Socioeconomic status, ABO phenotypes and risk of ischaemic heart disease: an 8-year follow-up in the Copenhagen Male Study
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Objectives The association of socioeconomic status with the risk of ischaemic heart disease is only partly explained by the uneven distribution of conventional risk factors. We tested the hypothesis that an uneven socioeconomic distribution of ABO phenotypes could contribute to the explanation.

Design A prospective study controlling for age and other relevant potential confounders: smoking, physical activity, wine consumption, height, weight, serum lipids, blood pressure, hypertension, type II diabetes, serum selenium concentration and soldering fumes exposure.

Setting The Copenhagen Male Study, Denmark.

Study participants Two thousand, nine hundred and ninety-three men aged 53–74 years without overt ischaemic heart disease.

Main outcome measure Incidence of ischaemic heart disease in an 8-year follow-up.

Results Two hundred and forty-two men (8.1%) had a first ischaemic heart disease event. There was no association between socioeconomic status and the ABO blood group phenotypes and, in accordance with this, ABO phenotype was not a confounder for the association of socioeconomic status with the risk of ischaemic heart disease. However, ABO blood group was a strong risk or effect modifier. Only among men with the O phenotype was socioeconomic status (social classes IV and V versus social classes I, II and III) associated with a significant excess risk (relative risk 1.7, 95% confidence interval 1.1 – 2.7 and \( P = 0.02 \) after adjustment for confounders; the corresponding relative risks among the A and B/AB phenotypes comparing low social classes with the higher social classes were 1.08 (\( P = 0.77 \)) and 1.08 (\( P = 0.89 \), respectively).

Conclusion ABO phenotypes did not contribute directly to the explanation of socioeconomic inequalities in the risk of ischaemic heart disease. However, the finding of ABO phenotypes being effect modifiers for the association of socioeconomic status with the risk of ischaemic heart disease may open up new possibilities of clarifying the roles of socioeconomic status and ABO blood group as cardiovascular disease risk factors. J Cardiovasc Risk 7:277–283 © 2000 Lippincott Williams & Wilkins.

Introduction We have previously shown that, in a cohort of almost 3000 men aged 53–75 years with a mean age of 63 years (the Copenhagen Male Study), factors related to lifestyle and occupation, together with personal clinical or paraclinical factors, were important mediating factors, explaining approximately 70% of the excess risk of ischaemic heart disease (IHD) of the lower social classes (social classes IV and V, i.e. mainly unskilled or semi-skilled workers). The relative risk (RR) of the lower classes suffering a first IHD event during a 6-year follow-up period was reduced from a significant 1.44 to a non-significant 1.12 after inclusion of these factors in a multivariable model [1].

Suggestive of an interplay of socioeconomic status and ABO blood group, a study by Beardmore and Karimi-Booshehri [2] showed that ABO genes were distributed differently in socioeconomic groups in England, a result consistent in both sexes, in natives and migrants and in very different parts of the country.

ABO blood group phenotypes have been associated with the risk of IHD [3–6]. Previous studies have given quite inconsistent results, the majority finding a higher risk in individuals with the A phenotype [3,4], an excess risk at least in part attributed to a somewhat higher serum cholesterol in phenotype A compared to the other phenotypes [4]. In apparent contrast, two British studies [4,5] found that towns dominated by the O phenotype had the highest risk of IHD and, in addition, a higher proportion of the O phenotype was found in a study of a consecutive series of coronary artery bypass candidates. Even the AB phenotype has been suggested as the ‘worst’ with respect to cardiovascular risk [7]. Accordingly, the role of ABO phenotypes remains controversial.
In an attempt to explain the association of socio-economic status with the risk of IHD further, we tested the hypothesis that an uneven socioeconomic distribution of ABO phenotypes could contribute to the explanation.

**Study participants and methods**

The Copenhagen Male Study was set up in 1970 as a prospective cardiovascular cohort study of 5249 men with a mean age of 48 years (range 40–59 years) [9,10]. In 1985–1986 a new baseline was established. All survivors from the 1970 study were traced by means of the Danish Central Population Register. Between June 1985 and June 1986 all survivors (except 34 emigrants) from the original cohort were invited to take part in this study. Three thousand, three hundred and eighty-seven (75%) men agreed and gave informed consent; their mean age was 63 years (range 53–75 years). The 1985–1986 study took place at Glostrup Hospital, University of Copenhagen. Each study participant was interviewed about a previously completed questionnaire and examined clinically; venous blood samples were obtained for determination of their ABO blood groups. Data from the 1985–1986 study were used for the present study except that information about physical activity and blood pressure from the 1970–1971 study was used; we have previously shown that these factors are associated with social class as well as the risk of IHD and have contributed to the explanation of social inequalities in the risk of IHD [1,11].

ABO phenotype determination including reverse typing was performed using a conventional haemagglutination technique in test tubes.

The men were classified into five social classes based on their level of education and job profile [12] according to the system of Svalastoga [13], which was later adjusted. The strata were defined as follows.

1. Class I: self-employed study participants with at least 21 employees and white-collar workers with at least 51 subordinates or study participants with an academic degree.
2. Class II: self-employed administrators with six to 20 subordinates and white-collar workers with 11–50 subordinates or study participants with an intermediate education.
3. Class III: self-employed study participants with one to five employees and white-collar workers with one to 10 subordinates.
4. Class IV: self-employed study participants without employees, white-collar workers without subordinates or without qualified work and skilled blue-collar workers.
5. Class V: unskilled blue-collar workers.

Typical jobs in the study cohort were as follows.

1. Class I: officer, civil engineer, office executive and head of department.
2. Class II: head clerk, engineer and non-academic architect
3. Class III: engine driver and train guard
4. Class IV: machine fitter in a telephone company and station foreman
5. Class V: unskilled labourer, mechanic and driver.

For presentation purposes social class was divided into two groups: low social class (classes IV and V) and high social class (classes I, II and III).

The study participants’ total weekly alcohol consumption was calculated from questionnaire items about their average alcohol consumption on weekdays and at weekends. Intakes of beer, wine and spirits were reported separately and one drink corresponded to 10–12 g ethanol. Overall, there were no social differences in total alcohol consumption, but social class differences in the consumption of wine contributed quite strongly to the explanation of social inequalities in the risk of IHD [1] and were included in the multivariable analyses in this study. The men classified themselves as never smokers, previous smokers or current smokers. Current tobacco smoking was calculated from information about the number of cigarettes, cheroots and cigars or the weight of pipe tobacco smoked daily. One cigarette was taken as equivalent to 1 g of tobacco, one cheroot as 3 g of tobacco and one cigar as 4 g of tobacco. As previously estimated by means of measurements of serum cotinine, the validity of tobacco smoking was high [14]. As regards leisure time physical activity, the men classified themselves as either physically active less than 4 h/week or physically more active. We have previously shown in an analysis of a large number of physical and chemical occupational exposures that exposure to soldering fumes was associated with low socioeconomic status as well as an increased risk of IHD and was quite a strong mediator of the association of low socioeconomic status with increased risk of IHD. Accordingly, we also included this factor in the analyses [1,15]. Information on occupational exposure to soldering fumes was obtained from the questionnaire. Long-term exposure was defined as frequent (several times a week) occupational exposure for at least 5 years.
In 1985–1986, blood pressure was measured in the right arm with the participant seated using a manometer developed by the London School of Hygiene [16]. Hypertension was defined as receiving antihypertensive treatment or having a systolic blood pressure ≥ 150 mmHg and a diastolic blood pressure ≥ 100 mmHg. Self-reported, non-insulin-dependent diabetes mellitus (NIDDM) was accepted, provided the diagnosis had previously been verified by a physician. None of the participants eligible for the prospective study had insulin-dependent diabetes.

Serum selenium levels were determined using a graphite furnace, atomic absorption, spectrophotometric method after a simple dilution with a solution containing nickel and nitric acid [17,18]. The solutions were analysed for selenium at 196 nm with a deuterium background corrector after single injections into the graphite furnace and a standard curve of reference serum was used for the calibration. All samples were analysed in duplicate. The coefficient of variation was 5.5%. Serum cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol were analysed using standardized methods [19]. We have previously shown that a triglyceride level within the range 1.6–2.5 mmol/l was associated with the highest risk in our study [19], so for analytical purposes triglycerides were entered in the analyses as a dichotomous variable and participants classified as being within this range or not. Low-density lipoprotein (LDL) cholesterol was determined according to Fridewald et al.’s [20] formula. Approximately 1.5% of the study population had a triglyceride level of >4.5 mmol/l, a level at which the indirect LDL cholesterol calculation becomes unreliable. However, exclusion of participants with a triglyceride level of >4.5 mmol/l from the study did not affect the results, so no exclusions were made based on triglyceride levels.

Eligibility
We excluded men who had a history of overt cardiovascular disease (acute myocardial infarction, angina pectoris, stroke or intermittent claudication) at baseline from the prospective study. We checked hospital records for all those who reported admission to hospital because of acute myocardial infarction before the start of the study. The diagnosis was accepted if at least two of the following symptoms/signs were recorded: retrosternal pain lasting more than 20 min, typical, serial electrocardiographic changes in more than two electrocardiograms and an acute increase in relevant serum enzymes (alanine aminotransferase, lactate dehydrogenase or creatinine phosphokinase MB). Information on angina pectoris, stroke and intermittent claudication was established from the questionnaire. Three hundred and forty-two men (10.1%) were excluded due to cardiovascular diseases or symptoms and 109 men (3.2%) due to missing data on either of the variables used for selecting participants for the prospective study.

A register follow-up was carried out with data available on morbidity and mortality between 1985–1986 and 31 December 1993. All men who had taken part in the 1985–1986 study were traced by means of the Danish Personal Register. Information on hospital admissions for non-fatal acute myocardial infarction and death certificate diagnoses within the follow-up period were obtained from official national registers and IHD diagnoses were codes 410–414 from the International Classification of Diseases (8th revision). Previous studies have demonstrated a high validity for Danish national registers [19].

Statistical analyses
All basic bivariate analyses, that is χ²-tests for heterogeneity and the multiple logistic regression analyses, were performed using the SPSS statistical software for Windows [21,22]. Odds ratios were estimated by taking natural log e raised to the regression coefficient for the variable of interest in multiple logistic regression models using backward elimination of the variables and the maximum likelihood ratio method [23]. Multiplicative interaction terms between the genetic risk group and relevant IHD risk factors were included in a final analysis in order to test whether the risk associated with identified lifestyle or personal risk factors was modified by genetic factors. The rationale of the method has been described by Kleinbaum et al. [24]. All main effects were adjusted for in the use of a backward elimination procedure. The finding of a significant interaction term had one of two major interpretations: either the strength of the association of the risk factor with the outcome was modified by or dependent on the genetic risk group of the participant or the strength of the genetic risk was modified by or dependent on the presence of the risk factor. The interaction terms consisted of the result of multiplication of the genetic factor (coded 1 for factor present and 0 for factor absent) and the risk factor (also coded 1 for factor present and 0 for factor absent). The interaction term could therefore hold one of two values, that is 1 meaning the presence of both factors or 0 meaning the presence of one of the factors or absence of them both. A priori, a two-sided probability value of P < 0.05 was taken as statistically significant.
Table 1: Distribution of ABO phenotypes and mean age (SD) according to social class

<table>
<thead>
<tr>
<th>ABO phenotype</th>
<th>n</th>
<th>%</th>
<th>Mean age (years)</th>
<th>Social class IV or V</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>1288</td>
<td>43.0</td>
<td>62.8 (5.1)</td>
<td>51.8</td>
</tr>
<tr>
<td>A</td>
<td>1259</td>
<td>42.1</td>
<td>62.8 (5.2)</td>
<td>50.0</td>
</tr>
<tr>
<td>B</td>
<td>326</td>
<td>10.9</td>
<td>62.4 (5.1)</td>
<td>50.3</td>
</tr>
<tr>
<td>AB</td>
<td>120</td>
<td>4.0</td>
<td>63.3 (5.3)</td>
<td>47.5</td>
</tr>
</tbody>
</table>

χ²-test for heterogeneity: P-value = 0.86.

**Ethics**
Each participant was informed that all personal data were confidential and gave written consent to participate. The study was approved by the Ethics Committee for Medical Research in the county of Copenhagen.

**Results**
Table 1 presents the ABO phenotype distribution according to social class; no significant association was found in a χ²-test for heterogeneity. An additional analysis using social class as a five-group variable gave a similar result (not shown).

Table 2 shows the cumulative incidence and relative risk (odds ratio) of a first IHD event for each of the ABO phenotypes according to social class. Low social class was only associated with a significantly increased risk of suffering a first IHD event during the 8-year follow-up for the O phenotype. The overall, age-adjusted odds ratio comparing low social classes with the higher social classes was 1.46 (95% confidence interval 1.1–1.9 and P = 0.006) and that comparing O phenotypes with the other phenotypes was 1.44 (95% confidence interval 1.1–1.9 and P = 0.007).

Table 3 shows which factors were predictive of IHD during the 8-year follow-up among men with the O phenotype and also among the other phenotypes. The two strongest factors were identical for both groups, that is diastolic blood pressure measured in 1971 and the serum triglyceride risk level (1.6–2.5 mmol/l) measured in 1985–1986; the odds ratios associated with these factors were almost identical. Only among O phenotypes were low height (arbitrarily defined as belonging to the shortest fifth of the sample) and low social class significantly associated with the risk of IHD and only among the other phenotypes were age, physical activity level and smoking significantly associated with the risk of IHD after adjustment for confounders.

Table 4 shows the results of a logistic regression analysis comprising all participants in the prospective study and including interaction terms for the factors which were significant predictors for O phenotypes but not the other phenotypes and factors which were significant predictors for the other phenotypes but not for O phenotypes. Two of these were significant: the interaction of social class (classes I, II and III coded as 0 and classes IV and V coded as 1) with ABO phenotypes (coded as O phenotypes = 1 and other phenotypes = 0) and the interaction of low height (lowest quintile, i.e. ≤169 cm coded as 1 and >169 cm coded as 0) with ABO phenotypes (coded as O phenotypes = 1 and other phenotypes = 0). At the time of removal from the backward elimination model the odds ratio of the main effect of social class was 0.87 (95% confidence interval 0.6–1.3 and P = 0.51).

**An additional analysis**
Men in low social classes (IV/V) with low height (≤169 cm, i.e. the shortest fifth) had the highest risk of IHD if they were also of O phenotype. An analysis of
Table 3 Relative strength of association with 8-year risk of IHD according to ABO phenotype

<table>
<thead>
<tr>
<th>Blood type</th>
<th>Odds ratio</th>
<th>95% confidence intervals</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>O phenotype ( (n = 1288)^a )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure in 1971 (risk associated with a 10 mmHg increase)</td>
<td>1.3</td>
<td>1.1-1.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides ( (1.6-2.5 \text{mmol/l versus rest}) )</td>
<td>1.9</td>
<td>1.2-2.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Low height ( (&lt; 169 \text{cm versus taller}) )</td>
<td>1.7</td>
<td>1.1-2.7</td>
<td>0.010</td>
</tr>
<tr>
<td>Wine intake versus no wine intake</td>
<td>0.6</td>
<td>0.4-0.9</td>
<td>0.010</td>
</tr>
<tr>
<td>Low social class ( (IV/VI) versus high social class ( I, II ) and ( III ) )</td>
<td>1.7</td>
<td>1.1-2.6</td>
<td>0.020</td>
</tr>
<tr>
<td>A, B and AB phenotypes ( (n = 1705)^b )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure in 1971 (risk associated with a 10 mmHg increase)</td>
<td>1.3</td>
<td>1.1-1.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides ( (1.6-2.5 \text{mmol/l versus rest}) )</td>
<td>1.9</td>
<td>1.2-2.9</td>
<td>&lt; 0.010</td>
</tr>
<tr>
<td>Low physical activity ( 1971 ) versus higher ( 1971 )</td>
<td>2.0</td>
<td>1.2-3.4</td>
<td>0.010</td>
</tr>
<tr>
<td>Physical activity ( \text{Age in years (5-year age groups in ascending order)} )</td>
<td>1.3</td>
<td>1.1-1.5</td>
<td>&lt; 0.010</td>
</tr>
<tr>
<td>Wine intake versus no wine intake</td>
<td>0.6</td>
<td>0.4-0.97</td>
<td>0.040</td>
</tr>
<tr>
<td>Smoking versus non-smoking</td>
<td>1.6</td>
<td>1.01-2.4</td>
<td>0.040</td>
</tr>
</tbody>
</table>

\(^a\) Not in the final model, \( P > 0.05 \): long-term soldering exposure, NIDDM, never smoking, physical activity in 1971, physical activity, hypertension, weight, HDL cholesterol, LDL cholesterol, systolic blood pressure, smoking, diastolic blood pressure, systolic blood pressure in 1971, selenium level and age.

\(^b\) Not in the final model, \( P > 0.05 \): LDL cholesterol, HDL cholesterol, selenium level, NIDDM, weight, systolic blood pressure in 1971, diastolic blood pressure, physical activity, hypertension, social class, systolic blood pressure, long-term soderting exposure, height and never smoking.

The variables are ordered according to the strength of their association with IHD after multivariable adjustment in a logistic regression model.

The distribution of risk factors in this subgroup showed that O phenotypes differed from non-O phenotypes with respect to only one factor: triglyceride level. Among O phenotypes, 29% belonged to the triglycerides' high-risk range \( (1.6-2.5 \text{mmol/l}) \) as compared with 20% among non-O phenotypes \( (P = 0.05) \).

**Discussion**

In accordance with the homogenous distribution of ABO phenotypes in the low and high social classes, ABO phenotypes did not contribute directly to the explanation of social inequalities in IHD risk. However, the association of low socioeconomic status with the risk of IHD was highly dependent on ABO phenotype, only men with phenotype O were at increased risk of IHD if they belonged to the lower social classes. ABO phenotypes thus appear to be effect modifiers for the association of socioeconomic status with IHD risk.

**Selection bias**

Could the findings have been the result of selection bias? The distribution of ABO phenotypes in the study did not deviate from previous Danish studies carried out on blood bank and medicolegal specimens [25]. This was the case even when participants excluded...
from the prospective study were included (not shown). Considering the excess risk of IHD found for men with the O phenotype, why was the proportion of men with the O phenotype not relatively smaller than expected in this cohort of relatively old men? In an attempt to estimate the degree of selection likely to have been caused by an excess risk of IHD among men with the O phenotype who were either in low social classes or had a low height we looked at mortality data from the initial baseline in 1970–1971 up to 1985. Some 695 had died from all causes, 217 due to IHD (ICD-8 410–412) corresponding to 4.1% of the initial cohort; 156 of those who had died due to IHD either belonged to lower social classes IV or V or the fifth of the sample with the lowest height. Assuming a proportion of O phenotypes of 40% and a relative risk of 1.8 for O phenotypes compared to the other phenotypes among these men (the relative risk found for this group using the 1985–1986 baseline; not shown), 49 of the 156 fatal IHD events could be ascribed to the O phenotype, that is less than 1% of the initial cohort might have been selected due to the O phenotype interplay with social class and height. Accordingly, genetic selection with respect to ABO phenotypes seems an unlikely explanation for our findings.

Low height, ABO phenotypes and risk of ischaemic heart disease

Low height was a quite strong risk factor among men with the O phenotype, with a significant excess risk of 70% found in men shorter than 170 cm. Height and social class were inversely correlated with the lower classes comprising a much larger proportion of men of short stature than the higher classes. Low social class using the method applied here based on education and job profile [12,13] and low height are both proxies of being socioeconomically relatively underprivileged. It is therefore interesting that significant independent interactions existed between social class and ABO phenotypes (O phenotypes versus other phenotypes) as well as low height and ABO phenotypes (O phenotypes versus other phenotypes).

Biological mechanisms

The biological rationale of our findings is uncertain. Nevertheless, coagulation defects have been repeatedly associated with cardiovascular risk [26] and several studies have found an association between coagulation factors and ABO blood group [27,28]; even a significant interaction of factor VIII activity, age and ABO phenotypes has been reported [29]. It has also been suggested that ABO phenotypes differ with respect to serum lipids [30] and even height. In a study by the Meade group [7], AB men were of shorter stature (on average 2 cm) than the other groups. Overall, we found no differences in the Copenhagen Male Study between the O phenotypes and the other phenotypes with respect to the cardiovascular risk factors under study, including height. However, in agreement with previous findings total serum cholesterol was slightly higher in A phenotypes (0.1 mmol/L) as compared with all other phenotypes (not shown); it was relevant in the context of this study that, among men of short stature in low social class, ABO blood group separated men with the O phenotype from the other phenotypes with respect to triglyceride level. This result indicated an underlying mechanism associated with lipid or carbohydrate metabolism or differences in dietary habits between men with phenotype O and the other phenotypes.

Interactions of relevance

In summary, there were two interesting new findings suggesting a gene × environment interaction.

1. A significant interplay of socioeconomic status, ABO phenotypes and risk of IHD was found, since only among men with the O phenotype was low social class associated with an increased risk of IHD.
2. The strength of the association of low height with the risk of IHD depended on ABO phenotype.

Conclusion

ABO phenotypes did not contribute directly to the explanation of socioeconomic inequalities in IHD risk. However, the finding of ABO phenotypes being effect modifiers for the association of socioeconomic status with the risk of IHD may open up new possibilities for clarifying the roles of socioeconomic status and ABO blood group as cardiovascular risk factors.

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