Toll the bell for another genetic association?

Bernard Keavney

Institute of Human Genetics, Newcastle University, UK

Online publish-ahead-of-print 6 October 2006

This editorial refers to 'Toll-like receptor 4 gene polymorphisms and myocardial infarction: no association in a Caucasian population' by W. Koch et al., on page 2524.

The reliable identification of common genetic differences between individuals, which could account for a proportion of a person’s susceptibility to atherosclerosis, has so far been difficult to achieve. The field has been characterized by non-replicable associations, often initially claimed in studies far too small to provide robust evidence, and subsequently contradicted by much larger studies. Indeed, it could be argued that after huge expenditure of time and effort by many groups, only the association between the ε-4 allele of the apolipoprotein E ε2/ε3/ε4 polymorphism and coronary heart disease risk has ‘cast-iron’ evidence in its favour both from large studies involving several thousands of disease cases, and from meta-analyses. Recently, however, there are more encouraging signs, as several groups present results obtained in studies large enough to confirm or refute the presence of the rather small-sized associations (relative risks of disease amounting to 15–20% or so), which are likely to be observed for most genetic effects. Koch et al.1 present results from substantially the largest study to investigate the association between polymorphisms of the Toll-like receptor 4 (TLR-4) gene and risk of MI.

TLR-4 is a member of a family of receptors that have critical functions in the function of the innate immune system (i.e. that arm of the immune system that requires no previous exposure to antigen to mount a full response). These receptors recognize evolutionarily conserved molecular patterns that are associated with particular classes of pathogen. The Toll-like receptors gain their name from the Toll gene of the fruit fly Drosophila (which has only innate immunity); Toll is a cell surface receptor which is essential for the production of antimicrobial peptides. In mammals, there are at least 12 different Toll-like receptors that recognize different unique structural components. The principal function of TLR-4 is to recognize the lipid A (endotoxin) moiety of Gram-negative bacterial lipopolysaccharide (LPS). LPS is the main inducer of shock and death in Gram-negative sepsis. The TLR-4 signal is transduced through a complex pathway involving the surface receptor CD14 and the downstream adaptor molecule myeloid differentiation factor 88 (MyD88), ultimately resulting in the nuclear translocation of NF-kappa-B and transcription of target genes for proinflammatory cytokines such as interleukin-6 and tumour necrosis factor alpha. TLR-4 is also activated by other exogenous ligands, such as chlamydial heat shock proteins, and endogenous ligands such as minimally modified LDL, HSP60, and fibronectin extra domain A.

Atherosclerosis is an inflammatory disease, the risk of which is correlated with plasma levels of a variety of markers of inflammation, and possibly with the presence of seropositivity for certain chronic infections such as Chlamydia and Helicobacter pylori. Low-level endotoxaemia was first shown by Wiedermann et al.2 to be associated with the subsequent development of carotid atherosclerosis. One attractive theory to explain these observations is that chronic infection, particularly by Gram-negative pathogens, subjects arteries to a long-term milieu of inflammation and oxidative stress, which is contributory to the development of atherosclerotic plaques. If so, diminished function of TLR-4 might be protective against atherosclerosis. There is supportive evidence for this concept from experiments in animal models of atherosclerosis: Michelsen et al.3 showed that ‘double-knockout’ mice lacking both the apolipoprotein E gene and either the TLR-4 or downstream MyD88 gene developed less severe atherosclerosis (a 28% decrease in atherosclerotic plaque in TLR-4/APOE double-knockouts) when compared with reference mice lacking only the apolipoprotein E gene. In 2000, Arbour et al.4 identified two genetic polymorphisms in humans, which changed the amino acid coded at position 299 (glycine rather than aspartate) and position 399 (isoleucine rather than threonine) of the TLR-4 protein. These polymorphisms tended to occur together on the same chromosome, at a frequency of about 6% of chromosomes in Caucasians (meaning that around 11% of the population would carry one copy of the rare alleles 299Gly and 399Ile, and 0.36% two copies). These authors tested the bronchospastic response to inhaled LPS in genotyped volunteers, and found that the dose response slope relating reduction in FEV1 to cumulative LPS dose was one-third as steep in heterozygotes and one-sixth as steep in rare homozygotes, that is, rare allele carriers were hyporesponders. Moreover, transfection assays showed that the 299Gly variant reduced NF-kappa-B activity following LPS stimulation by one-third, and abolished the interleukin-1-alpha response to LPS in airway endothelial cells. This moderate-to-low frequency polymorphism therefore appeared to have large functional effects on TLR-4 function.5

Subsequently, Kiechl et al.6 studied the effects of these TLR-4 variants on plasma inflammatory markers and...
carotid atherosclerosis in 810 subjects recruited from a general population. They found a lower risk of carotid atherosclerosis determined using a composite score [OR 0.54 (95% CI 0.32–0.98)], a 10% lower carotid intima–media thickness, and 10–20% lower levels of a number of plasma inflammatory markers including interleukin-6 and fibrinogen in the 55 carriers of the 299Gly allele in their cohort. Carriers of the TLR-4 variants also appeared to be more susceptible to severe infection. These results supported the idea of a ‘trade-off’ between the risks of acute infection and of atherosclerosis mediated through genetically determined inter-individual differences in the intensity of response to chronic infections. However, the number of carriers of the relatively uncommon TLR-4 variants in this study was quite small, and the confidence intervals correspondingly wide. With respect to exogenous ligands of TLR-4 (such as endotoxin and chlamydial proteins), a number of large-scale trials have now investigated whether antibiotic therapy given to individuals at high risk of cardiovascular events modifies future event rates, and these have been negative. However, these clinical trials do not address whether differential activity of TLR-4 in response to endogenous ligands (such as minimally modified LDL) could contribute to differences in atherosclerosis risk. Sufficiently large-scale genetic epidemiological evidence has the potential to address such questions regarding the causality of hypothesized novel pathways to disease, and this is the context of the study by Koch et al.

Koch et al. investigated the association of the Asp299Gly and Thr399Ile polymorphisms with MI in 3657 MI cases and 1211 Caucasian controls from South Germany. They find no association of the 299Gly allele with MI in their cohort. Carriers of the TLR-4 variants also appeared to be more susceptible to severe infection. These results supported the idea of a ‘trade-off’ between the risks of acute infection and of atherosclerosis mediated through genetically determined inter-individual differences in the intensity of response to chronic infections. However, the number of carriers of the relatively uncommon TLR-4 variants in this study was quite small, and the confidence intervals correspondingly wide. With respect to exogenous ligands of TLR-4 (such as endotoxin and chlamydial proteins), a number of large-scale trials have now investigated whether antibiotic therapy given to individuals at high risk of cardiovascular events modifies future event rates, and these have been negative. However, these clinical trials do not address whether differential activity of TLR-4 in response to endogenous ligands (such as minimally modified LDL) could contribute to differences in atherosclerosis risk. Sufficiently large-scale genetic epidemiological evidence has the potential to address such questions regarding the causality of hypothesized novel pathways to disease, and this is the context of the study by Koch et al.

Koch et al. investigated the association of the Asp299Gly and Thr399Ile polymorphisms with MI in 3657 MI cases and 1211 Caucasian controls from South Germany. They find no significant evidence of association [OR 0.86 (95% CI 0.70–1.05)] for the likely functional Asp299Gly variant. This study has two important strengths: first, its large size; and second the careful characterization of the cases and controls by clinical assessment, coronary angiography, and echocardiography. A meta-analysis involving a total of seven studies is also presented, and several important points emerge from that. First, Koch et al. have more than doubled the number of cases that have been studied for this association, taking the total number of cases studied to 5926. Second of the six previously published studies, four involved between 95 and 183 cases of coronary disease, and were therefore far too small to provide robust results. Third, a formal test for heterogeneity between the studies in the meta-analysis is highly significant (P = 0.01); the sources of this heterogeneity are not formally explored by Koch et al., but sources of heterogeneity that have been identified in previous genetic meta-analyses often include the tendency of smaller published studies to achieve more extreme results than larger studies (indicative of publication bias). The meta-analysis yields a combined odds ratio of 0.90 (95% CI 0.68–1.19) for MI among carriers of the 299Gly allele when compared with non-carriers. These wide confidence intervals may be explained by the fact that the only other sizeable study yet performed, that of Edfeldt et al., which included 1172 cases and 2689 controls, found a marginally significant association in the opposite direction to that expected from the pathobiological argument, that is, towards increased risk of cardiovascular disease in those carrying 299Gly.

So, is it time to ‘toll the bell’ for this genetic association? At present, it does not seem that there is sufficient evidence to consider the association refuted, despite the admirable study of Koch et al. The meta-analysis remains consistent with a reduction in the risk of MI of up to 30% in carriers of the 299Gly allele. This question exemplifies the difficulty of studying genetic variants of modest allele frequency, for which very large studies indeed are required to allow accurate inferences to be drawn, even if the phenotypic effect of the variant in question might be quite large. Indeed, as here, even the largest single studies may not be large enough to reach definitive conclusions. In this situation, meta-analysis could contribute much. However, as in the work of Koch et al., substantial unexplained heterogeneity, which limits the security of the inferences drawn, is frequently observed in genetic meta-analyses. Meta-analysis of individual participant data has the very significant advantage over literature-based meta-analysis, that sources of heterogeneity between studies can be explored and accounted for in much greater detail; the principal disadvantage of this approach is its labour-intensive nature, which means that it can only be applied very selectively. In the case of the TLR-4 Asp299Gly polymorphism, further data from other large studies will no doubt shortly appear; the presence of a proven functional polymorphism in a pathway of potential key importance would make this question attractive for such an individual participant data analysis.

Conflict of interest: none declared.

References


