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Power: not only a matter of numbers, but also of design

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In this issue, Hommels *et al.* reported a study on a possible relationship between the Asp²⁹⁹Gly polymorphism in the Toll-like receptor-4 (TLR-4) gene and advanced aortic atherosclerosis.¹ They did not find a significant relationship in their study, although others have reported a protective effect.^{2,3} The authors comment that the conclusion of their study should be taken with caution because of the small number of subjects. The study raises the question whether small studies are useful to study the relationship between common polymorphisms and disease.

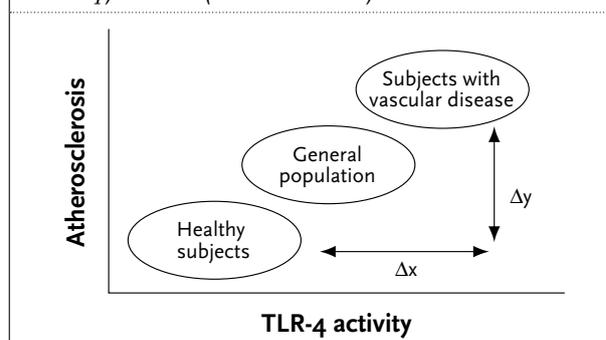
First of all, I would like to stress the enormous advantage of genetics in the search for causes of disease. Although the relationship between inflammation and cardiovascular disease has been known for many years, nongenetic observational epidemiological studies are hampered by confounding and reverse causality. For instance, if somebody does find a relationship between C-reactive protein (CRP) and cardiovascular disease in a case-control study, the question arises whether this relationship could be explained by an increase in CRP through cardiovascular disease or vice versa. Moreover, several other factors could explain such a relationship through confounding. Using the concept of Mendelian randomisation, Davey Smith and Ebrahim eloquently describe the advantages of the use of genetic determinants instead of plasma markers.⁴ Besides this conceptual advantage, using genetic determinants also has some practical advantages. When DNA is isolated, many genetic determinants are easily available via high throughput facilities and genetic measurements are less influenced by storage conditions compared with the measurement of plasma markers. However, an important drawback of genetic studies is that in complex disease the effect of a single polymorphism is usually small and often dependent on the genetic and environmental background it is evaluated in. This requires not only an adequate sample size but also an adequate study design.

STUDY DESIGN

The study of the effect of a polymorphism on the incidence of a certain disease requires an effect measure which could be defined as the ratio between a difference in a certain determinant (X) and the difference in a certain outcome (Y). In case of the study on TLR-4 and atherosclerosis, one would like to know whether a change in TLR-4 activity is accompanied by a change in (incidence of) atherosclerosis (*figure 1*).

In fact, such a study could start with a contrast in X or with a contrast in Y. In other words one could start by recruiting a group of people that show a contrast in atherosclerosis and look for TLR-4 activity, or vice versa. Hommels *et al.* started their study with a group of patients with hypertension who underwent scanning of the abdominal aorta. The question is whether the contrast in atherosclerosis is comparable with a case-control study as carried out by Ameziane *et al.* who studied patients with vascular disease on the one hand and healthy subjects (hospital employees and blood donors) on the other.³

Figure 1. The reliability of effect estimation of a possible relation between TLR-4 activity and atherosclerosis depends on the contrasts in X (TLR-4 activity) or in Y (atherosclerosis)



The contrast in atherosclerosis may be smaller when all patients have hypertension, which is a risk factor for atherosclerosis. In fact the authors made a comparison between subjects with advanced aortic atherosclerosis and less advanced aortic atherosclerosis.

Figure 1 shows that a comparison between subjects with vascular disease and a healthy population shows the strongest contrast. However, if a certain effect is found one is not sure that it is an effect of the determinant on the occurrence of disease or on the occurrence of healthiness. Therefore, it is recommended to take the control group from the general population.

A second point is the contrast in X. The hypothesis is that a change in TLR-4 activity is accompanied by a change in atherosclerosis. Because TLR-4 activity is difficult to measure, the authors measured the Asp²⁹⁹Gly TLR-4 polymorphism, which is a genetic determinant of TLR-4 activity. This is a meaningful approach, which is in some ways better than measuring TLR 4 activity, because studies that use genetic determinants are less prone to confounding. However, only the wild-type and heterozygote genotypes were found. The question is whether there is enough contrast between wild-type and heterozygote genotype in TLR-4 activity.⁵ If there is no difference in TLR-4 activity in wild-type and heterozygotes, the estimation of effect will strongly tend to no effect.

A third point is the question of confounding. Hommels *et al.* presented an uncorrected odds ratio. As stated before, studies that use polymorphisms as determinants are less prone to confounding than studies that use plasma markers. Therefore, the unadjusted estimate could be regarded as a good measure of effect. However, this does not rule out confounding. As shown in their table there are big differences in age. Therefore I would recommend also presenting adjusted odds ratios. This is comparable with the case of randomised trials with an uneven distribution of covariates. Randomisation should in theory result in an equal distribution of covariates, but if it does not this could allow confounding to occur. Another advantage of adjustment for age is that this would increase the contrast in atherosclerosis, which is strongly age dependent.

SAMPLE SIZE

The fourth point is on numbers. A genetic study of 123 subjects with a polymorphism that is supposed to give at the most a small effect is at least underpowered to find a significant effect. However, one should keep in mind that the point estimate of effect is not influenced by the sample size. Whether it is useful to publish the results of small studies is a matter of debate.

In a recent study Morgan *et al.* tried to validate the effects of multiple genes on atherosclerosis.⁶ The authors screened the literature and found 96 polymorphic genetic variants in 75 genes that were positively associated with atherosclerosis. They subsequently screened 85 polymorphisms in 70 genes. Using appropriate statistical techniques, including correction for multiple testing, they did not find a positive correlation between any of the tested genes and the risk of atherosclerosis. This study nicely illustrates the drawbacks of this type of research.

A lot of small studies taken together make a big one. The question is whether such pooled analysis suffers from bias because journals tend not to publish 'negative' results. Recently, Borm *et al.* showed that publication bias is not a serious issue in meta-analysis of trials and it could be argued that the same holds for studies on genetic determinants of disease.⁷ It could be argued that the same holds for studies on genetic determinants of disease, although it has been demonstrated that molecular genetic research is more sensitive to publication bias than clinical trials.⁸

In conclusion, I don't argue that we should stop publishing small studies, but it is important to be very reserved in the conclusions based on such small studies, whether 'negative' or 'positive' - as is done in the paper of Hommels *et al.* Firm conclusions should be based on multiple, large-scale studies.

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