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The role of the apolipoprotein E polymorphism in the prediction of coronary artery disease age of onset

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We investigated the role of the apolipoprotein (Apo) E polymorphism in the prediction of CAD age of onset in a sample of unrelated living male (n = 65) and female (n = 54) Caucasian subjects diagnosed with CAD. Cumulative distributions of age at the first diagnosis of CAD were estimated for each *Apo E* genotype and tested for homogeneity using the log-rank test. The *Apo* ε33 genotype was used as a reference group for all hypothesis tests. Analyses were performed separately in males and females. We found evidence suggesting that the presence of the *Apo* ε32 genotype in males is associated with a significantly earlier CAD age of onset. These results suggest that the *Apo E* polymorphism may be a gender-specific predictor of CAD age of onset.

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A central question of genetic studies of coronary artery disease (CAD) is whether knowledge about variation in the genome will be useful for the prediction of disease onset, rate of progression and severity (Sing et al. 1994). Traditional epidemiological studies of CAD have focused on identifying intermediate biochemical and physiological traits as well as environmental factors that may contribute to the prediction of presence of disease (Kannel & Gordon 1970). Measured genotype information has the potential to contribute to our ability to predict CAD onset, rate of progression and severity because genotypes are typically not altered by the disease process or time, may represent information about a biochemical trait that cannot be measured, and may be easier and less expensive to obtain than biochemical and physiological information (Sing & Moll 1990b).

Coronary artery disease is the result of progressive thickening of the arterial wall, due in part to lipid infiltration and smooth muscle cell proliferation, presumably in response to injury to the endothelium. Of all the established risk factors for CAD, aging, as an index of changes in metabolism and the environment, is the strongest predictor of atherosclerotic plaque initiation and progression. Evidence that changes in lipid metabolism are indexed by aging (Hazzard 1987, Kottke et al. 1991, Kritchevsky 1980, Sing et al. 1979) suggests that this biochemical system may influence the onset of CAD.

Genes that code for the important apolipoprotein components of plasma lipoproteins are considered to be candidates for determining interindividual differences in the risk of CAD (Sing & Moll 1990a). Of particular interest is the gene coding for apolipoprotein (Apo) E. The structure, function and metabolism of Apo E have been well characterized (Davignon 1993, Mahley 1988). Cross-sectional and longitudinal studies have demonstrated an association between allelic variation in the gene coding for Apo E and variation in risk of CAD (reviewed by Davignon 1993, Stengård et al. 1995). These studies provide evidence suggesting that the *Apo E* gene may be a prototypical CAD susceptibility gene.

In the present study, we investigated the role of the common *Apo E* genotypes in the prediction of CAD age of onset in living male and female subjects diagnosed with CAD. The cumulative distribution of age at first diagnosis of CAD was estimated for each *Apo E* genotype in males and females separately. Homogeneity among genotypes was tested using the log-rank test. We found evidence suggesting the *Apo* ε32 genotype is associated with a significantly earlier CAD age of onset than the *Apo* ε33 genotype in males but not in females.

Material and methods

Turner et al. (1989) provided details of the strategy used in the Rochester Family Heart Study (RFHS) to sample 276 multigenerational pedigrees representative of the Caucasian population of Rochester, Minnesota, USA. A sample of 119 unrelated, living subjects diagnosed with CAD from these pedigrees were studied. This sample consisted of eight ε32 females, seven ε32 males, 37 ε33 females, 34 ε33 males, seven ε34 females, 21 ε34 males, two ε44 females, and three ε44 males. Subjects with the ε44 genotype were combined with the ε34 genotype for the statistical analyses reported here. Inferences were not altered when the ε44 genotype was removed from the analyses.

All blood samples were collected in EDTA by venipuncture. Apo E isoforms were determined from frozen plasma samples by isoelectric focusing as described by Kamboh et al. (1988). *Apo E* genotypes were inferred from the Apo E isoform phenotypes. Each subject had a physical exam and a medical record evaluation at the time of enrollment into the RFHS. A clinical diagnosis of CAD was made if medical records indicated coronary surgery, myocardial infarction, definite angina, CAD, ischemic heart disease, or abnormal results from coronary angiography, an exercise multi-gated acquisition scanning, a treadmill exercise test, or an electrocardiogram. Age of onset of CAD was determined from the age at first diagnosis of CAD.

The goal of our statistical analysis was to determine whether CAD age of onset distributions were homogeneous across the common *Apo E* genotypes. The most common *Apo* ε33 genotype was used as the reference group for contrasts with the ε32 and ε34 + ε44 genotypes. All analyses were performed separately in males and females. The cumulative distribution of age at first diagnosis of CAD was estimated for each *Apo E* genotype. Homogeneity of the cumulative distributions across genotype strata was tested using the log-rank test (Kalbfleisch & Prentice 1980). The log-rank test statistic is asymptotically distributed as a Chi-square distribution with degrees of freedom equal to the number of strata tested minus one. The null hypothesis of no difference between cumulative distributions was rejected when $p \leq 0.05$.

Results

In both females and males, there were no statistically significant differences in mean age at the time of enrollment into the RFHS (ε33 females: 71.4 ± 8.6, ε32 females: 75.6 ± 4.5, ε34 + ε44 females: 71.1 ± 10.4, ε33 males: 69.9 ± 8.8, ε32 males: 62.6 ± 14.6, ε34 + ε44 males: 67.5 ± 11.0). In a pairwise comparison with the *Apo* ε33 genotype, no statistically significant difference in mean age of onset of CAD was detected between ε33 females (65.4 ± 10.3 years) and ε32 females (70.4 ± 7.3 years) or ε34 + ε44 females (67.7 ± 10.1 years). No statistically significant difference in mean age of onset was detected between ε34 + ε44 males (59.5 ± 11.2 years) and ε33 males (61.7 ± 10.9). However, there was a statistically significant difference in mean age of onset between ε32 males (52.1 ± 14.0 years) and ε33 males (61.7 ± 10.9 years).

The proportion of subjects with a first diagnosis of CAD by a particular age is summarized in Fig. 1 for each of the *Apo E* genotype and gender strata. The log-rank test of homogeneity indicates that male subjects with the *Apo* ε32 genotype had a statistically significant different cumulative distribution than subjects with the ε33 genotype (Fig. 1A). This observation is consistent with the statistically significant difference in the mean age of onset between these two groups. No other contrast between subjects with the ε32 or ε34 + ε44 genotypes and subjects with the ε33 genotype (Fig. 1B–1D) was significant at the 0.05 level of probability in either gender.

Discussion

Evidence from studies of coronary arteries in children and young adults suggest that atherosclerotic plaques begin to develop in children 10–15 years of age in approximately half of the population (Stary 1987). An important public health problem is to identify risk factors that predict an early CAD age of onset, with the ultimate goal of improving disease prevention. A fundamental question facing geneticists is whether information about the genome will be useful for predicting the onset, progression and severity of common chronic diseases like CAD (Sing et al. 1994). The results presented in this study suggest that variation in the *Apo E* gene may play a gender-specific role in the prediction of CAD age of onset. Because several of the gender and genotype strata had small sample sizes, this conclusion must remain a working hypothesis to be confirmed or refuted in subsequent studies.

Our finding that the *Apo* ε32 genotype is predictive of an earlier age of onset in males is consistent with two earlier reports of an association between the ε2 allele and risk of CAD (de Andrade et al. 1995, Eto et al. 1989). This finding is, however, inconsistent with the general conclusion that has been drawn from previous studies investigating the association between *Apo E* genotype and risk of CAD (reviewed by Davignon 1993). In most studies, the frequency of the ε4 allele was significantly higher in patients with confirmed CAD than in

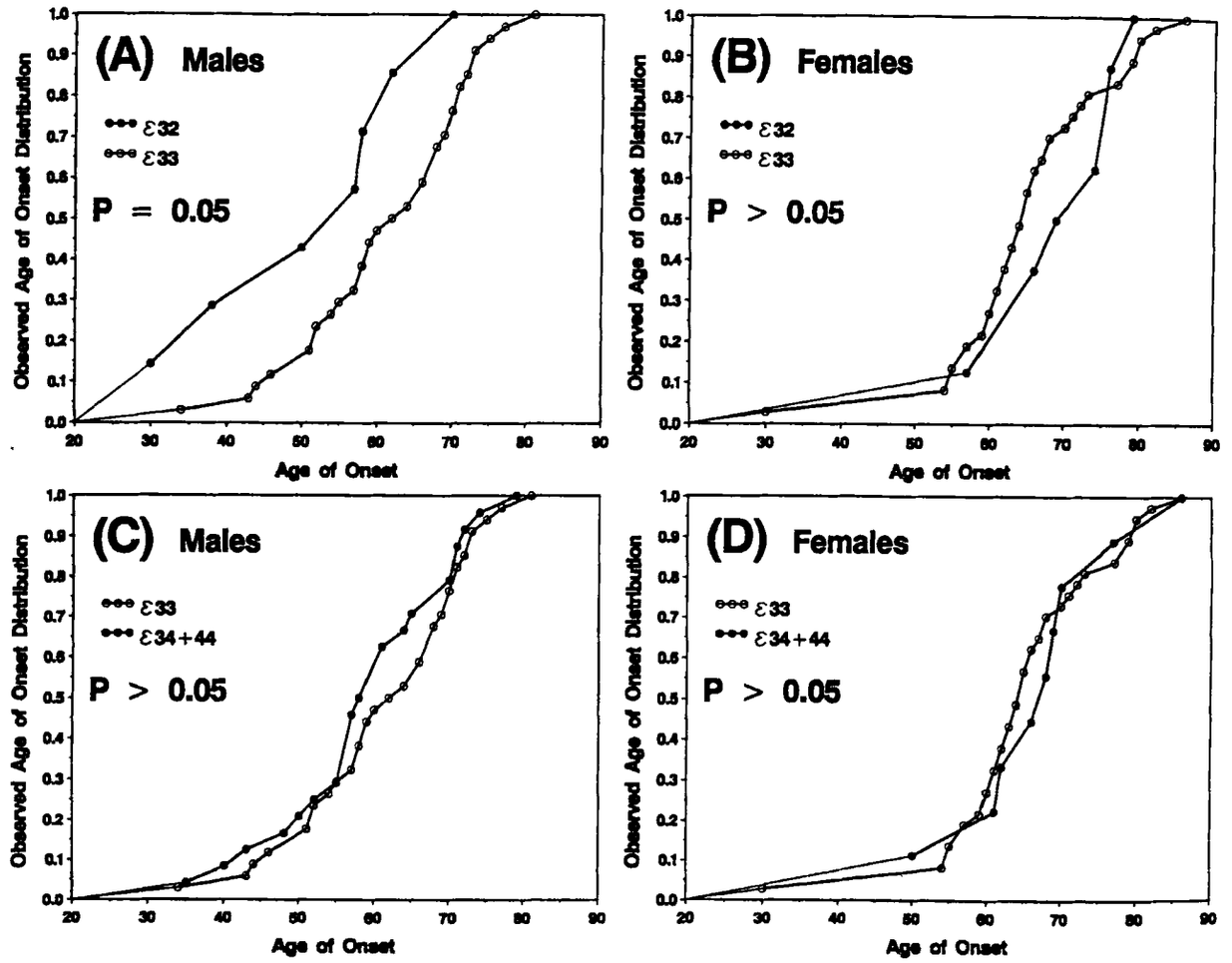

Fig. 1. Cumulative distributions summarizing the proportion of subjects with onset of CAD by a particular age. A, Distributions for ε32 and ε33 males. B, Distributions for ε32 and ε33 females. C, Distributions for ε33 and ε34+ε44 males. D, Distributions for ε33 and ε34+ε44 females.

healthy controls or in random population samples. A recent prospective study following two cohorts of elderly men concluded that the ε4 allele was a significant predictor of future CAD mortality in two Finnish populations (Stengård et al. 1995). Also, the ε4 allele has been associated with an earlier age of myocardial infarction (Cumming & Robertson 1984, Lenzen et al. 1986). Differences in disease classification, sampling design, the population of inference and/or the small sample size of the present study may explain inconsistent findings. It is possible that effects of one or more of the genotypes of the many susceptibility genes which influence CAD age of onset interact with Apo E effects in such a way that particular *Apo E* genotypes appear to have different marginal effects on risk of CAD in different contexts.

What role might the *Apo* ε2 allele play in susceptibility to an earlier age of onset of CAD? There is accumulating evidence suggesting that the *Apo* ε2 allele has atherogenic potential. The *Apo* ε2 allele may be associated with increased levels of triglyceride-rich lipoproteins which are believed to play a role in the development of atherosclerosis (Davignon 1993). A study by Reilly et al. (1991) in the same Rochester, MN population observed higher mean levels of triglycerides in ε32 males than ε33 males. Thus, the association between the *Apo* ε32 genotype and an earlier age of onset of CAD in males from the present study could be partly due to the influence of the ε2 allele on plasma triglyceride levels. This hypothesis is supported in part by a study of 5919 middle-aged men that found plasma triglyceride levels were a significant predictor of early onset CAD (Benfante et al. 1989). Studies of the relationship between plasma triglyceride levels and age of onset in different *Apo E* genotypes are needed to fully investigate this hypothesis.

The general utility of genomic information for

predicting common chronic disease onset, progression and severity is yet to be evaluated for the large number of candidate susceptibility genes that have been described. Gene by gene and gene by environment interactions are expected to influence how genomic information might improve prediction. The gender- and genotype-specific findings in this study add support to the hypothesis that the predictive value of a fraction of the genome type information is context dependent (Sing et al. 1996).

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