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A Study of Cardiovascular Risk in Heterozygotes for Homocystinuria

S. HARVEY MUDD,[1] RICHARD HAVLIK,[2] HARVEY L. LEVY,[3] VICTOR A. MCKUSICK,[4] AND MANNING FEINLEIB[2]

SUMMARY

Early atherosclerotic-like lesions and thromboembolic problems are prominent in homocystinuric patients. Recent evidence suggested that mild homocyst(e)inemia, such as is present in heterozygotes for homocystinuria due to cystathionine synthase deficiency, may cause a marked excess in early ischemic heart disease. To evaluate the risk due to mild homocyst(e)inemia, the frequencies of heart attacks and strokes in parents and grandparents of homocystinuric children were assessed in the present study. No statistically significant increases in the incidence of heart attacks or strokes were consistently detected. The data available are sufficient to virtually exclude an increase in the cardiovascular risk for homocystinuria heterozygotes of as much as fivefold compared to controls, and to make very improbable a relative risk of as much as threefold. Less than 5% of homocystinuria heterozygotes are likely to have a fatal or nonfatal heart attack by age 50. These results fail to suggest that mild homocyst(e)inemia is an important contributory factor in the overall incidence of cardiovascular disease.

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[1] Laboratory of General and Comparative Biochemistry, National Institute of Mental Health, Bldg. 32, Rm. 101, 9000 Rockville Pike, Bethesda, MD 20205.

[2] Epidemiology and Biometry Program, Division of Heart and Vascular Diseases, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205.

[3] The Joseph P. Kennedy, Jr. Laboratories of the Neurology Service, Massachusetts General Hospital, Children's Hospital Medical Center, Department of Neurology, Harvard Medical School, Boston, MA 02114.

[4] Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

INTRODUCTION

Cystathionine synthase deficiency is a relatively frequently encountered inborn error of metabolism characterized by homocystinuria, and by plasma accumulation of homocysteine, its oxidized disulfide, homocystine, and methionine. The chief cause of morbidity and early mortality among affected patients is their tendency to thrombosis and embolism, with resultant strokes and coronary occlusions [1]. Pathological changes similar to those in atherosclerosis have been described [2, 3]. Administration of homocysteine, or related compounds, has been reported to produce arteriosclerotic vascular lesions in rabbits [4, 5] (although this claim has been contested [6]) and in baboons [7]. Studies of children with alternative genetic diseases that produce homocystinemia without hypermethioninemia suggest these pathological changes may be secondary to plasma accumulation of homocyst(e)ine rather than to accumulation of methionine [3, 8–10].

The possibility that mild homocyst(e)inemia might play a broader role in the pathogenesis of coronary artery disease was raised by a recent Australian study of patients under age 50 with angiographic evidence of ischemic heart disease [11]. After a methionine load, the mixed disulfide of homocysteine and cysteine (a chemical derivative indicative of some degree of abnormal homocyst(e)inemia) was detected in 17 of 25 patients, but in only five of 22 control subjects free of known ischemic disease. In seven of the ischemic heart disease patients, but in only one of the controls [11], the concentrations of homocysteine-cysteine disulfide were comparable to those attained after similar loading of obligate heterozygotes for cystathionine synthase deficiency [12]. Such heterozygotes possess less than 50% of mean control specific activities of cystathionine synthase [1], and accumulate abnormally high plasma concentrations of homocysteine, or its derivatives [12, 13]. Together, these results suggest that heterozygotes for cystathionine synthase deficiency might be at greatly increased risk (at least sevenfold, possibly much more) of developing coronary artery disease by age 50. The rates of detection of cystathionine synthase deficiency in screening programs of newborns [1] indicate that heterozygotes for this recessively inherited condition are present in various populations at minimal frequencies of 0.5%–1.5%. If these heterozygotes are at serious risk of developing early coronary artery disease, an explanation would then be provided for a substantial portion of such disease currently unassociated with known risk factors.

Some of these considerations, as well as others, have been used to advance a "homocysteine theory of arteriosclerosis" [5] and to suggest the possible utility of a low methionine diet, perhaps accompanied by increased vitamin B_6 intake, in the prevention of arteriosclerosis in the population of the United States [14, 15].

To explore further the relationship between mild homocyst(e)inemia and coronary artery disease or stroke and to define the risk for heterozygotes for cystathionine synthase deficiency, we measured the rates of coronary attacks and strokes in parents and grandparents of cystathionine synthase-deficient patients. The natural parents are obligate heterozygotes, as are two of the grandparents. The rates of coronary attacks and strokes were compared with those in two control groups

comprised of relatives of children with either new-mutation achondroplastic dwarfism or with impaired phenylalanine metabolism. The results are reported here.

METHODS

Ascertainment, Study Populations, and Questionnaires

The basic plan of the present study was to obtain, by means of questionnaires, information about the health history of parents and grandparents of children with either homocystinuria due to cystathionine synthase deficiency, impaired phenylalanine metabolism, or new-mutation achondroplastic dwarfism. To permit maximum standardization of questionnaires, the study was limited to areas in which English is a principal language: the United States, Canada, the United Kingdom, Ireland, Australia, and New Zealand. Physicians who might know of families with a homocystinuric member were identified from reported cases and by contact with centers specializing in diagnosis and/or management of genetic diseases. Physician cooperation was solicited also by notices in appropriate ophthalmologic journals, in the newsletter of the Child Neurology Society, and by circulation of a letter to members of the American Association of Pediatric Ophthalmology and Strabismus. To ensure that only cystathionine synthase-deficient families would be included, physicians were asked to select families in which the affected proband(s) had cystathionine synthase deficiency proven by enzyme assay, or had either hypermethioninemia, dislocated optic lenses, or both, in addition to homocystinuria [1]. Forms were sent by physicians to responsible family members, requesting information on parents and grandparents concerning a number of chronic conditions and diseases, including heart attacks and strokes as well as years of birth and death, and cause of death if a family member was deceased. A representative section of the questionnaire has been deposited with, and is available through, the National Auxiliary Publications Service.* The respondent completed an informed consent that was returned to the attending physician. A numbered and anonymous copy of the questionnaire was returned to the central office for analysis. Family members were cautioned to complete only one form, since it was possible that the patient(s) had been seen by more than one physician. The major portion of the survey occurred during 1978.

During approximately the same period, a similar routine was used for acquiring data from comparison families with dwarfism or with impaired phenylalanine metabolism. The dwarf population consisted of apparent new-mutation cases of achondroplasia with a proband of current age 30 years or less available from a larger roster of referrals from the Mid-Atlantic area of the United States. The patients with impaired phenylalanine metabolism included chiefly those with classical phenylketonuria, some with hyperphenylalaninemia, and one recognized case of dihydropteridine reductase deficiency. These patients mostly came from the New England area and had been identified through screening programs.

Analysis and Statistics

Information from the returned forms was entered into the computer and verified by complete re-entry of data. A search for potential duplicate information, due to a single family having responded twice, detected one repeat, and the duplicate information was deleted. Preliminary analysis revealed that a disproportionately high number of grandparents had a reported unknown exact year of birth or death. The responding relative was then

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asked to estimate these dates within 10-year intervals or to give an approximate age at death. This information was added to the data set. Further, since the original form did not ask for the year of first heart attack, this additional information was solicited for the three groups of fathers and mothers.

Two interrelated statistical approaches were utilized to evaluate the data. First, age-stratified heart attack or stroke-death incidence rates were calculated for the four major relatives in each study group by the frequency of heart or stroke death over person-years of experience for three 15-year age periods [16]. Second, a life table was constructed [17] starting at an arbitrary age of 30 years, and based upon yearly experience, corrected for withdrawals for age in 1978 or death from other causes. This table was used to determine the cumulative probability of remaining free of an event. Rate ratios and confidence limits for data with person-time denominators were estimated [18]. Confidence limits for differences in probabilities of remaining free of an event, or for differences in cumulative incidences between case and comparison families, were derived from the standard errors calculated by Ederer's modification of the method of Greenwood [19] for each age interval. Subgroup analyses were also performed excluding individuals with known diabetes, or hypertension, or living outside of North America.

RESULTS

During this study, 260 families with one or more homocystinuric children received questionnaires. Of these, 203 (78%) responded. The response rates for the families of children with impaired phenylalanine metabolism and with new-mutation achondroplastic dwarfism were comparable: 81.1% and 74.3%. The numbers of participating family members as well as mean birth years are shown in table 1. The homocystinuria family members were, on the average, older than the comparison groups, and had 3–4 years more potential exposure than members of families with dwarf offspring, and 8–9 years more potential exposure than members of families with patients with impaired phenylalanine metabolism.

Heart Attacks

The number of relatives with histories of fatal and nonfatal heart attacks are shown in table 1. Because of age differences between the members of the three family groups, direct comparisons of frequencies were not appropriate. The results

TABLE 1

NO. FAMILY MEMBERS, MEAN BIRTH YRS, AND NO. FATAL AND NONFATAL HEART ATTACKS

	NEW-MUTATION ACHONDROPLASTIC DWARFISM			HOMOCYSTINURIA DUE TO CYSTATHIONINE SYNTHASE DEFICIENCY			IMPAIRED PHENYLALANINE METABOLISM		
RELATIVE	No.	Mean birth yr	No. heart attacks	No.	Mean birth yr	No. heart attacks	No.	Mean birth yr	No. heart attacks
Fathers	156	1932	4	195	1929	10	174	1938	3
Mothers	156	1936	1	199	1932	1	180	1941	2
Grandfathers	308	1903	93	385	1899	92	343	1908	106
Grandmothers	310	1907	40	391	1903	69	349	1911	48

of person-years analyses for three comparable age periods [16] are shown in table 2. The disease experience of the parents was limited by virtue of their ages and low event rates. There was no marked increase of heart attacks for either fathers or mothers of homocystinuric children (although fathers from 45–59 years had a slight increase). Similarly, neither grandfathers nor grandmothers showed striking increases. Indeed, for grandfathers from 45–59 years and 60–74 years, the rates of fatal heart attacks were lower in the homocystinuria group than in either control group.

The probabilities of remaining free of heart attacks were calculated (results available from the National Auxiliary Publications Service*). Generally, the confidence limits were wide, and no marked decreases in the probabilities of remaining free of heart attacks were revealed among relatives of homocystinuric children. Figure 1 graphically displays the cumulative incidences of heart attack mortality in the grandparents and provides a comparison of the data from this study to those derived from the Framingham experience [20].

To summarize the results with heart attacks, age intervals were combined for each homocystinuria relative to permit calculation of an overall rate. In table 3, these rates are compared with the corresponding rates for control relatives, taking the dwarfism and impaired phenylalanine groups together, and several confidence intervals for each rate ratio are presented.

To gain some insight into the effect of mild homocyst(e)inemia in groups free of hypertension and diabetes, known alternative risk factors for coronary disease, each of the above analyses was repeated after prior removal of individuals known to have one or both of these conditions. The resulting tables (available from the National Auxiliary Publications Service*) were not markedly different from those forthcoming from the previous analyses.

To ascertain whether geographical factors played a major role in the results obtained, the heart attack probabilities for only the members of the families from North America were compared with the control groups. No differences were observed. Further, the disease occurrence in Australian families was comparable to that in the remainder of the population.

Strokes

When probabilities of strokes were analyzed in a similar manner (tables available from the National Auxiliary Publications Service*) no marked differences were detected for either fathers, mothers, grandfathers, or grandmothers of homocystinuric children as compared with either control group. A life table analysis combining stroke mortality with total heart attacks as an end point and comparing fathers of homocystinuric patients with a pooled control group of both fathers of phenylalanine-impaired and dwarf offspring showed no major differences. (Results deposited with National Auxiliary Publications Service.*)

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TABLE 2

AGE-STRATIFIED INCIDENCE OF HEART ATTACKS

AGE INTERVAL (YEARS)	NEW-MUTATION ACHONDROPLASTIC DWARFISM			HOMOCYSTINURIA DUE TO CYSTATHIONINE SYNTHASE DEFICIENCY			IMPAIRED PHENYLALANINE METABOLISM		
	No. events	Person-years at risk	Rate/1,000	No. events	Years at risk	Rate/1,000	No. events	Years at risk	Rate/1,000
Fathers:									
30–44	0	1,619	0	1	2,245	0.4	2	1,400	1.4
45–59	2	741	2.7	8	1,098	7.3	1	341	2.9
60–74	2	103	19.4	1	211	4.7	0	62	0
Mothers:									
30–44	0	1,399	0	0	2,069	0	1	1,206	0.8
45–59	1	547	1.8	1	949	1.1	0	231	0
60–74	0	17	0	0	179	0	1	37	27.0
Grandfathers:									
30–44	1	4,497	0.2	3	5,176	0.6	6	4,962	1.2
45–59	19	4,100	4.6	15	4,724	3.2	20	4,285	4.7
60–74	34	2,265	15.0	28	2,988	9.4	30	2,060	14.6
Grandmothers:									
30–44	0	4,534	0	5	5,432	0.9	1	5,030	0.2
45–59	11	4,172	2.6	3	4,899	0.6	2	4,278	0.5
60–74	8	2,195	3.6	21	3,242	6.5	10	2,116	4.7

NOTE: For parents, both fatal and nonfatal heart attacks were scored. Because of lack of specific dates for nonfatal heart attacks for grandparents, only fatal heart attacks could be considered.

DISCUSSION

This study was designed to gain information on the extent to which heterozygotes for homocystinuria due to cystathionine synthase deficiency are at increased risk for coronary attacks and strokes, manifestations that are prominent features among affected patients with this enzyme deficiency [1]. The heterozygote population for homocystinuria was defined genetically by family constellation. Historical information on only the more accurately diagnosed fatal and nonfatal heart attacks was obtained rather than on the softer end points of coronary insufficiency or angina pectoris. Rates were compared with those in two groups of control

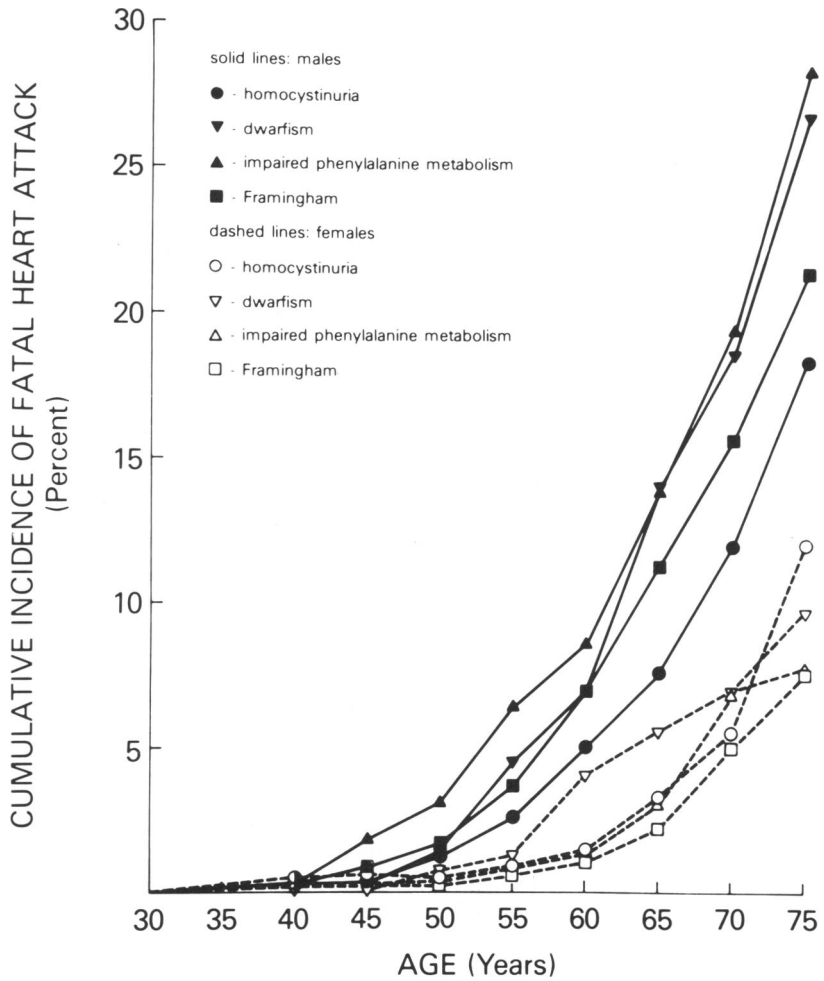

FIG. 1.—Cumulative incidence of fatal coronary attacks as a function of age for each group of grandfathers and grandmothers of affected patients and for subjects in Framingham study. Lines were derived from life tables as explained in text. Data for subjects in Framingham study [20] were treated similarly.

TABLE 3

RATE RATIOS AND CONFIDENCE INTERVALS FOR HEART ATTACKS

CONFIDENCE INTERVALS	RATE RATIOS		
	Fathers 1.43	Grandfathers 0.66	Grandmothers 1.33
90%	0.64–3.21	0.49–0.88	0.87–2.02
95%	0.55–3.75	0.47–0.93	0.80–2.19
99%	0.40–5.09	0.42–1.03	0.69–2.57
99.9%	0.28–7.21	0.37–1.17	0.57–3.08

NOTE: Heart attacks scored as in table 2. For each homocystinuria relative, age intervals were combined to permit calculation of overall rate that was compared with similar rate for control relatives, combining dwarfism and impaired phenylalanine metabolism groups. Insufficient heart attacks occurred among mothers to permit this sort of calculation. For grandmothers, rate ratios were inconsistent across age strata, so group maximum-likelihood estimate is only an approximation.

families in which alternative genetic diseases usually diagnosed early in childhood also occurred. Families with *new-mutation* achondroplasia were selected because this group should be unbiased by genetic factors. The majority of patients in the impaired phenylalanine metabolism group had phenylketonuria, a recessive condition associated, as is cystathionine synthase deficiency, with mental retardation in the untreated. Neither of these control groups has any known predilection to atherosclerotic lesions or coronary heart disease.

Results for the fathers of homocystinuric children are of particular interest. Our results suggest that the relative risk for homocystinuria fathers of suffering heart attack is 1.43 times that of the combined control fathers (table 3), a relative risk that falls short of statistical significance at the $P < .05$ level. The 95% limits for the rate ratio in fathers vs. combined controls were from 0.55 to 3.75 (table 3) (i.e., one may be 95% confident that the true relative risk is not less than 0.55 and not more than 3.75). One can be 99.5% confident that the true relative risk is not greater than 5.09 (table 3).

Heart attacks were infrequent in homocystinuria mothers, as well as in mothers of each control group. The results show that the absolute risk for female homocystinuria heterozygotes is low until *at least* an age of approximately 50, but too few events occurred to permit valid estimates of risk ratios for homocystinuria mothers as compared with control mothers.

Grandparents were included in the study to provide information upon older groups in which the frequency of coronary attacks and strokes was, as expected, higher. Although information was obtained on almost twice as many grandfathers and grandmothers as upon fathers and mothers, the grandparental groups have the disadvantages that only half of the individuals are expected to be heterozygotes and that the historical information about them was probably less accurate. For grandfathers of homocystinuric patients, the risk ratio compared with combined controls was 0.66 with 95% confidence limits of 0.47 to 0.93. For grandmothers compared with combined controls, the rate ratio was 1.33 with 95% confidence limits of 0.80 to 2.19. Most importantly, in each instance, the chances

that the cardiovascular risk for homocystinuria grandparents was increased as much as 2.5-fold could be excluded with a great deal of confidence. (A 2.5-fold increase for the total group would result from a fourfold increase for the half of the grandparents who were actually heterozygotes.)

In evaluating these results, certain limitations of this study should be borne in mind. First, because the cardiovascular diagnoses were arrived at by the questionnaire method, the validity of these diagnoses is subject to more uncertainty than would have been the case had direct physical and electrocardiographic examination been possible. However, the fact that the cumulative incidences of fatal heart attacks determined for both men and women in the control groups for our study were in reasonable agreement with those based upon the Framingham experience (fig. 1) supports the validity of the results obtained by the questionnaire method. Second, with about a 20% loss in response rate, some unidentified participation biases may have possibly been operating. We regard this possibility as unlikely in view of: (1) the similar response rates among the homocystinuria group and the control groups, (2) the fact that intensive follow-up efforts were made by the study staff to encourage uniform participation among all families contacted, (3) the fact that the two separate control populations were contacted by different staff personnel, thereby minimizing any systematic bias, and (4) the reasonable agreement between the present results and those from Framingham (alluded to above). Third, because of the indirect approach used, a class of clinically confirmed young patients with ischemic heart disease similar to those studied by the Australian investigators [11] could not be identified. The hypothesized relationship could possibly be limited to such individuals because of a more subtle interaction between mild homocyst(e)inemia and a particular cardiovascular end point, or because of another contributing risk factor such as diet high in protein or saturated fat and cholesterol. However, diets in Australia and in the United States are not markedly dissimilar [21].

Taken together, the overall results of this study virtually exclude a risk for homocystinuria heterozygotes that is increased as much as fivefold over the control rate. Very probably the relative risk is not increased as much as threefold. In any case, the absolute risk for homocystinuria heterozygotes is such that no more than 5% of such individuals are likely to have either a fatal or nonfatal coronary attack by age 50. To increase the confidence with which the relative cardiovascular risk due to mild homocyst(e)inemia may be specified, it would be advantageous to repeat a study of the present sort with larger groups. However, it will be difficult to do so until greater numbers of cystathionine synthase-deficient patients have been diagnosed, or until a simple and unequivocal method becomes available for identification of heterozygotes in the population at large. We believe that the great majority of identified cystathionine synthase-deficient families in the geographical areas surveyed received questionnaires in connection with our study. For the moment one may conclude that the probability is low that naturally occurring variations in homocyst(e)inemia contribute importantly to the cardiovascular risk of the population at large, and this study fails to provide support for hypotheses that postulate such an association [14].

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Erratum

In the paper "Estimation of Environmental and Genetic Components of Quantitative Traits with Application to Serum Cholesterol Levels" by Simpson et al. (*Am J Hum Genet* 33:293–299, 1981), there are two errors. The equation on page 294 should read:

$$\rho_3 = \frac{\rho_1^2 + \rho_2^2 - 2\rho_1\rho_2\rho_4}{1 - \rho_4^2} .$$

The last sentence in the last full paragraph on page 295 should read: "For ascending families in which the mother and/or father is not studied, and descending families in which the spouse is not studied, appropriate marginal distributions are obtained."