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GENES, ENVIRONMENT AND PATHOGENS: AN EVOLUTIONARY PERSPECTIVE ON THE CAUSES OF CHRONIC DISEASES

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SUMMARY

Chronic diseases are commonly studied through quantification and accumulation of risk factors. To reliably lead to an understanding of disease causation, however, the role of risk factors in the process of pathogenesis must be evaluated. This evaluation must consider in an integrated way the full range of possible causes of chronic disease, which can be categorised as genetic, infectious and non-infectious environmental. To understand how to prevent disease causal risk factors need to be distinguished from risk factors that are spurious correlates of causal processes, and primary causes need to be distinguished from exacerbating causes. Insights from evolutionary biology provide a foundation for this process by distinguishing feasible causal hypotheses from infeasible ones. This paper applies this approach to the epsilon-4-associated diseases as a paradigm for chronic diseases, and then considers atherosclerosis in more detailed as an illustration of epsilon-4-associated diseases. The most parsimonious conclusion is that the epsilon-4-associated diseases in general and atherosclerosis in particular are for the most part infectious diseases that are exacerbated by the documented environmental and genetic risk factors.

INTRODUCTION: CATEGORIES OF CAUSATION

Diseases can be attributable to genetic, infectious, and environmental causes. Although diseases are often referred to as though they belong to one of these categories, it is generally recognised that more than one of these categories of causal factors generally contribute to each disease (Figure 1). Cystic fibrosis, for example, is referred to as a genetic disease but life-threatening crises result from respiratory infections with pathogens such as *Streptococcus pneumoniae* or *Pseudomonas aeruginosa*. All three categories of causal factors generally contribute to infectious diseases (defined broadly in this paper to include all examples of internal parasitism); infections with *Mycobacterium tuberculosis*, for example, can range from asymptomatic to lethal depending on genetic susceptibility and a person's nutritional status. The corollary of this generalisation is that the identification of genetic or non-infectious environmental influences on disease cannot be used as evidence against infectious causation, because such influences are expected among infectious diseases. Nevertheless, researchers often commit this logical error when they dismiss infectious causation on the basis of the evidence consistent with genetic causes or non-infectious environmental causes. This error is especially counterproductive when the evidence that is consistent with genetic causation is also consistent with infec-

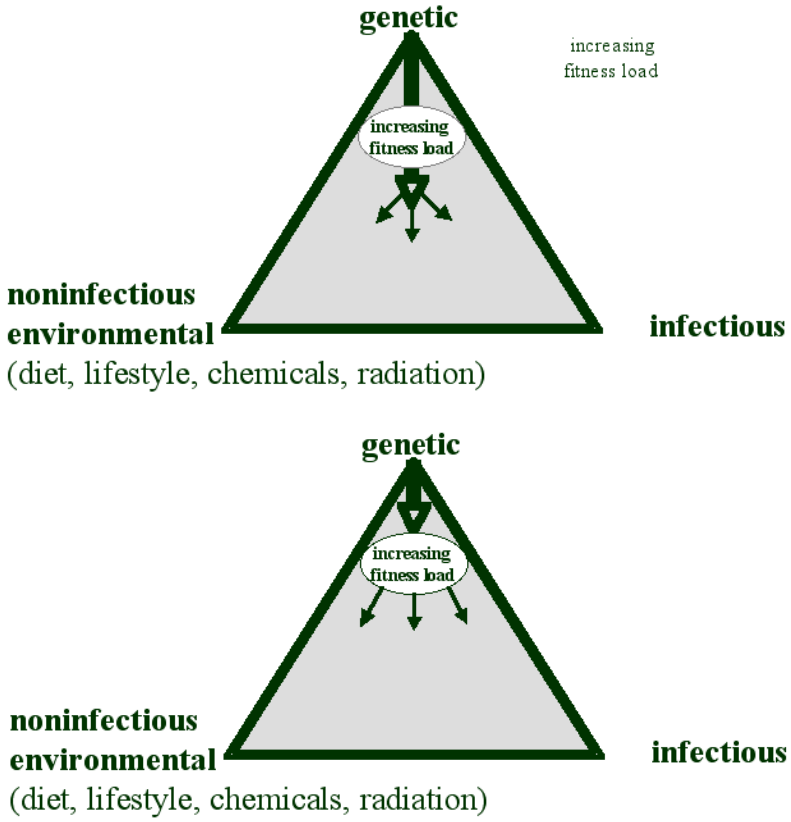

Figure 1: The triad of disease causation. The diagram emphasises that more than one category of disease causation is in operation for any particular disease. The location of a disease within the triangle corresponds the relative importance of the three categories. The further from the apex the less the relative importance of the designated category of causation. The arrow thus signifies decreased importance of genetic causation relative to the influences in the other categories. Increased fitness load (defined in text) implies reduced importance of genetic causation, because mutation alone can maintain a genetic basis only for diseases with very low fitness loads.

tious causation, as is the case for example, for schizophrenia (*Ledgerwood* et al., 2003).

The tendency for candidate causes of disease to be correlated with other variables and the difficulty of distinguishing correlation from causation has contributed to the tendency for researchers to couch discussions of chronic diseases in terms of risk factors rather than causes. This tendency can be counterproductive, however, by clouding the different roles of different risk factors. Some risk factors will be primary causes, which initiate the disease process, whereas others will be secondary causes, which exacerbate the disease process. Other risk factors may not play a causal role at all, but may simply be correlated with primary or secondary causes.

The identification of primary causes is critical for the eventual control of disease, because blocking of a primary cause eliminates the disease, whereas blocking of a secondary cause does not. *M. tuberculosis* is the primary cause of tuberculosis because tuberculosis cannot occur without *M. tuberculosis* infection.

The same claim cannot be made for host genetic or non-infectious environmental factors that exacerbate *M. tuberculosis* infections. Tuberculosis can occur among people who are not particularly genetically susceptible to *M. tuberculosis* infection or who have not been exposed to particular environmental factors that exacerbate tuberculosis infection, if other factors such as high dosage or temporary immunosuppression allow *M. tuberculosis* infection to progress to tuberculosis. *M. tuberculosis* is therefore considered the primary cause of tuberculosis, and tuberculosis is considered an infectious disease, even though human genetic variation and non-infectious environmental factors may influence the manifestations of *M. tuberculosis* infections. The problem of tuberculosis can be eliminated if *M. tuberculosis* infection is blocked. Categorising tuberculosis as an infectious disease emphasises this point. Analogously, mutations in the cystic fibrosis trans-membrane conductance regulator gene are primary causes of cystic fibrosis, whereas *S. pneumoniae* is a secondary causes, because eradication of *S. pneumoniae* would not eradicate cystic fibrosis.

MOVING FROM RISK FACTORS TO CAUSATION

Consideration of risk factors is safe, because the term "risk factor" implies only correlation and not causation, and correlations can generally be demonstrated more easily and definitively than causation. This generalisation is especially valid for chronic diseases. But risk factors are studied with hopes of intervening to control disease; the most important risk factors are therefore those that play a causal role. Careful study and logic are required to assess which risk factors are parts of a causal process and which are not. Unfortunately, although reference to risk factors is often made with sufficient care to avoid jumping from correlation to causation, the repeated identification of risk factors without assessment of the feasibility of a causal mechanism has created a vacuum that has led the popular media and the medical literature to blur the distinction between risk factors and causes of illnesses. This blurring then influences the hypotheses that are evaluated, with some risk factors being favoured targets of research effort because their causal role is presumed. When the feasibility of causal hypotheses is directly addressed, however, some of these risk factors are often found to be inadequate as primary causes. When considered against the full spectrum of possible causes, such risk factors may be exacerbating or ameliorating influences or merely correlates.

These considerations provide an important framework for understanding infectious disease because causation of disease is not well understood for about half of all human diseases. The importance of this framework is evidenced by the track record in identifying infectious causes of disease over the past three decades. The recognition of new examples of infectious causation has not slowed during this time and evidence suggests that infection may be a primary cause of a large proportion of the most important diseases of unknown cause (*Cochran* et al., 2000).

Assessment of the relative importance of infectious, genetic, and environmental causes requires the integrated application of insights from several different disciplines of biology. The concept of evolutionary fitness is particularly useful in distinguishing genetic primary causes from infectious and non-infectious primary causes. Evolutionary fitness is a measure of genetic contribution into the succeeding generations. When applied

to genes, the fitness of an allele refers to the change in its representation over time relative to alternative alleles. The negative effects of a disease on the passing on of the presumptive genetic basis for the disease are referred to as the fitness load of the disease (*Cochran* et al., 2000). If the negative effects of a disease are so great that genetic instructions for the disease could not be maintained through time by mutation, then genetic factors are not feasible as primary causes.

The main caveat is that compensating advantages of disease-causing alleles could allow the maintenance of severe genetic diseases at moderately high frequencies. Sickle cell anaemia provides the classic example. Where falciparum malaria is common, sickle-cell anaemia can be maintained at a frequency that is over two orders of magnitude greater than the frequency that could be maintained simply by new mutations, because individuals who are heterozygous for the sickle-cell allele are protected against falciparum malaria (*Vogel* and *Motulsky*, 1997).

Such genetic diseases are maintained at high frequency because their genetic basis provides a "self-destructive defence" against an infectious disease. All such diseases recognised to present have distinctive characteristics: they result from mutations that cause a protein product to lose normal function, they are inherited by simple Mendelian ratios, they confer protection against infection in heterozygous form, and they occur in high frequency in restricted geographic regions or in certain ethnic groups, and in low frequency in other populations. Several diseases besides sickle cell anaemia share such characteristics category, for example, thalassemia, cystic fibrosis, haemochromatosis, glucose-6-phosphate dehydrogenase deficiency, Tay Sachs, Gauchier's disease (*Cochran* et al., 2000). These diseases probably all represent rapid, "quick-and-dirty" evolutionary response to recent threats or environmental challenges. Although it is not known for most of these diseases whether this challenge is posed by an infectious disease, infectious diseases are prime candidates for generating the selective pressure that favours many of them. The responsible infectious challenges may new in the sense that the infectious agents recently entered the human population or because they represent ever-changing threats as they co-evolve in response to human defences.

Although the known self-destructive defences share these characteristics in common, the model of self-destructive defences has been applied indiscriminately to explain how damaging genetic diseases could be maintained at relatively high frequencies even when the diseases do not share these characteristics. As a consequence, considerations of disease causation generally fail to appreciate the severe restrictions that the fitness load of a disease imposes on the feasibility of genetic causation of disease. For most of the common and damaging chronic diseases of unknown cause, the fitness load is too high to allow the maintenance of the disease simply by mutation (*Cochran* et al., 2000). Hypotheses of genetic causation for these diseases must therefore be evaluated critically to assess how they could be feasible in the context of the observed fitness load. In practice, however, hypotheses of genetic causation are generally accepted uncritically on the basis of family studies, particularly when the concordance for the illness among identical twins is high. By themselves these family studies do not demonstrate genetic causation because other non-genetic causes of disease may correlate with genetic causes. The *in utero* environment for monozygotic twins, for example, is more similar than is the in utero environment for dizygotic twins because monozygotic twins share a common gestational sac and placenta more often than do dizygotic twins.

Monozygotic twin may therefore not only share more genes in common but more infectious agents *in utero*. High monozygotic twin concordances and lower dizygotic twin concordances are therefore consistent with genetic causation but are not sufficient to demonstrate genetic causation. Rather than revealing the degree of genetic causation they provide a ceiling on the extent of genetic causation--low-to-moderate concordance indicates that some environmental cause, either infectious or non-infectious, is playing a major role. Such considerations call into question, for example, the commonly held belief that schizophrenia is largely a genetic disease (*Ledgerwood* et al., 2003).

GENETIC PREDISPOSITIONS TO INFECTION: THE EPSILON 4 ALLELE

This framework of inquiry can be applied to every disease of unknown cause. For illustrative purposes this paper will consider the diseases that have been associated with the epsilon 4 allele of the Apolipoprotein E gene, namely atherosclerosis, stroke, Alzheimer's disease, and severe cases of multiple sclerosis (*Hardy*, 1995; *Ji* et al., 1998; *Urakami* et al., 1998; *Evangelou* et al., 1999; *Ilveskoski* et al., 1999; *Mahley* and *Huang*, 1999; *Love* et al., 2003). The epsilon 4 allele is a provide a particularly informative example, because the discovery of its association with these diseases is considered one of the great medical advancements of human genetics and the rich body of knowledge that is now available for the epsilon-4-associated diseases. A balanced approach to consideration of the causes of these epsilon-4-associated diseases may therefore provide a general model for evaluation of genetic associations with human disease as well as a better understanding of the contribution of human genetics to the health sciences.

The epsilon-4-associated diseases have their greatest negative impacts on health after direct reproduction; they therefore probably have had much of their effect on fitness through reductions in resources available to children and other relatives such as grandchildren. Still a substantial portion of the negative effects of the epsilon-4-associated diseases on fitness load occur during reproductive periods for especially for males, because males continue to have children into sixth and seventh decades of life and because debilitating and lethal cases of epsilon-4-associated diseases (particularly heart attacks, strokes, and multiple sclerosis) often occur in the fifth decade of life or even earlier.

The fitness load of the epsilon 4 allele resulting from the negative effects of Alzheimer's disease, multiple sclerosis, atherosclerosis, and stroke is probably well over 1% (*Cochran* et al., 2000). Although this 1% figure is a rough estimate, it is about two orders of magnitude above the percentage that could be maintained by mutation. This assessment indicates that there must be some compensating benefit or that this disadvantage of the epsilon 4 allele has not always been present throughout the existence of *Homo sapiens*. It seems doubtful, however, that the negative effects of the epsilon 4 associated diseases could have been negligible if the risk of developing them at a given age had been present human history and pre-history. Less death and incapacitation from these illnesses due to early death from other causes was probably substantially compensated for by more severe consequences on survival of offspring and reduced birth control in older people in previous centuries.

The distribution of the epsilon 4 allele among human populations and primate species indicates that the epsilon 4 allele itself could not be intrinsically bad or defective. Epsilon 4 is maintained in all human populations at frequencies from about five to 40% (*Corbo* and *Scacchi*, 1999; *Fullerton* et al., 2000). If epsilon 4 were an inherently inferior allele it could not be maintained at such frequencies over long periods of time. Phylogenetic analyses indicate, however, that it has been maintained over long periods of time and that epsilon 4 is the ancestral allele, with the epsilon 4 being more similar to the epsilon alleles of chimpanzees and other primates than are the other epsilon alleles of humans (*Fullerton*, 2000); it could not have predominated in primates for millions of years, if it were defective. In humans its frequency is about 5-15% in populations that have had been living in agricultural or urban setting for thousands of years but at frequencies of about 20-40% in populations that have been living as hunter/gatherers until the 20th century. This difference indicates that the allele has been declining as humans shifted away from hunting and gathering over the past 10,000 years.

One commonly accepted explanation for the persistence of epsilon 4 is that humans have only recently lived long enough to experience the negative effects of the epsilon 4-associated diseases, which often occur after the third decade of life. But this argument does not apply to multiple sclerosis, which typically occur during or before the third decade of life. With a prevalence of up to 0.3% in regions, it alone could have exerted a substantial selective pressure against epsilon 4. Another problem with this short-life argument is that its fundamental assumption does not hold up to the evidence. Studies of hunter/gatherer societies have found a high probability of survival into older age groups, contradicting the widely held but poorly supported belief that hunter-gatherers rarely lived past 40. The probability of surviving from the onset of reproduction to age 65 was about 70% among the San (a.k.a. "Bushmen" of southern Africa) (H.C. Harpending, unpublished data). Even among the more violent Ache of South America survival over this interval was about 45% (*Hill* and *Hurtado*, 1996). Evidence from tooth wear also suggest that humans have regularly lived into and beyond their fourth decade of life during the 20,000 years prior to the onset of agriculture (*Caspari* and *Lee*, 2004).

Another hypothesis to explain the current presence of the epsilon 4 allele has been derived from the function of the epsilon 4 protein: transport of cholesterol and lipids. Application of the "thrifty genotype" hypothesis for diabetes (*Neel*, 1962) to the epsilon 4 diseases suggests that the epsilon 4 allele may be too good rather than too bad (*Corbo* and *Scacchi*, 1999). That is, its high efficiency of transport of lipids and cholesterol was beneficial during times when nutrients were scarce, but epsilon 4 is transporting too much lipid and cholesterol now that diets are so much richer than they were in the past. This hypothesis, however, has two problems. The first is that even hunter-gatherer populations do not have epsilon 4 frequencies that approach 100%. If the frequency of epsilon 4 dropped as a result of the rich diets in agricultural settings then we would expect that hunter/gatherer should have virtually 100% epsilon 4. But epsilon 4 among hunter-gatherers generally lies between 20% and 40%. The shift away from epsilon 4 therefore must have begun well before the shift to rich agricultural diets.

Phylogenetic analyses indicate the other two major epsilon alleles, epsilon 3 and epsilon 2, were derived evolutionarily from the epsilon 4 allele in humans and increased in frequency over the past 200,000 years (*Fullerton* et al., 2000);

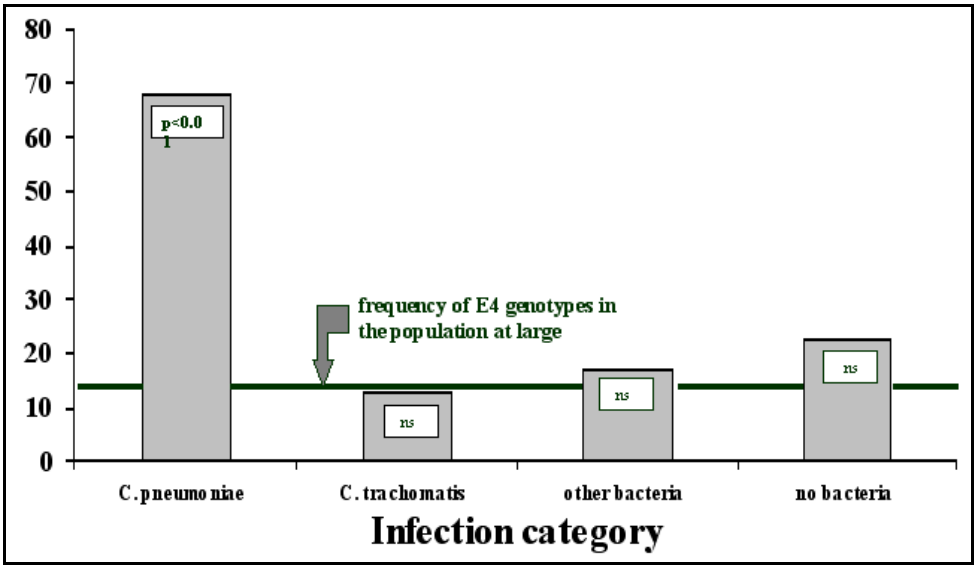

Figure 2: Association of *Chlamydia pneumoniae* infection with epsilon 4 genotype in the synovial tissue of arthritis patients. Patients who were positive for *C. pneumoniae* DNA were much more likely to have the epsilon 4 allele than the general population, but epsilon 4 was not significantly more common among patients who were positive for DNA from *Chlamydia trachomatis* or other bacteria, nor among those who were negative for all of the tested pathogens. The abbreviation, ns, indicates that the frequency of epsilon 4 genotypes was not significantly greater than the frequency in the general population from which the arthritis patients belonged. Data are from *Gérard* et al. (1999).

that is, epsilon 2 and epsilon 3 have increased at the expense of epsilon 4 during the time interval over which *Homo sapiens* is seen as a distinct from *H. erectus*. Although the timing of this increase cannot be determined with accuracy, the phylogenetic analyses together with the substantial presence of epsilon 2 and epsilon 3 in hunter gatherers indicates that the shift began long before the shift from hunting and gathering to agriculture.

The frequencies of epsilon 4 among the few hunter-gatherers with rich diets also argue against the thrifty allele hypothesis as an explanation of epsilon 4's detrimental effects. If rich modern diets were responsible, one would expect that hunter-gatherer populations with rich diets should have low epsilon 4 frequencies. Inuits, however, who have eaten a rich for many thousands of years have a relatively high frequency of epsilon 4, one that is comparable to other populations of humans whose ancestors lived indigenously, in low densities in North and South America and (*Corbo* and *Scacchi*, 1999).

Another problem with the thrifty allele hypothesis is that different defective functions must be envisioned for each epsilon-4-associated disease. It can offer a hypothetical explanation for the association of rich diets with atherosclerosis and stroke, but it does not offer a mechanism for the association between epsilon 4 and Alzheimer's and multiple sclerosis. The association between epsilon 4 and these other diseases has been explained by generating hypotheses based on mechanistic aberrancies other than lipid deposition (*Henderson*, 2004). Such post-hoc hypothesis, still have the same weaknesses mentioned for the

association between epsilon 4 and fat deposition in atherosclerosis; for example, a variant on the thrifty allele hypothesis proposes that the negative effects of epsilon 4 on Alzheimer's disease arise in response to the carbohydrate rich diets associated with agriculture (*Henderson*, 2004). But, as argued above, the negative effects of epsilon 4 must have been in action many thousands of years before the beginning of agriculture to explain the epsilon 4 allele frequencies of hunter-gatherers.

These considerations lead to the conclusion that epsilon 4 must have conferred vulnerability to some other cause of the epsilon-4 associated diseases that was present when humans were still hunter-gatherers, but became more important for humans in agricultural settings. Genetic vulnerability to infectious agents, for example, would tend to be more problematic as human populations become larger and denser and hence could maintain higher levels and intensities of infection. Genetic vulnerabilities to infectious agents appear to be a pervasive cause of allelic associations with disease (*Abel* and *Dessein*, 1997; *Cochran* et al., 2000). This genetic-vulnerability-to-infection hypothesis is consistent with the overall trends, because it suggests that the pathogen pressure would increase in agricultural societies but would have existed before the onset of agriculture.

GENETIC PREDISPOSITIONS TO INFECTION

Chlamydia pneumoniae is one of the most plausible candidates for such a process because it is the only infectious organism that is implicated as a cause of each of the epsilon 4-associated diseases. Accordingly, research on arthritis patients has demonstrated the predicted association between the epsilon 4 allele and *C. pneumoniae* infection (*Gérard* et al., 1999; see Figure 2). This finding suggests that epsilon 4 somehow increases the vulnerability to *C. pneumoniae* infection. *In vitro* studies of growth in macrophages of different genotypes provide further support: *C. pneumoniae* grow most prolifically in cells that are homozygous for epsilon 4, moderately well in epsilon 4 heterozygotes and much less well in macrophages of other epsilon genotypes (A.P. Hudson, unpublished data). These findings provide a theoretical basis for integrating the entire spectrum of epsilon 4-associated diseases. In contrast to the inadequacy of the "thrifty genotype" hypothesis as an explanation of the full array of epsilon 4 associated disease, the hypothesis that epsilon increases vulnerability to *C. pneumoniae* readily explains the entire spectrum of available evidence, because a given pathogen can cause very different pathologies in different tissues. The different epsilon-4-associated diseases may therefore result from the different effects of the increased vulnerability to epsilon 4 infection in different tissues. By this argument, *C. pneumoniae* in the brain causes damage that manifests itself as Alzheimer's disease (*Balin* et al., 1998). If brain damage occurs through an autoimmune mechanism triggered by *C. pneumoniae* (*Lenz* et al., 2001), it may be manifested as multiple sclerosis. Invasion of the endothelial lining of arteries and subsequent accumulation and oxidation of lipid is manifested as atherosclerosis (*Byrne* and *Kalayoglu*, 1999; *Kalayoglu* et al., 1999)

The association between epsilon-4-diseases and *C. pneumoniae* offers a specific hypothesis to explain the pattern of epsilon 4 allele frequency in human populations. Because *C. pneumoniae* is spread as a respiratory tract pathogen by coughing, the exposure should increase with increasing population density.

Those peoples who have been living in high densities and close quarters for more millennia should have had the greatest exposure to high frequencies and dosages of infection and thus should have experienced the strongest selective pressure against epsilon 4 thus explaining why Inuits have high epsilon 4 frequencies like other populations who until recently have been hunter-gatherers, and why people with a long ancestry in the Mediteranean and China have low frequencies of epsilon 4 (*Corbo* and *Scacchi*, 2000).

This line of logic also helps explain seemingly discordant findings about suites of related diseases. Different forms of Alzheimer's disease, for example, are associated with different risk factors. Early-onset Alzheimer's disease (also known as "familial Alzheimer's disease") is a genetic disease in the traditional sense. Presently mutant alleles of three different genes code are responsible for three variants of early-onset Alzheimer's disease: the beta amyloid precursor protein gene on chromosome 21, the presenilin-1 gene on chromosome 14, and the presenilin-2 gene on chromosome 1 (*Clark* et al., 1996; *Pastor* et al., 2003). The epsilon 4 allele exacerbates the variants of early onset Alzheimer's Disease that are attributable to mutations in the amyloid precursor protein. The evidence from individuals with presenilin mutations is mixed with one large study indicating exacerbation whereas smaller studies have documented no significant effect (*Haan* et al., 1994; *van Broeckhoven* et al., 1994; *Sorbi* et al., 1995; *Lendon* et al., 1997; *Romero* et al., 1999; *Pastor* et al., 2003). In the large study (*Pastor* et al., 2003), rural residence was associated with later onset of Alzheimer's disease. This result is consistent with an influence of infection on development of early-onset Alzheimer's disease, because the incidence of infections with respiratory pathogens such as *C. pneumoniae* are typically greater in urban environments where populations are more congested and tend to spend more time indoors. The clarification of this different causal mechanism is thus suggesting that Alzheimer's disease is actually a spectrum of related but distinct diseases. Late-onset Alzheimer's disease, which is much more common the early-onset Alzheimer's disease, appears to be one or more infectious diseases for which *C. pneumoniae* is a primary cause and epsilon 4 an exacerbating cause (*Balin* et al., 1998). Early-onset Alzheimer's disease is thus a collection of genetic diseases for which epsilon 4 is sometimes an exacerbating cause, particularly when they co-occur in the same individuals. Although *C. pneumoniae* has never been studies as an exacerbating cause of early onset Alzheimer's disease, the association between epsilon 4 and *C. pneumoniae* raises this possibility as a hypothesis for future study.

The emphasis on infectious causation and the genetic variability in susceptibility to infection may also help clarify some confusion over infectious causation of other members of the suite of epsilon 4-associated diseases. Epidemiological patterns, fitness costs, and the low monozygotic twin concordance associated with multiple sclerosis, for example, implicate infectious causation (*Gilden*, 1999). Although the initial report (*Sriram* et al., 1998) of an association between *C. pneumoniae* and multiple sclerosis was roundly dismissed by experts, a recent independent confirmation of this finding lends much strength to the hypothesis (*Munger* et al., 2003). This association is bolstered by the finding that a *C. pneumoniae* specific peptide cross reacts serologically with a portion of myelin basic protein (an antigen that stimulates the autoimmune response of multiple sclerosis) and causes an MS-like disease in rats (*Lenz* et al., 2001). *C. pneumoniae* is most strongly associated with severely progressive

multiple sclerosis (*Munger* et al., 2003), as is epsilon 4 (*Evangelou* et al., 1999; *Hogh*, 2000; *Fazekas* et al., 2001; *Enzinger* et al., 2004); this parallel accords with the evidence that epsilon 4 increases vulnerability to *Chlamydia pneumoniae*. Indeed this association between severe multiple sclerosis and *C. pneumoniae* was predicted on the basis of the association between severe multiple sclerosis and the epsilon 4 allele (*Ewald* and *Cochran*, 2000).

These arguments offer a broad causal perspective on epsilon 4-associated chronic diseases. Rather than viewing epsilon 4 as a deleterious allele that damages cardiovascular and neuronal tissue by disregulating the transport and reactivity of lipids and cholesterol, this new perspective considers the epsilon 4 allele to be an Achilles heel that makes the person vulnerable to *C. pneumoniae* infection. This argument thus casts *C. pneumoniae* infection, but not epsilon 4, as a primary cause of the epsilon-4-associated diseases.

Unlike the thrifty allele hypothesis, this genetic-vulnerability-to-infection hypothesis is consistent with the available information on distribution of apolipoprotein E alleles in different populations. Whereas the thrifty allele hypothesis links the onset of a disadvantage associated with the epsilon 4 allele with the onset of rich agricultural food supplies, the genetic-vulnerability-to-infection hypothesis links the disadvantage with events that that favour pathogen transmission and could have occurred long before the onset of agriculture. This disadvantage of epsilon 4 could have arisen before the onset of agriculture if human populations were increasing, people were living increasingly in interior dwellings, or lifespan was increasing.

The epidemiology of *C. pneumoniae* fits this scenario especially well. It is a respiratory tract pathogen that appears to be present in all human populations; it therefore can persist across a broad range of population densities, technologies, and social structures. The persistence in small populations probably is attributable in part to its ability to cause persistent infections in humans. The intensity of exposure to *C. pneumoniae*, however, surely must have been less in hunter-gatherer societies where *Chlamydia* coughed into the outside air tend to be quickly diluted and destroyed by solar radiation. Conversely as population density increases it is probably transmitted to individuals more frequently or in higher doses. As is generally the case for pathogens it would undoubtedly persist more continuously in larger human populations because the chance of local extinctions would decrease. These transitions should tend to increase the negative effects of *C. pneumoniae* per vulnerable individual in the population and hence the relative fitness disadvantage associated with epsilon 4. Any increase in human lifespan would also tend to increase the negative fitness effects of *C. pneumoniae* on vulnerable people, because most of the life-threatening disease for which *C. pneumoniae* is a suspect tend to occur in the later decades of life, typically after the fourth decade. With longer lifespans, negative events that would occur in these later decades of life would have a greater negative impact on the fitness of diseased individuals. In accordance with this hypothesis, recent evidence from tooth wear suggests that around 30,000 years ago--about 20,000 years before the onset of agriculture--human population size and longevity increased substantially, with a five-fold increase in the proportion of people living past age 30 (*Caspari* and *Lee*, 2004). Perhaps this change contributed to the disfavouring the epsilon 4 allele by increasing the negative effects of *C. pneumoniae* or other epsilon 4-associated pathogens. During the agricultural period this disfavouring of epsilon 4 may have increased

further as a result of further increases population size and interior dwelling, thus explaining the differences in allele frequencies between modern hunter-gatherers and populations with a long agricultural tradition.

INTERPLAY OF ENVIRONMENTAL RISK FACTORS AND INFECTION

The preceding overview illustrates the importance of considering genetic associations with disease in the context of infectious causes. Non-infectious environmental risk factors may similarly influence the expression of diseases in ways that only make sense if underlying infectious causes are recognised. Atherosclerosis provides a particularly important example of this problem, because atherosclerosis is so damaging and because so much is known about risk factors associated with atherosclerosis. As discussed above, the association between epsilon 4 and *C. pneumoniae* resolves some of the questions raised by the geographic patterns of the epsilon alleles. Integrating hypotheses of infectious causation similarly resolves questions that are raised by analyzing each of the major non-infectious environmental risk factors for atherosclerosis. As is the case with epsilon 4 allele, non-infectious environmental risk factors for atherosclerosis may be not only consistent with infectious aetiologies but difficult to explain without invoking infectious aetiologies (see also: *Saikku*, 1995; *Leinonen* and *Saikku*, 1999).

Smoking

Tobacco smoking is a risk factor for atherosclerosis (*Berenson* et al., 1998; *Zieske* et al., 1999). But is smoking is a primary cause of cardiovascular disease or an exacerbating influence? It is reasonable to propose that harmful components of tobacco smoke directly damage the linings of arteries causing them to accumulate fat and cholesterol. But smoking also contributes to pulmonary infection; it is, for example, associated with *C. pneumoniae* infection which infect macrophages in the lungs that subsequently spread systemically (*Saikku*, 1995; *von Herzen*, 1998; *Mizooka* et al., 2003). Either hypothesis--direct damage from smoke or indirect damage through exacerbation of respiratory tract infections--could explain the evidence from studies of smokers.

The direct-damage-from-smoke hypothesis, however, seems problematic as an explanation for the increased risk for cardiovascular disease associated with exposure of non-smokers to smoke from smokers. This exposure, termed "passive smoking," has been implicated as a risk factor for atherosclerosis (*He* et al., 1999) and atherosclerosis-associated diseases such as stroke (*Bonita* et al., 1999). The increased risk associated passive smoking is about one-third of the increased risk associated with smoking (*He* et al., 1999) even though passive smokers inhale only about 1% of the amount of smoke that is inhaled people who smoke 20 cigarettes per day (*Pechacek* and *Babb*, 2004). The risk associated with passive smoking seems way out of proportion to the small amount of smoke inhaled by passive smokers relative to smokers (*Bailar*, 1999). Lab tests have documented negative effects of small amounts of smoke on the functioning of platelets, vascular endothelium, myocardial exercise tolerance, antioxidants and lipid metabolism (*Valkonen* and *Kuusi*, 1998; *Howard* and *Thun*, 1999). These findings lend some credence to the hypothesis that second-hand smoke could contribute directly to atherosclerosis, but these effects do not negate the difficulties inherent in interpretations that presume that the negative effect per unit of smoke is

much greater at very low doses than at high doses. If the effect of smoke were largely from generation of interactive intermediates one would expect a more liner relationship as has been found between tobacco smoke and lung cancer (*Pechacek* and *Babb*, 2004). An exponentially increasing dose-response curve would also be reasonable if the defences that are effective at low exposures to smoke become overwhelmed at high exposures. From an evolutionary perspective the hypothesised effects of extremely small amounts of inhaled smoke on life-threatening disease seem especially unlikely for humans, who have spent most of their evolutionary history in smoky environments (*Ewald* and *Cochran*, 2000). Although seemingly illogical, it is possible that cigarette smoke contains some particular compounds for which humans have not evolved the ability to detoxify at low levels and for which increasing doses are progressively much less damaging, but the disproportionately large effects of second hand smoke relative to the smoke inhaled by smokers remains a paradox if the effects on passive smokers are direct effects of smoke.

But this paradox is resolved if the relevant effects of smoke are indirect, occurring through exacerbation of infectious causes of atherosclerosis. The risk to passive smokers may thus arise because of exposure to the more florid or more frequent infections of smokers rather than to the second-hand smoke itself (*Ewald* and *Cochran*, 2000). This hypotheses seems especially feasible because it assumes only that the infection-proneness of smokers would increase the transmission of *C. pneumoniae* or some other pathogen sufficiently to elevate the risk among those exposed to second hand smoke by one-third. Smoking suppresses immune function and is associated with elevated rates and intensities of a variety of infectious diseases as well as diseases suspected of being caused by infection (*Sopori*, 2002). Associations between exposures to second-hand smoke and increased frequencies of respiratory tract infections have been documented (*Takala* and *Clements*, 1992; *Vadheim* et al., 1992; *Arnold* et al., 1993; *Sorpori*, 2002). A similar argument applies to *Porphyromonas gingivalis*, which is a cause gingivitis and periodontal disease and is present in atherosclerotic lesions (*Stoltenberg* et al., 1993; *Haraszthy* et al., 2000; *Eggert* et al., 2001; *Kuroe* et al., 2004). Smoking is strongly associated with periodontal disease, which in turn is strongly associated with stroke and myocardial infarction. Evaluations of associations between smoking and *P. gingivalis* have given mixed results, though overall it appears that the prevalence of *P. gingivalis* at different sites in the mouth is greater among smokers than non-smokers, even though the presence or absence of *P. gingivalis* differs little if at all between smokers and non-smokers (*Haffajee* and *Socransky*, 2001). Non-smoking partners of smokers are also more likely to have exacerbated periodontal disease (R.J. Genco, unpublished data), presumably because *P. gingivalis* and other causal organisms are transmitted by kissing or other salivary contact to the non-smoking contacts of smokers. A recent study of the relationship between smoking, infection, and the development of atherosclerosis showed that the atherosclerosis was significantly associated with smoking only when an indicator of chronic infection (particularly chronic obstructive pulmonary disease, chronic bronchitis, or periodontitis) was present (*Kiechel* et al., 2002). This finding accords with the idea that it is the second hand pathogens are the culprit rather than the second hand smoke, because these illnesses are associated with pathogens that are candidate causes of atherosclerosis: the pulmonary diseases are associated with *C. pneumoniae* and periodontitis is as-

sociated with oral bacteria, particularly *P. gingivalis, Actinobacillus actinomycetemcomitans,* and *Bacillus forsythus.*

Lipids

Lipid accumulation in atherosclerotic lesions has long been recognised as a hallmark of atherosclerosis. This association has led to the conclusion that high fat diets contribute directly to atherosclerosis. Although this hypothesis is intuitive--too much input of fat leads to too much accumulation of fat in the arteries--it has been readily accepted over the past 30 years with scanty evidence (*Taubes*, 2001) and almost no consideration of alternative hypothesis that could explain the development of atherosclerotic lesions in the absence of high fat diets. Nor has the evidence allowed the parsing of the lipid hypothesis to distinguish exacerbating effects of lipids from initiating effects. Initiating effects have been hypothesised to involve reactive lipid compounds that may damage to the endothelium, but this idea raises other concerns about causality: If reactive lipids are important initiators of damage, why is there so much variability from person to person in the atherosclerosis that is uncorrelated with lipid intake? And if this variation is due to variation in the degree of disregulation and reactivity of lipids, why is vulnerability so variable from person to person?

An evolutionary perspective places considerable weight on these questions because people who are genetically vulnerable to such disregulation and damage should have had this vulnerability weeded out by natural selection. The resolution is that some environmental factor must be generating the disregulation. *C. pneumoniae* is a candidate because it induces cellular oxidation of low-density lipoproteins and lipid accumulation in macrophages, which are thus transformed into foam cells (*Kalayoglu* et al., 1999; *Muller* et al., 2003). This finding is important because the presence of foam cells is a hallmark of the early stages of atherosclerosis. Because *C. pneumoniae* is lipophilic, hyperlipidaemia and obesity may contribute to atherosclerosis as an exacerbating response to *C. pneumoniae* as a primary cause. This hypothesis also applies to *P. gingivalis*, which can similarly stimulate lipid aggregation in macrophages and their transformation into foam cells (*Miyakawa* et al., 2004).

Alcohol

Another problem with the lipid hypothesis is that high fat diets are not associated with high rates of cardiovascular disease in some geographic areas. In France, for example, diets are high in fat but cardiovascular disease is only approximately one-third the rate in other western countries with comparable fat intake (*Gorinstein* and *Thrakhtenberg*, 2003). This anomaly in the association between high fat diets and atherosclerosis is attributed to relatively high intake of wine in France. Although it was originally thought that wine might uniquely suppress development of atherosclerosis, it is now clear that alcoholic beverages generally provides protection. The emerging view is that alcohol and some phenolic compounds found in wine and beer may have beneficial effects; the evidence mustered in defence of these mechanisms, however, is mostly indirect, being documentations of effects on lipid characteristics that are presumed to be causes of atherosclerotic damage. Lipids researchers are generally presuming that any direct effects of alcohol are on lipid metabolism (*Gorinstein* and *Thrakhtenberg*, 2003) even though infectious agents, such as *C. pneumoniae* are known to alter lipid sequestration and metabolism. Because alcohol has antimicrobial effects, research into the effects of alcohol on atherosclerosis must consider indirect effects of alcohol on atherosclerosis via effects on candi-

date microorganisms. It is important to assess for example whether alcohol levels that occur in the blood could inhibit the growth of *C. pneumoniae* in macrophages and the transformation of *C. pneumoniae*-infected macrophages into foam cells.

Garlic

Garlic is another dietary component that has attracted interest because of its apparent beneficial effects on cardiovascular disease and other chronic ailments. Evidence of beneficial effects has led to studies that have attempted to determine the particular biochemical and physiological influences of the components in garlic. Effects on serum lipids, blood pressure and platelet aggregation have been studied (*Rahman*, 2001; *Brace*, 2002). Though some studies have reported beneficial effects on these manifestations of disease, results from different investigators have been contradictory (*Brace*, 2002). Most randomised, placebo-controlled studies, for example, have not support the proposed suppressive effect of garlic on serum lipids (*Brace*, 2002). Evidence for a suppressive effect on blood pressure and platelet aggregation are also inconclusive (*Brace*, 2002). Like alcohol, garlic is known to have powerful antibacterial effects (*Billing* and *Sherman*, 1998; *Ankri* and *Mirilman*, 1999; *Harris* et al., 2001; *Lee* et al., 2003). Assessments of the value of garlic in protecting against atherosclerosis depend on the extent to which a mechanism of action can be demonstrated. The evidence referred to above suggests that the failure to demonstrate such a mechanism of action to date may result from an inappropriate focus. Research has investigated direct effects of garlic on correlates of atherosclerosis but not on infectious agents that may be the primary cause of atherosclerosis. Studies of effects of garlic on the growth and survival of the pathogens that have been implicated in atherosclerosis are needed. As is the case with studies of alcohol, such studies need to address whether even slight inhibition of pathogens *in vivo* could result in protection by allowing immune responses to better suppress the pathogens. Such studies also need to consider whether any physiological effects of garlic (e.g., on lipid levels) represent direct effects of garlic on human physiology or indirect effects that are brought about by suppressing the effects of lipid-altering pathogens.

Iron

High iron levels have been associated with atherosclerosis (*deValk* and *Marx*, 1999). As is the case with garlic, most of the research on this association has investigated whether iron directly influences some biochemical interaction with cells. One hypothesis proposes that iron ions oxidise fat, which in turn damages the arteries. Evolutionary considerations cast doubt on this sort of explanation. Humans have probably had substantial iron and fat in their diet throughout our evolutionary history, because humans hunted animals for food. Adaptations for protecting against damage from iron-induced reactivity of fats should be well in place. These mechanisms could not have evolved in response to iron supplements found in vitamin pills, but comparisons of dietary iron (e.g., from red meat) with iron supplements indicate that regulatory mechanisms control dietary iron less effectively. So it is dietary iron--the form that humans should have evolved to cope with--that is the bigger problem.

Iron may influence the progression of atherosclerosis indirectly by enhancing the growth of pathogens (*Sullivan* and *Weinberg*, 1999). Bacteria, like human cells, need iron. If iron levels are not too high within the body, our iron sequestering proteins can bind the free iron, keeping it from the pathogens. Bacteria also produce iron-sequestering

proteins to usurp it for their own use before host sequestration mechanisms make the iron unavailable. If iron levels rise through excess iron in the diet, the ability of the body to sequester iron may be compromised, allowing the bacteria to acquire it, reproduce and consequently cause more damage. This argument leads to the hypothesis: the association between increased iron intake and exacerbations of atherosclerosis results from an indirect enhancing effect of iron on the pathogens that cause atherosclerosis. This hypothesis is related to the lipid hypotheses mentioned above. Unless the full range of alternative hypothesis is considered an association between atherosclerosis and red meat diets might be accepted as evidence of lipid induced disease. A broader consideration emphasises not only the feasibility but also the parsimony of hypotheses that consider interaction of pathogens with lipids and iron as a mechanism by which red meats and lipids may exacerbate atherosclerosis.

Inflammation

Associations between atherosclerosis and indicators of inflammation, such as C-reactive protein (CRP) have led to an emphasis by some on the role of inflammation in the pathogenesis of atherosclerosis. Although this association clarifies the pathogenesis of atherosclerosis, one of the most important parts of a full causal explanation is the mechanism by which the inflammatory process is switched on. This important aspect is generally glossed over in descriptions of the role of inflammation in atherosclerosis, but it is central to an understanding of the primary causes of the disease. The initiating event is now often ascribed to collections of factors such as the oxidised lipoprotein-cholesterol complex, injury, and infection (*Willerson* and *Ridker*, 2004). Reference to "injury" does not resolve the problem of primary causation but rather raises the question, "What causes the injury?" Similarly, reference to oxidised lipoprotein-cholesterol complexes raises the question, "What causes the oxidation, and why is it so variable from person to person?" In contrast, reference to infection does point toward a hypothesis of primary causation, because infectious processes may cause injury and production of reactive intermediates that contribute to oxidation of compounds such as lipoprotein-cholesterol complexes. Accordingly, CRP is elevated in persistent *C. pneumoniae* and *P. gingivalis* infections (*Huittinen* et al., 2003; *Craig* et al. 2003; *Kuroe* et al., 2004).

One source of confusion pertains to the tightness of the correlation between a risk factor and measurements of disease. The tightness of such correlations is particularly important in identifying clinical markers of progression to damaging illness. But it is less relevant to discussions about primary causation, because exacerbating causes that are downstream in the process of causation may be more tightly correlated with resulting damage than the primary causes that set the entire process in motion. This generalisation is especially relevant to chronic diseases for which the process of causation may take place over years or decades. CRP, for example, is a stronger predictor of severe cardiovascular events than in *C. pneumoniae* infection. CRP may therefore be a better indicator of risk, even if the elevated CRP is initiated by *C. pneumoniae* infection, but the tight correlation between CRP and cardiovascular damage tells us little about the primary causes of atherosclerosis. Linking CRP with infection offers a more complete explanation. *C. pneumoniae* infection has been associated with elevated CRP in early and late phases of cardiovascular disease, and risk of coronary events is greater when *C. pneumoniae* positivity is associated with elevated CRP (*Huittinen* et al., 2003; *Tasaki* et al., 2004). These find-

ings are consistent with a primary causal role for *C. pneumoniae*. Elevated CRP may serve as an indicator for those *C. pneumoniae* infections that are more likely to cause damaging effects much as elevated histamine serves as an indicator for bee stings that are more likely to cause damaging effects. The fact that elevated histamines is more strongly associated with life-threatening anaphylactic shock than the presence of a bee-sting does not argue against bee stings being the primary cause of anaphylactic shock. In the same way, a stronger association between elevated CRP and atherosclerosis does not logically lead to the conclusion that CRP should be considered a cause of atherosclerosis to the exclusion of *C. pneumoniae* (or any other pathogen) as the primary cause.

This argument also bears on interpretation of protective effects of non-steroidal anti-inflammatory drugs such as aspirin. Use of these drugs has been associated with protection against several chronic illnesses including cardiovascular events and Alzheimer's (*Etminan* et al., 2003; *Nilsson* et al., 2003). Although some attribute the beneficial effect of aspirin on cardiovascular events to blood thinning and beneficial effects of aspirin on other chronic diseases to other mechanisms, it is more parsimonious to consider that aspirin alters some common pathological process. The inflammatory process is the obvious choice. If the anti-inflammatory effect is the important commonality, bringing infectious causation into the picture alters interpretations by implicating an alteration of the infectious process, namely the inflammatory response to it. Because chronic diseases are those that are not controlled by the immunological defence, it is reasonable to expect that the immunological defence will be particularly damaging in chronic infections as compared with acute infections because the inflammatory response evolved largely as a defence against infection. If it is successful the infection will tend to be short-lived and any disease it causes will be acute. Accordingly effects of aspirin are generally beneficial for chronic diseases but generally detrimental for acute infectious disease (*Ewald*, 1994).

IMPLICATIONS FOR THE FUTURE

If effects of all the non-infectious risk factors for coronary artery disease are combined, only about half of the overall risk can be explained (*Muhlestein*, 2002). This finding indicates that research needs to look beyond the current list of non-infectious risk factors. The association with epsilon 4 has led some researchers to believe that a search for more genetic determinants is warranted. Current evidence indicates, however, that epsilon 4 creates a genetic vulnerability to infectious causes of atherosclerosis and the other epsilon-4-associated diseases rather than directly causing the damage that characterises these diseases. Evolutionary considerations of fitness load similarly indicate that genetic causes of these diseases--particularly atherosclerosis and multiple sclerosis--will be of minor importance relative to other causes. Detailed consideration of non-infectious environmental risk factors for atherosclerosis illustrates how these risk factors are better explained in the context of infection. This integrative perspective offers several lines of inquiry that can improve our understanding of the primary and exacerbating causes of disease through the testing of hypotheses that specify environmental and genetic influences on infectious processes.

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