the most important ailment in this population.

A standard examination is given the members of each set simultaneously by different physicians. Historical data are obtained about the twin and his family. This includes a chronological listing of residence and employment since 1945, and major diseases of the subject's spouse and all first degree relatives. An estimate is made of the degree of association and relative dominance of the twins in childhood, school, and adult life. Eye and hair charac-

teristics are recorded. Marital history with a comparison of the wives' characteristics is obtained. The medical history includes all previous hospitalizations, serious illnesses, medications, smoking history, and a review of respiratory and cardiovascular symptoms. Physical activity is estimated in terms of a 24-hour enumeration of sleep, job, and extra-curricular activities.

Determination of height, weight, skin fold thicknesses, and blood pressure are made as part of a comprehensive physical examination. Special studies consist of a 13-lead ECG, chest X-ray, spirometric studies, and peripheral pulse oscillography. Analysis of blood specimens include lipoprotein quantification and paper electrophoresis, complete serotyping for zygosity determinations, and glucose measured one hour after a 50g glucose load. Two dietary histories are obtained: one consisting of a 24-hour recall and the other of a dietary frequency interview.

One of the major assets of this study is the availability of the resources of the Framingham Heart Study, which is a prospective investigation conducted by the National Heart Institute of a population of 5209 adults who have been examined biennially since 1948. Using the Framingham facilities ensures uniform examination of all of the twins by experienced cardiovascular epidemiologists who will adhere to a specified and well-tested protocol. Furthermore, the results of the examination done on the twins can be validated against the previously obtained information on a general population sample of similar age examined by identical procedures during the past two decades.

An important aspect of this study will be to examine hereditary influences on serum cholesterol, triglycerides, and specific lipoprotein concentrations, as well as the relationship of these lipids to the patient's diet and other risk factors. The four major lipoprotein fractions (chylomicrons, very low density lipoproteins, α - and β - lipoproteins) show varied abnormalities in hyperlipoproteinemia, and certain familial and acquired elevations of these plasma lipoproteins appear to be associated with coronary and peripheral vascular disease (Fredrickson et al, 1967). The biochemical techniques used in the determination of these lipoprotein fractions have been described by Levy and Fredrickson (1968).

The data obtained will be analyzed by a variety of techniques. The primary method will be the analysis of variance procedures described by Osborne and De George (1959) and others (Falconer, 1960; Kempthorne and Osborne, 1961; Partanen et al, 1966). In previous studies several investigators tried to analyze the effects of separate environmental components by looking at twins who were discordant for recognized factors. Thus, Cederlöf et al (1966, 1967*a*, 1967*b*) have looked at the risk of heart disease in smoking and nonsmoking MZ twins. Other investigators have looked at the variability of blood pressure and cholesterol in twins living together and those currently living apart. The present study will try to expand on these techniques by identifying sets discordant for other recognized environmental influences. This will be done on the basis of the physical activity history, the dietary history, the occupational history, the marital history and from subjective intrapair evaluations by the twins themselves. It is hoped that sufficient numbers of discordant sets

will be obtained to be able to use this powerful method to accurately separate the effects of environmental and genetic factors.

A final word should be mentioned about the follow-up procedures to be used in this Twin Study. Although it is too early to make plans for follow-up of the twins through subsequent examinations, the National Academy of Science and National Research Council will maintain mortality follow-up for this group. Thus, at a minimum, mortality data will be available which can be correlated with the cross-sectional measurements obtained at this stage in the study. This double-barreled approach of studying risk factors cross-sectionally and prognosis in relation to heart disease prospectively should make this a very powerful study for elucidating the role of genetic factors in cardiovascular diseases.

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