



Epidemiology / Épidémiologie

Coronary heart disease aetiology: associations and causality

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Abstract

Coronary heart disease (CHD) prevention has largely benefited in the past from the development of epidemiological research. However, the opposition association–causation is currently raised from observational data. We successively review, from some important examples, the classical methodological approach for discussing causality in epidemiology. The easy identification of DNA polymorphisms has prompted new CHD aetiological research in the past 10 years. Causality of the associations presents some special characteristics when genes are involved: necessity of replication, Mendelian randomization..., which might prove to be important in future research. **To cite this article:** P. Ducimetière, F. Cambien, *C. R. Biologies 330 (2007)*.

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Résumé

Étiologie de la maladie coronaire : associations et causalité. La prévention de la maladie coronaire a été fondée en grande partie sur le développement de la recherche épidémiologique. Cependant, les résultats d'observation posent la question de la causalité des associations. Nous passons en revue, à partir d'exemples importants, l'approche méthodologique classique permettant de discuter de la causalité en épidémiologie. Au cours des 10 dernières années, l'identification aisée des polymorphismes de l'ADN a permis de relancer la recherche étiologique sur la maladie coronaire. La causalité des associations présente des caractéristiques particulières lorsqu'elles concernent des polymorphismes génétiques : nécessité de reproduire les résultats, « randomisation mendélienne »..., qui peuvent se révéler importantes dans les recherches futures. **Pour citer cet article :** P. Ducimetière, F. Cambien, *C. R. Biologies 330 (2007)*.

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1. Introduction

Discussing the nature of associations derived from observations is a classical theme of epidemiology and science in general [1]. An explosion of observational population studies took place in the 30 last years and yielded new trails in the aetiology of common chronic

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diseases, some of them leading to important public-health consequences. However, lately, some scepticism may be noted concerning the capabilities of epidemiology to further help to disentangle multiple aetiological factors of the so-called complex diseases [2]. The molecular genetics revolution has revived the interest for aetiological work and a new generation of epidemiologists is drawn to genetic investigations. In fact, the experience now accumulated raises some interrogations that sustain the old debate about causes and associations. The publication in 2006 of two special reports by the International Journal of Epidemiology from two papers respectively by R.C. Lewontin [3] and A.V. Buchanan et al. [4] is an illustration of such a topicality.

Coronary heart diseases (CHD) constitute an important subset of chronic complex diseases and they are often considered as prototypes in that field. It is now recognized that the current longevity increase in western populations is largely due to the decline of their incidence and lethality and that these evolutions have tremendous public-health implications. Conversely, some developing countries are experiencing a rise in coronary diseases, which indicates that epidemiological transition is nearly complete among their population. It is not surprising that the epidemiological approach, which aims at studying health in populations, underwent a huge expansion. However, knowledge on the biological processes that determine the development of atherosclerosis in the arteries and further, the triggering of clinical complications, also improved considerably. A high-level synergy between the two approaches was certainly a key factor in the progress to date.

In this context, epidemiological work has applied its own methods, using its own concepts of aetiology and causation [5]. Strictly speaking, the aetiology of clinical events such as myocardial infarction, acute coronary syndrome, sudden death, or stable angina... is sought for, but in fact, it encompasses the whole life of individuals, including the silent phase of atherosclerosis development. Looking epidemiologically for more specific end-points, like the arterial process itself, became recently more feasible at least for some arterial territories and it contributes largely to the understanding of the aetiology of the CHD nebula.

We will try below to review schematically some of the main aetiological factors of CHD from a methodological point of view. In a first part, we will examine the so-called classical approach of causality before the molecular genetics era. In a second part, we will attempt to see how the identification of human DNA polymorphism may have modified this approach and may open new perspectives.

2. Classical approach

Many factors have been found associated with CHD risk in various observational study settings and considered as risk factors for the disease; however, most of them are not considered as causal factors. In some intuitive way, causality implies that one should demonstrate that modifying the factor implies a change in the disease occurrence. Conceptually simple, these notions are difficult to apply practically for a series of reasons: measures of association are generally biased due to the study structure and population on the one hand, and to the role of confounding factors on the other hand – population experiments are often unfeasible for ethical or practical reasons. Moreover, when the factor under study is a quantitative biological measurement, interventions, even pharmacological ones, which specifically target changes in this particular factor, rarely exist.

It is not surprising in such conditions that causality of a CHD risk factor has never been decided from a critical experiment, but rather from an accumulation of convergent data, observational and interventional, which progressively strengthen the credibility of the causality hypothesis. This process, which can be related to ‘Informal Bayesianism’ [4], is guided by a well-known set of nine criteria proposed by Bradford Hill [6] in 1965, which became a classic of epidemiology textbooks. It is important to note that no condition among the nine is sufficient, whereas only temporality of the association seems necessary.

2.1. Blood lipids

An elevated blood cholesterol level (more exactly cholesterol in low-density lipoproteins or LDL-cholesterol) is considered as one cause of coronary atherosclerosis and its clinical complications. It is remarkable that this conclusion, which was reached more than 30 years ago, has remained an established scientific fact until now. It has been illustrated since by a vertiginous number of reported pieces of evidence, by their extraordinary coherence and their consistency with an ever-growing number of biological underlying mechanisms. The most recent literature would certainly permit to check the applicability of Bradford Hill’s criteria to the cholesterol story. The most striking element appears to be the extraordinary congruence of the quantitative measures of the cause–effect relationship between a cholesterol change and the subsequent variation in relative CHD incidence, whatever the particular setting for their estimation: ‘ecological correlations’, individual

observations in diseased subjects or from general populations, data from migrants, experimental changes...

Clearly, results from controlled prevention trials are particularly convincing and many data are now available on statin use. Their biological effect as blockers of the endogenous cholesterol synthesis, leading to rapid and important LDL cholesterol lowering, is the essential part of their preventive action, although other mechanisms have also been evoked. A recent meta-analysis concerning 14 controlled prevention trials with statins [7] has shown a 21% decrease in the so-called major coronary events for a 1 mmol/L LDL-cholesterol lowering, with a relatively small heterogeneity index between primary and secondary prevention studies and according to the level of numerous prognostic factors including initial LDL-cholesterol. It should be noted that the first drug prevention trial with enough power, which used cholestyramine [8] with a completely different and less efficient cholesterol-lowering mechanism, indicated an extrapolated 30% reduction in CHD risk associated to a 1 mmol/L LDL-cholesterol decrease. This estimate was of the same order of magnitude (20–30%) as that, not experimental, which was observed in the intervention and placebo groups of the trial and in numerous population-based cohorts in the world [8].

In fact, in this relationship, the LDL-cholesterol level should be considered as a ‘causal marker’, or more precisely as an integrative marker of the causal biological system that regulates cholesterol uptake by peripheral cells. Although other markers might be proposed, no independent contributory effect – both observational and experimental – to the CHD risk has been consistently shown, beyond that given by LDL-cholesterol alone.

It is interesting to contrast this state of evidence with that concerning the blood high-density lipoprotein cholesterol level (HDL-cholesterol). Its association [negative] with the coronary risk, although strong and generally observed at the individual level within many populations, presents some different features: absence of ecological relationship, absence of specificity... The presently available results from preventive trials using fibrates are not sufficient to estimate with precision the specific part of risk reduction that might be ascribed to the HDL-cholesterol elevation they cause. This part, however, appears to be small [9].

Whereas the so-called ‘reverse cholesterol transport’ is likely involved among the atherosclerosis mechanisms, the role of HDL-cholesterol as a causal integrative marker is clearly questioned. Many candidates enter the list: lipid transport proteins, enzymes... but the consistency of the associations is not presently established. It is however possible that very soon, new pharmaco-

logical classes, which aim at increasing reverse cholesterol transport like CETP inhibitors or LCAT expression enhancers, might prove their efficacy [10]. Some preventive opportunities directly concern Apoprotein A1, which seems to play a pivotal role in the system [11]. It is very likely that an integrative marker other than HDL-cholesterol will be identified and that causality of its relationship to CHD risk will be open to new epidemiological investigations.

2.2. Behavioural factors

The study of behavioural factors in relation with CHD risk is rather tricky. On the one hand, most often, behaviours are not suitable to quantitative measurements: their expression is highly variable among populations and they are very likely associated with numerous confounding factors. On the other hand, experimental behaviour changes, when feasible, are difficult to obtain, which explains that preventive trials are generally non-informative, rather than negative, because only small mean changes of the risk factor were obtained between the intervention and the control groups. In these conditions, comparing the magnitude of an observational association with that of an experimental one between a behavioural factor and CHD risk is barely feasible. It is particularly the case for the role of life-long dietary habits. The causality of the so-called ‘diet heart concept’, which implies cholesterol and saturated fat intake, relies much more on the undisputed causality of LDL-cholesterol as an intermediate phenotype rather than upon direct pragmatic evidence, at least at the individual level.

The unfeasibility of controlled trials is sometimes used as an argument against causality, even though the evidence is clearly in favour of a causal role. Tobacco smoking is a good example that illustrates the fact that Hill’s criteria do not constitute a closed list of necessary and sufficient conditions for causality. The status of alcohol consumption is less clear because some doubts subsist on the alleged causal effects of moderate intake on CHD risk, especially when drinking behaviours are not taken into account [12]. In spite of careful adjustments in the analysis of observational data, the possibility of confounding, especially by psychosociological factors with potential cumulative effects during the whole life of the individuals, cannot be ruled out.

In fact, this possibility has been recently raised in order to explain the consistent dissents between observation and experiment in the association of antioxidant vitamin consumption and CHD. It is important to note

that these trials did not directly study dietary intake, but vitamin supplementation (single or multiple). Several large cohort studies have shown robust negative associations between an estimated ‘usual’ dietary intake of carotenoids, vitamins C and E, and CHD risk in men and women [13,14]. These associations were generally confirmed by observations using blood marker levels [15, 16]. The protective role of these vitamins could be anticipated by the impact of oxidative mechanisms, particularly on LDL particles, in the atherosclerotic process. The absence of preventive effect – with in some cases a suspicion of a harmful one – appeared in several trials that used relatively high doses of vitamins. Two more recent large trials using cocktails of vitamins with nutritional dosages did not disclose any effect on cardiovascular diseases and mortality [17,18]. Similar data from a supplementation trial in China [19] seem to indicate that this lack of effect is not specific to the western world but might also apply in populations with prevalent vitamin deficiencies. As suggested by Lawlor et al. [20], these dissents on causality might be explained by truly protective social and environmental long-lasting confounding factors associated with dietary patterns in observational studies, but convincing demonstrations still seem necessary before this interpretation could prevail.

2.3. Treatment adverse effects

Pharmacoepidemiology is naturally a growing field of interest, but most investigations rely on observational data, and causality of the reported associations is thus a major issue. However, the question can finally be solved when enough data are available. A causal relationship between low blood cholesterol level (possibly provoked by dietary changes or hypolipidemic drugs) and cancer risk was hypothesized following the observation of a negative association in several cohort studies. Strong but indirect arguments have been advanced to refute causality and to suggest an inverse causality bias [21]. Published statin trials data are now large enough to permit to rule out completely the hypothesis [22].

Recent studies on the cardiovascular risk associated with hormonal replacement therapy have given rise to the same kind of divorce between observation and experimentation [23]. The observed protective effect can be partly explained by different psychosocial characteristics of women taking the treatment, but an allocation bias due to a lower probability of doctor’s prescription in women who already present some risk factors (high cholesterol for instance) is likely to occur [24].

3. Associations with DNA polymorphisms and causality

By the end of the 1980s, it became apparent that linkage analysis methods, which have been successfully developed in the study of monogenic diseases, were not adapted to unravel the aetiology of complex diseases, with coronary heart disease as an important example [25]. The implication of multiple genes in the multiple mechanisms that intervene in their pathogenesis is likely, and the power of linkage analysis is very low in this case, contrary to association studies, at least when they are of sufficient dimension.

The fact that several known metabolic pathways are involved in the atherosclerosis process and in the determinism of its clinical complications allowed one to screen a large number of candidate genes as possibly associated with CHD risk. The working hypothesis over the last 15 years has been, more or less implicitly, that among them, a relatively small number, still to be discovered, might ‘explain’ a large part of the ‘heritability’ of the disease. The technology permitting rapid and reliable identification of DNA polymorphisms developed considerably in that period, and scientific literature gives evidence of unbridled research efforts. They led to the building of extensive biological banks containing, particularly, DNA of cases and controls, an unusual scheme in CHD observational research, but which appeared more suitable in the case where an ‘inverse causality’ bias might be considered as excluded. In parallel, the experimental study of the biological functionality of the selected polymorphisms became crucial for interpreting their pattern of association.

Consequently, we are witnessing the rise of a new category of CHD risk factors – polymorphisms of candidate genes –, which clearly implies an extension of the classical conception of causality. Indeed, their association with the disease cannot integrate any comparison with human experimental data and relevance to public health, and disease prevention is no longer direct, but it might depend on their potential interactions with modifiable environmental factors or drugs.

3.1. Polymorphisms and CHD risk

An association between one polymorphism of one candidate gene with a biological phenotype [plasma protein level for instance] is generally due to its linkage disequilibrium with one or several functional polymorphisms, which are responsible for the phenotypic effect. This is a special case of confounding, which implies that allele combinations (haplotypes) should become fac-

tors of interest. More generally, the association of one candidate gene polymorphism with the disease may be confounded by the association of other genes with the disease, linkage disequilibrium being only one possible mechanism of confounding. As a consequence, although data analysis techniques for taking confounding into account are mostly similar to those of classical epidemiology, elements of knowledge specific to molecular genetics should also be introduced and, particularly, at both the stages of definition of analysis strategy and of interpretation of results.

The published report in 1992 of a strong association of an Ins-Del polymorphism of the Angiotensin1 conversion enzyme (ACE) gene with myocardial infarction in the ECTIM case-control study [26] appeared as an illustration of the new possibilities offered by the extension of association studies to genetics. Whereas this polymorphism is not functional, it has been repeatedly shown to be associated with circulating enzyme activity. It is however remarkable that the association with CHD as such did not prove to be reproducible and meta-analysis of many reports published since then concluded to a possible mean small effect but with a large between-study heterogeneity [27]. This polymorphism has also been found to be associated with several other diseases or pathological states, but, as far as we know, no reproducible pattern of associations is presently convincing.

Apart from a small number of examples that we will discuss later, the absence of robustness of the reported associations between polymorphisms of candidate genes and CHD risk (and perhaps susceptibility to many other complex diseases) seems empirically compelling [28]. Many classic sources of variability might participate in that heterogeneity, including chance, lack of power, unknown confounders, publication bias... Variable genetic stratification has been invoked as an important source of heterogeneity in associations with polymorphisms. However, in these studies, subjects of common origin are generally selected and this effect, though existing at least theoretically, seems to have a minimal impact.

Difficulties seem in fact deeper and surely more intrinsic. Their outline is exposed in the seminal paper by RC Lewontin [3], first published in 1974, challenging the concept of heritability estimation in the setting of selection experiments in animals. He considered the case where the phenotype is a function of one genetic allele and the environment is considered as one generic variable. This function, called 'norm of reaction', is particularly complex with numerous non-linearities, interactions, feedback effects, redundancies... A local linear regression analysis at the centre of gravity of the ob-

servations can exhibit as well a positive, negative, or null marginal association of the genetic trait, depending on the values of the norm of reaction for surrounding observations. Consequently, an association observed in one sample is very unlikely replicated in another sample. In Lewontin's language, this means that 'heritability' of the trait is not intrinsic when measured by linear techniques. The only exception would be that the allele effect in the norm of reaction is constant whatever the environment value, i.e. it follows the classical analysis of covariance model.

This way of reasoning is easily transposed to observational data with numerous unobserved genes that intervene on the phenotype value in addition to the environmental variables. Complexity of this determination at the individual level is extreme, as genes may be supposed to have non-linear and mutual interactive effects. Any sample estimate of the effect of one isolated polymorphism cannot be robust, as its true value is unpredictably changing according to individuals. Replication can only be expected in special situations when marginally constant individual effects of the polymorphism are present or, more generally, when a set of interactive effects of the polymorphism with some other genetic or environmental variables is constant among individuals.

At least two relatively frequent polymorphisms of candidate genes have been somewhat reproducibly associated with CHD risk: the epsilon polymorphism of Apoprotein E (at least for the epsilon 4 allele) [29] and the 677C-T polymorphism of the MTHFR gene [30], as shown by these meta-analyses that were able to put into evidence small relative risks (1.2 to 1.4). In both cases, the association seems to be mediated by the level of an integrative blood phenotype, LDL-cholesterol, and homocysteine, respectively. Causality of the relationships however cannot be symmetrically discussed, as experimental data on the effects of reducing homocysteine levels in humans (by folate supplementation) are not complete.

3.2. 'Mendelian randomization'

Looking for the consistency of the mutual associations between gene polymorphisms, biological phenotype levels, and complex disease risk has been naturally of permanent concern as soon as large-scale genotyping became available [25]. Indirectly, this search for consistency might potentially contribute to identify modifiable environmental or drug factors that could be causally related to the disease, and this application of causality reasoning has been made popular by G. Davey Smith under the name of Mendelian randomization [31,32].

A first formulation was given by M. Katan [33] in order to add an argument pro or against causality in the relationship between low cholesterol and cancer risk. Apoprotein E polymorphism being reproducibly associated with circulating cholesterol level, an inverse relation between the epsilon4 allele and cancer should be disclosed if causality holds, but it was not the case. Indeed, the random transmission of genotypes of individuals, conditional to those of their parents, leads to consider a priori that confounding factors and inverse causality, which introduced biases in the cohort estimates of association, are excluded from the genetic setting that mimics randomization. Moreover, causality should imply some degree of consistency between direct risk estimates from the polymorphism association and those derived from the effect on the associated phenotype. Nevertheless, several complementary hypotheses are necessary and, in this example, any other aetiological link between Apoprotein E and the cancer process, directly or by association with other genes, should be excluded. Discussion on causality should therefore be specific and depend mainly on existing knowledge on mechanisms [34].

Many data analyzes of this type are currently published and some of them concern the role of inflammation in coronary disease. Experiments in humans on this topic are presently hard to imagine, whereas robust associations have been established between some simple biological parameters of inflammation and CHD risk. Any indication on the possibly causal nature of these associations might be useful for a better definition of preventive interventions, possibly using drugs.

The most studied polymorphism of beta fibrinogen gene is G-455-A in the promoter region, and the most recent analysis [35] reported an increase of 11.7 mg/L (95% confidence interval 9.9–14.2) of fibrinogen attached to the presence of allele A and a CHD relative risk of 0.98 (0.92–1.04). The causality hypothesis would imply that the relative risk associated with an increase of 1 g/L fibrinogen be 0.81 (0.46–1.40), whereas a meta-analysis of this relation [36] yielded 1.8 (1.6–2.0). These results are not in favour of the causality of the association between fibrinogen level and CHD risk. The same analysis has been applied to the association of CRP level, another ubiquitous marker of inflammation, with the same type of results and conclusion [37].

It is remarkable that in one recent cohort study [38], an haplotype of four genes involved in the IL18 biological system has been found associated with both cardiovascular mortality and IL18 serum level, consistently with the direct association between these last two variables. However, as it has already been emphasized,

the robustness of the association of the multilocus polymorphism with mortality must be established before any firm interpretation can be drawn.

4. Conclusion

Reports of observational associations of DNA polymorphisms with CHD risk and risk factors have multiplied in the scientific literature. The interrogation about causality, which was central to classical aetiological work, seems to be less crucial with polymorphism association because, if it is real, causality of the involved gene is likely, to say the least.

Thus, interrogation focused more on the robustness of the reported associations, marginally but, as we may hypothesize, also interactively with other genes or environmental factors.

Some strategies for further research in this direction are clearly required. For example, it has been proposed that multilocus biological systems be investigated as a whole for association with CHD, with the aim of identifying new integrative phenotypes that might prove to be causal [39], but necessity of replication cannot be avoided. Evidently, it is also a requisite for those studies that emphasize the importance of detecting gene–environment interactions, and epidemiologists know how replication of interaction findings is hazardous.

Finally, we should add that more global genomic and proteomic investigations are currently underway in order to bypass the limitations introduced by the selection of candidate genes. Their results will certainly give new dimensions to the association–causation dilemma and perhaps open new avenues for the future of CHD aetiological research.

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