Hum Genet (2000) 107 : 466–475
DOI 10.1007/s004390000394

ORIGINAL INVESTIGATION

Kim E. Zerba · Robert E. Ferrell · Charles F. Sing

Complex adaptive systems and human health: the influence of common genotypes of the *apolipoprotein E* (*ApoE*) gene polymorphism and age on the relational order within a field of lipid metabolism traits

Received: 14 July 1999 / Accepted: 22 August 2000 / Published online: 13 October 2000

Abstract We analyzed the influence of age, *apolipoprotein E* (*ApoE*) genotype, and their interaction on the variation of each of all possible pairwise correlations among plasma levels of ApoE, ApoB, total cholesterol, triglyceride, and HDL cholesterol. Our cross-sectional study sample included 1,876 individuals (979 females and 897 males) from the Rochester, MN population, unselected for health, with a common *ApoE* genotype of ε_{32}, ε_{33}, or ε_{43}, and ranging in age from 5 to 90 years. We conducted analyses on data from female and male subjects separately, using a hierarchical set of generalized additive models. The age changes in the correlations were estimated using a 30-year sliding window across the age range. There were qualitative differences between genders in the age at which the peaks in the correlations occurred. For female subjects, peaks in correlations were mostly in the middle and older age windows, whereas in males, peaks were mostly in the younger and middle age windows. We found for both genders that for each of the possible pairwise correlations, the influence of age was significantly dependent on *ApoE* genotype (all $Pr<0.0001$). We also found for female and male subjects that the ε_{32}- and ε_{43}- specific age changes in the correlations were each significantly different from those for the ε_{33} genotype ($Pr<0.0001$), with two exceptions for males (marginally significant differences, $P<0.08$). We conclude that the influence of *ApoE* genotypic variation extends far beyond the levels of the gene product, to the dynamics of the relational order among measures of lipid metabolism with age. Moreover, age and common *ApoE* genotype are not independent predictors of the gender-specific changes in relational order that we observed among these measures of lipid metabolism. These results have implications for the development and application of therapeutic approaches to treat human disease and our enhanced understanding of the role of genetic variation in the dynamic actions of complex adaptive systems with age that occur in response to environmental change. These dynamic actions emerge as the phenotypes that are measures of human health in the population at large.

K. E. Zerba (✉)
Bristol-Myers Squibb, Pharmaceutical Research Institute,
Non-clinical Biostatistics, PO Box 5400,
HW19-2.01, Princeton, NJ 08543-5400, USA
e-mail: kim.zerba@bms.com,
Tel.: +1-609-8186647, Fax: +1-609-8186060

K. E. Zerba · C. F. Sing
Department of Human Genetics, University of Michigan,
Ann Arbor, Michigan, USA

R. E. Ferrell
Department of Human Genetics, University of Pittsburgh,
Pittsburgh, Pennsylvania, USA

Introduction

Genetics, molecular biology and biochemistry are revealing an unrivaled degree of intricate internal biological detail that is best described as complex. Genes are central to most research programs to simplify this biological complexity. Current research on human diseases focuses on characterizing those genetic variations that predict risk of disease and those that may reveal potential avenues of therapy. Most of these efforts assume a mechanical, reductionist paradigm for modeling causation (Rosen 1991; Salthe 1993), and that genetic variation is the root, transeunt cause (Emmet 1992) of deviations of individuals from health. The central genetic dogma of the 20th century assumed that genetic variations result in variations in gene products which, in turn, are functionally responsible for interindividual variations in health.

More than 98% of the human disease burden is represented by the diseases that have a complex, multi-factorial etiology and cannot be explained by independent variations in single genes (McKeown 1979; Strohman 1993). These diseases illustrate that variations in DNA sequences, by themselves, cannot be considered to be direct causes (Goodwin 1994a; Lewontin 1992a; Rose 1997). Protein production is a non-linear, dynamic process that requires the multi-way feedback interactions of DNA, proteins and cellular organelles influenced by the context of the cell and

tissue type, the organ system (Cohen and Rice 1996), and the external environmental factors defined by the individual (Holdredge 1996; Levins and Lewontin 1985; Lewontin 1996; Maturana and Varela 1980; Maturana and Varela 1992; Zerba and Sing 1993). Causation is a consequence of the non-linear, dynamic interactions between genes, proteins, cellular organelles, cells, tissues, and organ systems as well as of the interactions between each of these classes of agents. Both sets of interactions are also influenced by the exposures to external environments specific to the individual that are indexed by time and ecological space.

This alternative view of reality assumes that the individual is the fundamental unit of organization of interacting agents from which health emerges as a dynamic property during the life cycle. The individual is thus the immanent cause and effect of itself through its actions (Emmet 1992; Goodwin 1994b; Levins and Lewontin 1985; Lewontin 1996) in the context of its ontogenetic history. The genome encompasses only one of many classes of agents involved in the functioning of the complex adaptive system (Gell-Mann 1994; Salthe 1993; Sing et al. 1996; Sing and Reilly 1993) represented by the individual. These agents participate in an autopoietic, self-organizing, dissipative, and cognitive network represented by the dynamic pattern of the set of relationships among metabolic processes that produce many of the agents (Capra 1996; Maturana and Varela 1980; Maturana and Varela 1992; Yates 1993). The network is organized hierarchically and heterarchically (Yates 1993) into fields of agents defined by domains of relational order in which the state of any agent at a particular time is a defined function of the states of neighboring agents (Goodwin 1994b). An example of a field in human physiology is lipid metabolism, which defines a domain of relational order within and among the genes, gene products, dietary and stored lipids, organs and tissues involved in lipid metabolism. The network is organizationally closed, to define and maintain coherence of the individual, but thermodynamically open to the flow of energy needed to sustain the metabolic needs of the individual through time (Capra 1996).

Variations in genes involved in lipid metabolism may contribute to interindividual variation in the interactions within and among the fields of agents that are involved in determining health. Molecular composition in general, however, provides no information about the principles by which the emergent dynamic processes of life are organized during ontogeny (Goodwin 1989). The unique genome type and other inherited particulars at the time of conception are limited to providing the initial conditions for the complex adaptive system that is each individual (Zerba and Sing 1993) and his/her context-dependent autopoietic capacities (Goodwin 1994a). This multitude of context-dependent possibilities for each individual is often defined as the norm of reaction (Lewontin 1992b).

Traditional views of health have considered physiological processes as being inherently stable (homeostatic) and predictable, such as heart rate (Goldberger 1992). This view suggests that disease results from a loss of inherent stability. Life processes, however, are inherently changing and precise predictability of states of biological systems in time is impossible (Prigogine 1989; Prigogine and Stengers 1984). Recognition of these two biological facts has led to the alternative view that healthy physiological systems are inherently homeodynamic to maintain coherence of a functioning system, but richly patterned in time so that individuals can adaptively function in inherently unstable and unpredictable environments (Glass and Mackey 1988; Goodwin 1997; Kauffman 1993; Yates 1993).

Recognition that the causes of health cannot generally be reduced to genes and that health may be a derivative of biological stability suggests a broadened scope of inquiry that may provide a richer understanding of the dynamic properties of the individual, including health, that emerge during the life cycle. The role of genetics in this broadened understanding shifts the focus of study to the influence of genetic variation on the relational order within and among the fields of agents of individual complex adaptive systems (Goodwin 1994a; Reilly et al. 1994). This relational order may also be influenced by age because of the dynamics of variation in internal conditions and external spatial contexts of individuals that occur during ontogeny (Zerba et al. 1996; Zerba and Sing 1993).

In this paper, we report on the influence of the three common genotypes of the gene (*ApoE*) that codes for apolipoprotein E (ApoE) and age on the relational order within a field of five measures of lipid metabolism (ApoE, ApoB, total cholesterol, triglyceride and HDL cholesterol) in a sample of 1,876 individuals (979 females and 897 males) unselected for health status with *ApoE* genotype ε_{32}, ε_{33} or ε_{43}, from the Rochester, MN population. The structural gene for ApoE is on chromosome 19 and is polymorphic with three common alleles in most populations (ε_2, ε_3, ε_4) that combine to produce six genotypes (Davignon et al. 1988). The three genotypes used in this study are the most common *ApoE* genotypes in most populations. The *ApoE* polymorphism is one of the few examples where there is evidence of variability in the functional characteristics of the gene product among common allelic variations (Rall et al. 1982). Many studies have established that allelic variation in the gene coding for ApoE makes an important contribution to the prediction of interindividual phenotypic differences in traits that are measures of agents involved in lipid metabolism (Davignon et al. 1988; Sing et al. 1985; Sing et al. 1992). On average, the less frequent *ApoE* ε_2 allele is associated with lower and the ε_4 allele with elevated plasma LDL-cholesterol levels compared with the most common ε_3 allele (Davignon et al. 1988; Sing and Davignon 1985; Xhignesse et al. 1991). LDL-cholesterol is among the most atherogenic of the lipoproteins and the *ApoE* polymorphism has been associated with the initiation and progression of CAD particularly for individuals carrying the ε_4 allele (Hixson and Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group 1991; Stengård et al. 1995).

Most human studies have been presented as if the *ApoE* genotype and allelic effects on agents of lipid metabolism were independent of the effects of age (Zerba et

al. 1996). Plasma apolipoprotein and lipid levels, however, change with age in the population at large (Kottke et al. 1991). Moreover, the relationships between *ApoE* genotypic and allelic variation and plasma levels of the *ApoE* gene product are dynamic with age (Reilly et al. 1990; Zerba et al. 1996). In addition, variation among common genotypes of the *ApoE* polymorphism has a significant influence on the relationships among measures of lipid metabolism, including ApoE, in the population at large (Reilly et al. 1994). The potential, however, for the influence of *ApoE* genotypic variation on the interrelationships of plasma ApoE with other agents involved in lipid metabolism to change with age, is unexplored.

As simple measures of relational order, we estimated the Spearman's rank correlations among plasma levels of ApoE, ApoB, total cholesterol, triglyceride, and HDL cholesterol for each of the three common *ApoE* genotypes in a sample of individuals ranging in age from 5 to 90 years from the Rochester, MN population. We found for both genders that for each of the possible pairwise correlations, the influence of age on the correlations was significantly dependent on the common *ApoE* genotypes. We also found for both genders that the ε_{32}- and ε_{43}- specific age changes in the correlations were significantly different from those for the ε_{33} genotype, except for two comparisons among these genotypes in the male subjects. These results have important implications for the development and application of therapeutic approaches to the treatment of human disease and our enhanced understanding of the role of genes in the dynamic actions of the complex adaptive systems that emerge as the phenotypes of human health.

Methods

Sample

Our study included 1,876 individuals, 979 female and 897 male, with a common *ApoE* genotype, measures of plasma ApoE, ApoB, total cholesterol, triglyceride, and HDL cholesterol level and age recorded at the time of sample collection. The sample sizes for the *ApoE* genotypes were 113 and 121 for ε_{32}, 616 and 535 for ε_{33}, and 250 and 241 for ε_{43}, for female and male subjects, respectively. These individuals were sampled from three- and four- generation pedigrees ascertained through elementary school children of the Rochester, Minn. population as part of the Rochester Family Heart Study (RFHS: described by Moll et al. 1989; Turner et al. 1989). These pedigrees are representative of multi-generation pedigrees from the Rochester, Minn. population at large.

Laboratory Measures

Blood samples were collected by venipuncture and put into EDTA. *ApoE* isoforms were determined by isoelectric focusing using the method described by Kamboh et al. (1988). Since the isoforms correspond directly to differences at amino acid residue sites 112 and 158 (Rall et al. 1982), we used the isoform typings to infer the *ApoE* genotype for each individual. Plasma ApoE and ApoB levels were measured by radioimmunoassay (Kottke et al. 1991). Levels of total plasma cholesterol and triglyceride were measured by standard enzymatic methods. HDL cholesterol level was measured according to the procedure of Izzo et al. (1981).

Statistical analyses

Plasma apolipoprotein and lipid levels were adjusted for laboratory assay date by linear regression (Kaprio et al. 1991) and the grand mean added back to the residuals. All subsequent analyses were conducted on female and male subjects separately, a necessity that has been established by the work of Reilly at al. (1991, 1992, 1994).

Pearson product-moment correlations are sensitive to non-normal bivariate distributions. We tested each trait for deviations from univariate normality using the sample size transformed Shapiro-Wilk statistic (Royston 1982; Shapiro and Wilk 1965), W, and the transformation of W. We found significant deviations from normality in all cases (Table 1). Consequently, we used the non-parametric Spearman's rank correlation, ρ (Sokal and Rohlf 1981), as the measure of the relationships among the plasma apolipoprotein and lipid levels.

We used a 30 year fixed-span sliding window to subsample the age range (Zerba et al. 1996) and obtain an estimate of the dynamics of relational order among the traits with age. The midpoints of adjacent windows differed by 1 year. All windows were symmetric about window midpoints such that midpoints of the windows at the ends of the age distribution were 15 years from the actual limits of the age distribution. For each age window and for each *ApoE* genotype, we estimated all possible Spearman's ρ between pairs of traits and total correlations. Total correlation was estimated as the sum of the absolute value of all of the correlations among trait pairs for a sample (Reilly et al. 1994). Significance levels were set at α=0.05 for all tests of statistical significance.

The *ApoE* genotype-specific plots of the correlations with age were non-linear. Therefore, a series of non-parametric hierarchical generalized additive models (Hastie and Tibshirani 1986; Hastie and Tibshirani 1990) was used to examine the influence of age, *ApoE* genotype and their interaction on the correlations. The full model included the correlation in each age window as the depen-

Table 1 Descriptive statistics

Gender	Trait	$\bar{Y}$	s	$\hat{g}_1$	$\hat{g}_2$	Normality test (W)
Female (*n*=979)	ApoE	5.20	2.49	2.5317	16.42	0.8676***
	ApoB	75.88	16.24	1.2297	3.06	0.9317***
	Total cholesterol	180.39	42.99	0.9649	1.79	0.9507***
	Triglyceride	114.96	88.99	10.5559	198.28	0.5618***
	HDL cholesterol	50.02	12.48	0.6934	0.95	0.9670***
Male (*n*=897)	ApoE	5.07	2.95	4.9712	46.80	0.7159***
	ApoB	75.98	15.80	0.7120	0.49	0.9542***
	Total cholesterol	177.07	43.72	1.1454	4.57	0.9505***
	Triglyceride	133.71	194.86	20.4609	509.15	0.2725***
	HDL cholesterol	43.36	11.28	0.9544	1.94	0.9512***

***Pr<0.001

Table 2 Spearman's rank correlations between measures of lipid metabolism for female subjects (above diagonal, n=979) and male subjects (below diagonal, n=879)

	ApoE	ApoB	Cholesterol	Triglyceride	HDL cholesterol
ApoE	–	0.35***	0.45***	0.35***	0.11***
ApoB	0.41***	–	0.71***	0.53***	–0.11**
Cholesterol	0.45***	0.78***	–	0.53***	0.20***
Triglyceride	0.39***	0.54***	0.54***	–	–0.36***
HDL cholesterol	–0.06[a]	–0.20***	–0.01[a]	–0.53***	–

0.01>Pr≥0.001; *Pr<0.001
[a]Not significant: Pr≥0.05

dent variable with *ApoE* genotype and *ApoE* genotype-specific non-parametric loess regressions (Cleveland and Devlin 1988; Hastie and Tibshirani 1990) on age window midpoint as the independent variables. The sliding window samples were not independent, however, the theory does not exist for the incorporation of such dependence among samples in generalized additive models.

In loess regression the data are used to specify the form of the model by fitting a curve to the data points locally so that at any point the curve at that point depends only on the observations at that point and a specified span of neighboring points. The loess regression estimates result in a smoothing of the observed variability in *ApoE* genotype-specific correlations across age. The degree of smoothing is dependent on the proportion of the total of data points (span) used in the localized smoothing estimations.

The span we used for the loess regressions was based on the observed data sample rather than an automatic span selection algorithm. The sliding window algorithm described above, used to subsample the age range and estimate the correlations in each age window, is a type of data span selection algorithm similar to that used in a loess regression. This sliding window procedure is a fixed span on age, but a variable span on the number of individuals in each age window. We estimated the proportion of the total number of individuals in each gender sample for each age window. We then estimated the average proportion over all age windows for each gender separately. The average proportion for female subjects was 0.349 and that for male was 0.340. These proportions were the spans used for each gender in the loess regressions. The choice of span for the generalized additive model analyses, therefore, represents about the same span, on average, of the sliding window subsampling done on the original data.

Using dummy variables, we tested (1) for significant interactions between age and *ApoE* genotype on the variability in the correlations and (2) the equality of the loess regressions on age for each of the ε_{32} and ε_{43} genotypes with that of the ε_{33} genotype by fitting reduced models without the appropriate interaction terms and comparing the sums of squares of the full with the respective reduced models in the context of the appropriate degrees of freedom for each model (Hastie and Tibshirani 1990). The degrees of freedom for the loess regression parameter estimates were determined as described by Hastie and Tibshirani (1990). The interactions between age and *ApoE* genotype were statistically significant for every correlation, (with two exceptions that were marginally significant (P<0.08); see below), so we did not explore the nature of the separate influence of age and *ApoE* genotype on the correlations.

We estimated the 95% pointwise confidence bands about the predicted correlations for each full generalized additive model (Hastie and Tibshirani 1990). These confidence bands were estimated as pointwise rather than global simultaneous confidence bands since the latter for generalized additive models may be too conservative. Hastie and Tibshirani (1990) showed that under random resampling of data, most of the resulting estimated functional forms of the models were contained in the pointwise confidence bands of the original model. We also computed 1,000 bootstrap (Efron and Tibshirani 1993) estimates of the predicted loess regression relationships of the genotype-specific correlations with age by resampling the residuals with replacement from the full generalized additive model. For each bootstrap, these residuals were then added to the original predicted correlations to provide a new bootstrapped set of observed correlations. For each of these bootstrapped sets of observed correlations we then estimated the predicted genotype-specific loess regression lines.

Results

The laboratory assay date influence was small. R^2 x100 values for the linear date of assay adjustments were all less than 0.7. Descriptive statistics of the laboratory assay date adjusted plasma apolipoprotein and lipid values for female and male subjects are presented in Table 1. All distributions were positively skewed and kurtotic. Triglyceride was extremely skewed and kurtotic. We observed significant deviations from univariate normality in all cases (Pr<0.001).

The correlations between traits for each gender sample are presented in Table 2. All correlations were significantly different from zero except that for total cholesterol with ApoE and HDL cholesterol for male subjects.

The relationships between the *ApoE* genotype-specific correlations and age are presented in Fig. 1. The relationships between the *ApoE* genotype-specific total correlations and age are presented in Fig. 2. The estimates for female and male subjects are in the left and right columns of panels in each figure, respectively. The patterns of age change in the correlations appear distinct for each genotype and gender. The pointwise 95% confidence intervals for the genotype-specific loess regressions are narrowly confined around each predicted line (dashed black lines). These results are reinforced by the similar close relationships of the 1,000 bootstrap estimates of the loess regressions to each genotype-specific predicted line (gray masses of lines).

For each gender and each of the possible pairwise correlations and total correlation, (1) the generalized additive full model that included *ApoE* genotype and *ApoE* genotype-specific loess regressions of correlations on age fit significantly better than a model with only *ApoE* genotype and a common loess regression on age [variation among the three common *ApoE* genotypes had a significant impact on the influence of variability in age on the variability in the correlations (all Pr<0.0001)] and (2) the ε_{32}- and ε_{43} genotype-specific loess regressions of the correlations on age were each significantly different from those of the ε_{33} genotype [all Pr<0.0001, except for those in male subjects for the ε_{32} vs ε_{33} ApoB with triglyceride correlation and the ε_{43} vs ε_{33} cholesterol with HDL cholesterol correlation (marginally significant; P=0.06 and 0.08, respectively)]. For female subjects, all R^2 values for the full gen-

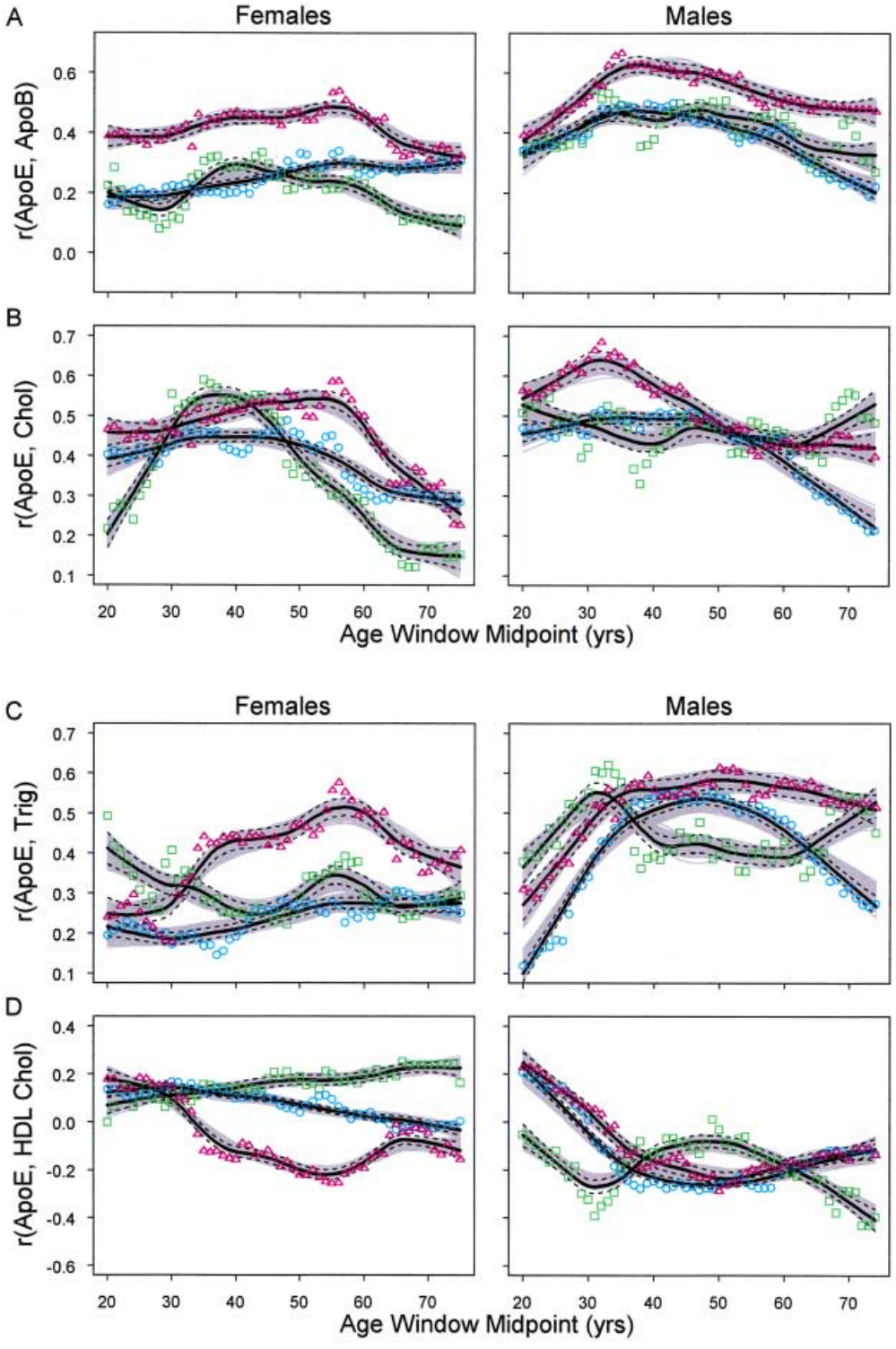
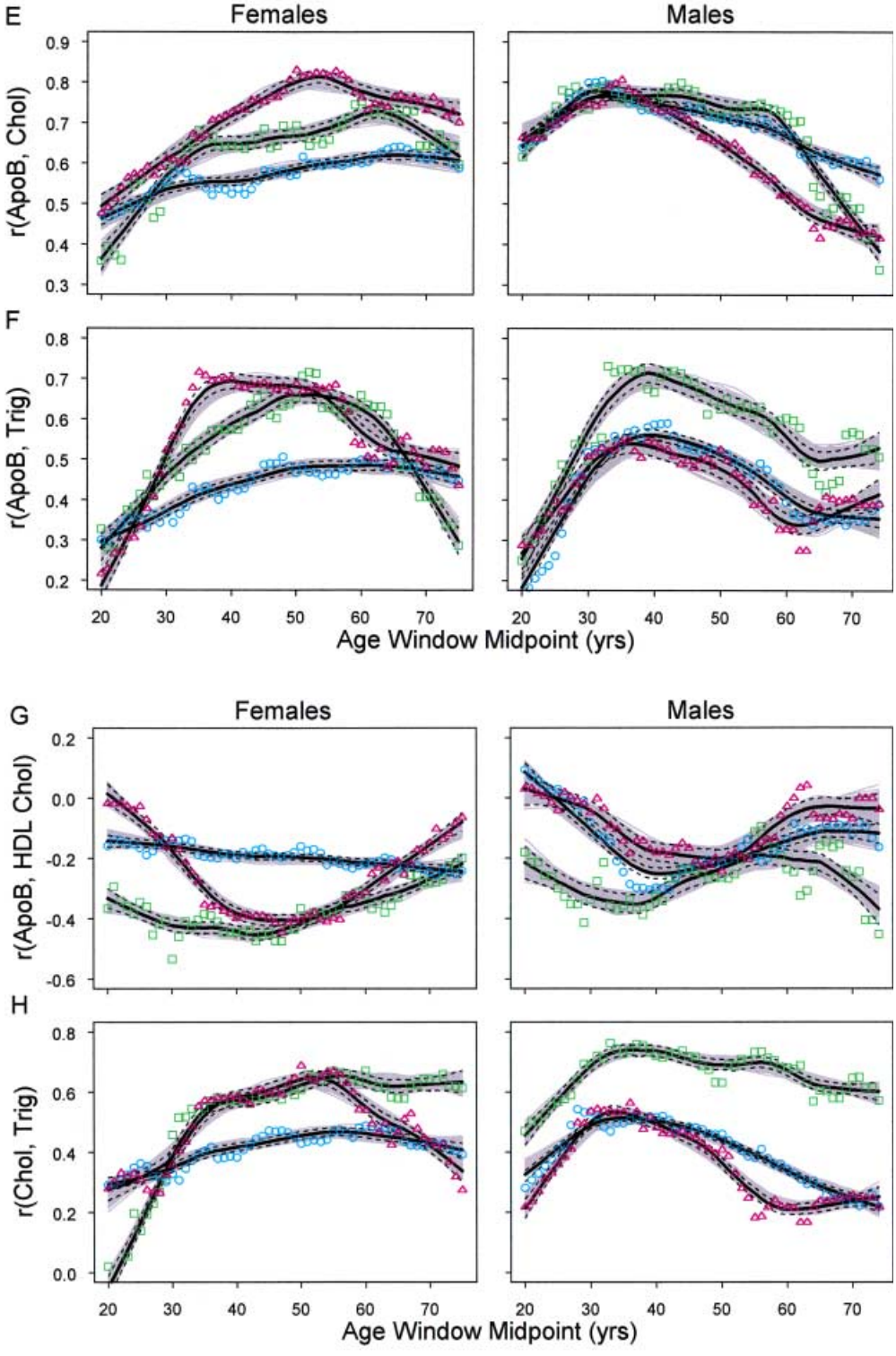


Fig. 1A–J The relationships between the Spearman's rank correlations among measures of lipid metabolism and age. **A** ApoE with ApoB; **B** ApoE with cholesterol; **C** ApoE with triglyceride; **D** ApoE with HDL cholesterol; **E** ApoB with cholesterol; **F** ApoB with triglyceride; **G** ApoB with HDL cholesterol; **H** Cholesterol with triglyceride; **I** Cholesterol with HDL cholesterol; **J** Triglyceride with HDL cholesterol. The correlation estimates for female and male subjects are in the left and right columns of the panels, respectively. The ε_{32} genotype is represented by *green squares*, the ε_{33} genotype is represented by *blue circles*, and the ε_{43} genotype is represented by *purple triangles*. The 95% pointwise confidence bands for the full generalized additive models of the dependence of the correlations on *ApoE* genotype and age window midpoint are represented by the *dashed black lines*. The predicted correlations from the models are represented by *thick black lines in the center of each confidence band*. The *gray masses of lines* represent 1,000 bootstrap estimates of the predicted loess regressions

eralized additive models that included *ApoE* genotype x age interactions were all greater than 0.90 whereas for males all but two R^2 values were ≥0.90, and those two were≥0.87. These results indicate that with two exceptions in the male subjects, age and common *ApoE* genotype are not independent predictors of variability in the correlations.

Total correlations were generally higher in the middle to older age windows (Fig. 2), with the exception of the ε_{32} genotype total correlation in males that peaked in the younger and oldest age windows, and the ε_{33} correlation in females that increased with increasing age windows. In contrast, the lowest correlations for the other genotypes were observed in the youngest and oldest age windows.

Fig. 1E–H

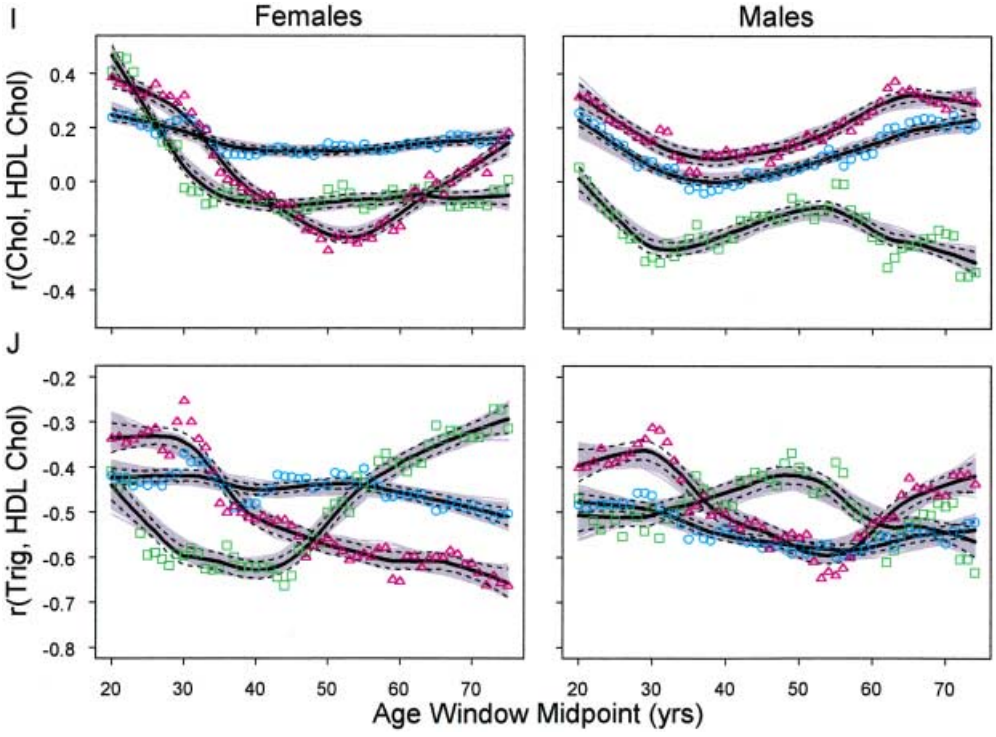


Discussion

Our study shows that the correlations among plasma levels of five measures of lipid metabolism and total correlations among the suite of measures are dynamic with age in a sample of individuals from the Rochester, Minn. population. The dynamic patterns of changes in these relationships with age were significantly influenced by variation among common genotypes of the *ApoE* gene polymorphism.

There were some similarities among the *ApoE* genotypes in the dynamics of age-related change in the some of the correlations, however, in all cases for female and in

Fig. 1I, J



all but two marginally significant cases in male subjects, the estimated statistical functional forms of these relationships for the ε_{32} and ε_{43} genotypes were significantly different from those for the ε_{33} genotype. Moreover, the patterns of change in correlations with age reflected differences between genders in the peaks of the correlations.

We observed increases and decreases in magnitudes of correlations as well as changes in the signs of some of the correlations with age. All of these changes appeared nonlinearly with age. A decrease in the magnitude of the correlation could be interpreted as a potential indicator of decreased stability of biological relationships among a pair of lipid metabolism traits. This decreased stability could, in turn, be interpreted as a potential indicator of decreased health associated with senescence. Such a view is concordant with traditional views of health (Goldberger 1992). The gender-specific peaks in total correlations that we observed in the middle to older age windows for most genotypes with lower total correlations in the youngest and oldest age windows, however, are in conflict with this interpretation. These results are supported by two other studies from this same population in which correlations among plasma levels of measures of lipid metabolism were generally higher in parents than in children (Nelson et al. 1999; Reilly et al. 1994).

For each individual, lipid metabolism represents a domain of relational order that is in constant flux and involves thousands of simultaneous biochemical reactions. Our cross-sectional sample, however, only captures a single state of the plasma level of each of the measures of lipid metabolism studied for each individual. Each age window-*ApoE* genotype-gender-specific correlation thus only represents the correlation in distributions of possible states of plasma levels of a particular pair of measures of

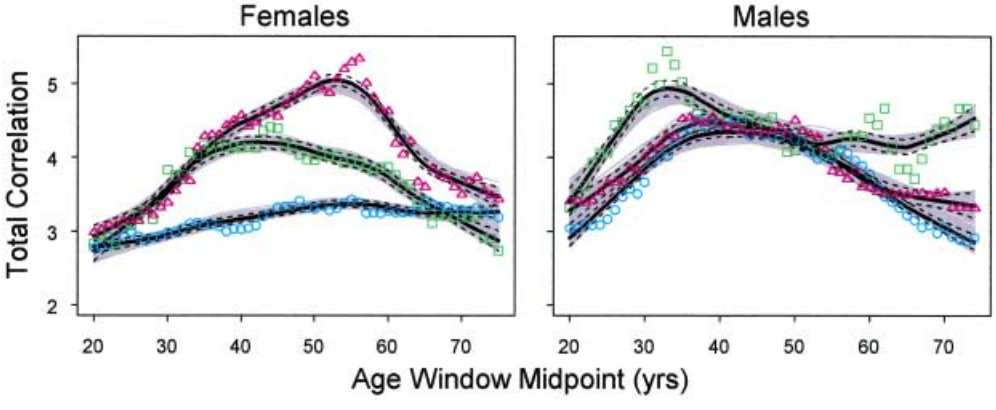


Fig. 2 The relationships between the total correlations and age. Symbols for the *ApoE* genotypes, confidence bands about the regressions, predicted correlations, and bootstrap estimates of the loess regressions correlations are as described in Fig. 1

lipid metabolism for that particular subgroup of individuals. The usefulness of such a correlation from a cross-sectional sample is that it probably reflects, on average, the potential correlation of possible states of plasma levels of that pair of measures of lipid metabolism over time for any particular individual in that subgroup. The observed *ApoE* genotype-gender-specific dynamic patterns of changes in correlations with age reflect the dynamic changes in lipid metabolism relational order in response to age-associated environmental changes experienced, on average, by individuals in the Rochester, Minn. population.

Our results have implications for the development and application of therapeutic agents to treat CAD. For example, the atherogenic potential of LDL cholesterol is widely recognized. For years it has been a controversial issue as to whether triglyceride has significant atherogenic potential (Austin 1989, 1991; Austin et al. 1998; NIH 1993). Triglyceride, however, is now considered as having as great or greater atherogenic potential as that of LDL cholesterol (Krauss 1998). In contrast, HDL cholesterol is generally considered as being protectively anti-atherogenic because of its involvement in reverse cholesterol transport. The general inverse relationship between plasma levels of triglyceride and HDL cholesterol is widely recognized, and there has been intense interest in the atherogenic nature of high plasma levels of LDL cholesterol and triglyceride particularly when high triglyceride levels coincide with low levels of HDL cholesterol (for example, Burchfiel et al. 1995; Deslypere and Jackson 1998; Lamarche et al. 1993; Manninen et al. 1992). Simultaneous pharmacological modulation of plasma triglyceride and HDL cholesterol levels to normal levels with the drug fenofibrate has been reported to be an effective therapeutic approach to treatment of dyslipidemic individuals (Chapman et al. 1998; Packard 1998). Our results suggest that differences among individuals in common *ApoE* genotype in the strength of the correlation between plasma levels of triglyceride and HDL cholesterol with age (Fig. 1J), especially for females, may contribute to differences in the effectiveness of such therapy on individuals in the general population.

The rich patterns of changes in correlations with age that we observed suggests that it is naïve to focus on the relationships of just one or a few pairs of traits as measure(s) of general health, just as it is naïve to focus on one gene-one trait relationships as independent measures of health. Recognizing the interrelated nature of the vast array of agents of lipid metabolism as well as among other related domains of cardiovascular physiology, Davignon (1998) and Krauss (1998) also expressed this view in discussions on the development of rational avenues for management of the lipid levels in CAD. It is our view that we also simply know too little, at present, about the role of genetic variation in determining the non-linear dynamics of the biological relationships among measures of lipid metabolism in individual complex adaptive systems, and the ways in which changes in the relational order result in adaptive changes in the health of individuals in the general population during the life cycle. The high degree of predictability of the observed variability in the correlations between traits by the interactions between age and *ApoE* genotype, however, suggests that it may be possible to define each trait as a function of the other traits in the field of lipid metabolism, and to investigate the role of *ApoE* genotypes as parameters, sensu Webster and Goodwin (1996), in physiological models of age-dependent changes in relational order among measures of lipid metabolism.

Our study shows that the influence of *ApoE* genotypic variation extends far beyond the levels of the gene product, to the dynamics of the relational order of measures of lipid metabolism with age. The challenge for future studies will be to examine the degree to which such influence is robust to additional DNA sequence variability that may underlie these common genotypes and the manner in which changes in relational order are reflective of the health of individuals in the population. Moreover, the cross-sectional nature of our study is only suggestive of the potential influence of genetic variation on the relational order among this suite of measures of lipid metabolism. Longitudinal studies of individuals are best suited to confirm these results and enhance our understanding of the role of genetic variability in the dynamic interactions between traits that occur in response to changes in exposures to environments distributed in time and ecological space (Zerba and Sing 1993).

In summary, our study documents that variation in the *ApoE* gene has context-dependent effects on the relationships between measures of lipid metabolism in addition to the well-established invariant and context-genotypic effects on the levels of these same measures of health considered separately. These findings are consistent with the reality that neither genes nor environments, but their interactions, are the causes of interindividual variation in the behavior of the complex adaptive system of traits that determine variation in health in the population at large.

Acknowledgements This work was supported by National Institutes of Health grants HL-39107, HL-58240, and HD-32197. We thank Dr. Andy Clark for critical comments which improved the paper.

References

Austin MA (1989) Plasma triglyceride as a risk factor for coronary heart disease. Am J Epidemiol 129:249–259

Austin MA (1991) Plasma triglyceride and coronary heart disease. Arterioscl Thromb 11:2–14

Austin MA, Talmud PJ, Luong LA, Haddad L, Day IN, Newman B, Edwards KL, Krauss RM, Humphries SE (1998) Candidate-gene studies of the atherogenic lipoprotein phenotype: a sib-pair linkage analysis of DZ women twins. Am J Hum Genet 62: 406–419

Burchfiel CM, Laws A, Benfante R, Goldberg RJ, Hwang LJ, Chiu D, Rodriguez BL, Curb JD, Sharp DS (1995) Combined effects of HDL cholesterol, triglyceride, and total cholesterol concentrations on 18-year risk of atherosclerotic disease. Circulation 92:1430–1436

Capra F (1996) The web of life: a new scientific understanding of living systems. Anchor Books, New York

Chapman MJ, Guerin M, Bruckert E (1998) Atherogenic, dense low-density lipoproteins. Pathophysiology and new therapeutic approaches. Eur Heart J 19 [Suppl A]:A24–30
Cleveland WS, Devlin SJ (1988) Locally weighted regression: an approach to regression analysis by local fitting. J Am Stat Assoc 83:596–610
Cohen J, Rice SH (1996) Where do biochemical pathways lead? In: Collado-Vides J, Magasanik B, Smith T (eds) Integrative approaches to molecular biology. MIT Press, Cambridge
Davignon J (1998) Advances in drug treatment of dyslipidemia: focus on atorvastatin. Can J Cardiol 14 [Suppl B]:28B–38B
Davignon J, Gregg RE, Sing CF (1988) Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 8:1–21
Deslypere JP, Jackson G (1998) A lipid hypothesis: prediction, observation and the triglyceride/HDL gap. Curr Med Res Opin 14:65–78
Efron B, Tibshirani RJ (1993) An Introduction to the Bootstrap. Chapman & Hall, New York
Emmet D (1992) The passage of nature. Temple University Press, Philadelphia
Gell-Mann M (1994) The quark and the jaguar: adventures in the simple and the complex. Freeman, New York
Glass L, Mackey M (1988) From clocks to chaos: the rhythms of life. Princeton University Press, Princeton
Goldberger AL (1992) Applications of chaos to physiology and medicine. In: Kim JH, Stringer J (eds) Applied Chaos. Wiley, New York, pp 321–331
Goodwin B (1994a) How the leopard changed its spots: the evolution of complexity. Scribner, New York
Goodwin BC (1989) Organisms and minds as dynamic forms. Leonardo 22:27–31
Goodwin BC (1994b) Toward a science of qualities. In: Harman W, Clark J (eds) New metaphysical foundations of modern science. Institute of Noetic Sciences, Sausalito, pp 215–250
Goodwin BC (1997) Temporal organization and disorganization in organisms. Chronobiol Int 14:531–536
Hastie T, Tibshirani R (1986) Generalized additive models. Stat Sci 1:297–318
Hastie TJ, Tibshirani RJ (1990) Generalized Additive Models. Chapman & Hall, New York
Hixson JE, Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group (1991) Apolipoprotein E polymorphisms affect atherosclerosis in young males. Arterioscler Thromb 11:1237–1244
Holdredge C (1996) Genetics and the manipulation of life: the forgotten factor of context. Lindisfarne Press, Hudson
Izzo C, Grillo F, Murador E (1981) Improved method for determination of high density lipoprotein cholesterol: isolation of high density lipoproteins by use of polyethylene glycol-6000. Clin Chem 27:371–374
Kamboh MI, Ferrell RE, Kottke BA (1988) Genetic studies of human apolipoproteins. V. A novel rapid procedure to screen apolipoprotein E polymorphism. J Lipid Res. 29:1535–1543
Kaprio J, Ferrell RE, Kottke BA, Kamboh MI, Sing CF (1991) Effects of polymorphisms in apolipoproteins E, AIV, and H on quantitative traits related to risk for cardiovascular disease. Arterioscl Throm 11:1330–1348
Kauffman SA (1993) The origins of order: self-organization and selection in evolution. Oxford University Press, New York
Kottke BA, P. MP, Michels VV, Weidman WH (1991) Levels of lipids, lipoproteins, and apolipoproteins in a defined population. Mayo Clin Proc 66:1198–1208
Krauss RM (1998) Triglycerides and atherogenic lipoproteins: rationale for lipid management. Am J Med 105:58S–62S
Lamarche B, Despres JP, Pouliot MC, Prud'homme D, Moorjani S, Lupien PJ, Nadeau A, Tremblay A, Bouchard C (1993) Metabolic heterogeneity associated with high plasma triglyceride or low HDL cholesterol levels in men. Arterioscler Thromb 13:33–40
Levins R, Lewontin R (1985) The dialectical biologist. Harvard University Press, Cambridge
Lewontin RC (1992a) Biology as ideology: the doctrine of DNA. Harper Collins, New York
Lewontin RC (1992b) Genotype and phenotype. In: Fox Keller E, Lloyd EA (eds) Keywords in Evolutionary Biology. Harvard University Press, Cambridge, pp 137–144
Lewontin RC (1996) Evolution as engineering. In: Collado-Vides J, Magasanik B, Smith T (eds) Integrative approaches to molecular biology. MIT Press, Cambridge, pp 1–10
Manninen V, Tenkanen L, Koskinen P, Huttunen JK, Manttari M, Heinonen OP, Frick MH (1992) Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. Circulation 85:37–45
Maturana HR, Varela FJ (1980) Autopoiesis and cognition: the realization of the living. Reidel, Dordrecht
Maturana HR, Varela FJ (1992) The tree of knowledge: the biological roots of human understanding. Shambhala Publications, Boston
McKeown T (1979) The role of medicine: dream, mirage or nemesis? Princeton University Press, Princeton
Moll PP, Michels VV, Weidman WH, Kottke BA (1989) Genetic determination of plasma apolipoprotein AI in a population-based sample. Am J Hum Genet 44:124–139
Nelson MR, Kardia SLR, Ferrell RE, Sing CF (1999) Influence of Apolipoprotein E genotype variation on the means, variances, and correlations of plasma lipids and apolipoproteins in children. Ann Hum Genet 63:311–328
NIH (1993) Consensus Development Panel on Triglyceride, High-Density Lipoprotein, and Coronary Heart Disease. Triglyceride, high-density lipoprotein, and coronary heart disease. JAMA 269:505–510
Packard CJ (1998) Overview of fenofibrate. Eur Heart J 19 [Suppl A]:A62–65
Prigogine I (1989) The philosophy of instability. Futures 21:396–400
Prigogine I, Stengers I (1984) Order out of chaos: man's new dialogue with nature. Bantam Books, New York
Rall SC, Jr., Weisgraber KH, Mahley RW (1982) Human apolipoprotein E: the complete amino acid sequence. J Biol Chem 257: 4171–4178
Reilly SL, Kottke BA, Sing CF (1990) The effects of generation and gender on the joint distributions of lipid and apolipoprotein phenotypes in the population at large. J Clin Epidemiol. 43: 921–940
Reilly SL, Ferrell RE, Kottke BA, Kamboh MI, Sing CF (1991) The gender-specific apolipoprotein E genotype influence on the distribution of lipids and apolipoproteins in the population of Rochester, MN. I. Pleiotropic effects on means and variances. Am J Hum Genet 49:1155–1166
Reilly SL, Ferrell RE, Kottke BA, Sing CF (1992) The gender specific apolipoprotein E genotype influence on the distribution of lipids and apolipoproteins in the population of Rochester, Minnesota. II. Regression relationships with concomitants. Am J Hum Genet 51:1311–1324
Reilly SL, Ferrell RE, Sing CF (1994) The gender-specific apolipoprotein E genotype influence on the distribution of plasma lipids and apolipoproteins in the population of Rochester, MN. III. Correlations and covariances. Am J Hum Genetics 55: 1001–1018
Rose SPR (1997) Lifelines: biology beyond determinism. Oxford University Press, New York
Rosen R (1991) Life itself: a comprehensive inquiry into the nature, origin, and fabrication of life. Columbia University Press, New York
Royston JP (1982) An extension of Shapiro and Wilk's W test for normality to large samples. Appl Stat 31:115–124
Salthe SN (1993) Development and evolution: complexity and change in biology. MIT Press, Cambridge
Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52:591–611

Sing CF, Davignon J (1985) Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. Am J Hum Genet 37:268–285

Sing CF, Reilly SL (1993) Genetics of common diseases that aggregate, but do not segregate, in families. In: Sing CF, Hanis CL (eds) Genetics of Cellular, Individual, Family and Population Variability. Oxford University Press, New York

Sing CF, Boerwinkle E, Moll PP (1985) Strategies for elucidating the phenotypic and genetic heterogeneity of a chronic disease with a complex etiology. In: Chakraborty R, Szathmary EJE (eds) Diseases of Complex Etiology in Small Populations: Ethnic Differences and Research Approaches, Vol 194. Liss, New York, pp 39–66

Sing CF, Haviland MB, Templeton AR, Zerba KE, Reilly SL (1992) Biological complexity and strategies for finding DNA variations responsible for inter-individual variation in risk of a common chronic disease, coronary artery disease. Ann Med 24:539–547

Sing CF, Haviland MB, Reilly SL (1996) Genetic architecture of common multifactorial diseases. In: Cardew G (ed) Variation in the Human Genome. Ciba Foundation Symposium 197. Wiley, Chichester, pp 211–232

Sokal RR, Rohlf FJ (1981) Biometry: the principles and practice of statistics in biological research, 2nd edn. Freeman, New York

Stengård JH, Zerba KE, Pekkanen J, Ehnholm C, Nissinen A, Sing CF (1995) Apolipoprotein E polymorphism predicts death from coronary heart disease in a longitudinal study of elderly Finnish men. Circulation 91:265–269

Strohman RC (1993) Ancient genomes, wise bodies, unhealthy people: limits of a genetic paradigm in biology and medicine. Perspect Biol Med 37:112–145

Turner ST, Weidman WH, Michels VV, Reed TJ, Ormson CL, Fuller T, Sing CF (1989) Distribution of sodium-lithium countertransport and blood pressure in Caucasians five to eighty-nine years of age. Hypertension 13:378–391

Webster G, Goodwin B (1996) Formation and transformation: generative and relational principles in biology. Cambridge University Press, New York

Xhignesse M, Lussier-Cacan S, Sing CF, Kessling AM, Davignon J (1991) Influences of common variants of apolipoprotein E on measures of lipid metabolism in a sample selected for health. Arterioscl Throm 11:1100–1110

Yates FE (1993) Self-organizing systems. In: Boyd CAR, Noble D (eds) The logic of life: the challenge of integrative physiology. Oxford University Press, New York, pp 189–226

Zerba KE, Sing CF (1993) The role of genome type-environment interaction and time in understanding the impact of genetic polymorphisms on lipid metabolism. Curr Opin Lipidol 4:152–162

Zerba KE, Ferrell RE, Sing CF (1996) Genotype-environment interaction: apolipoprotein E (*ApoE*) gene effects and age as an index of time and spatial context. Genetics 143:463–478