

On Causes

Hippocrates, Aristotle, Robert Koch, and the Dread Pirate Roberts

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But let us inquire what are the causes of these things which happened to them. —Hippocrates¹

The idea of risk factors for vascular disease has evolved from a dichotomous to continuous hazard analysis and from the consideration of a few factors to mechanistic investigation of many interrelated risks. However, confusion still abounds regarding issues of association and causation. Originally, the simple presence of tobacco abuse, hypertension, and/or hypercholesterolemia were tallied, and the cumulative score was predictive of subsequent coronary artery disease.² Since then, dose responses have been defined for these and other factors and it has been suggested that almost 300 factors place patients at risk; these factors include elevations in plasma homocysteine. Recent studies shed interesting light on the mechanism of this potentially causal relationship, which was first noted in 1969.³ Aside from putative effects on vessel wall dynamics, there is now direct evidence that homocysteine is atherogenic. Twenty-fold increases in plasma homocysteine achieved by dietary manipulation of apoE^{-/-} mice increased aortic root lesion size 2-fold and produced a prolonged chronic inflammatory mural response accompanied by elevations in vascular cell adhesion molecule-1 and tumor necrosis factor- α .⁴ In long term follow-up, homocysteine levels elevated by dietary supplementation with methionine or homocysteine promoted lesion size and plaque fibrosis in these atherosclerosis-prone mice early in life, but without influencing ultimate plaque burden as the animals aged.⁵ A number of mechanisms were proposed by which homocysteine achieved this effect, including promotion of inflammation, regulation of lipoprotein metabolism, and modification of critical biochemical pathways and metabolites including nitric oxide (NO).⁶

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In the present issue of *Circulation*, Stühlinger et al⁷ advance these mechanistic insights one critical step further by defining homocysteine's effects at an enzymatic level. The group led by Lentz published an association between levels of the endogenous inhibitor of NO synthase, asymmetric dimethyl arginine (ADMA), and homocysteine in cultured endothelial cells and in the serum of cynomolgus monkeys.⁸

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Such an association is interesting because the L-arginine-NO synthase pathway seems to be a critical component in the full range of endothelial cell biology and vascular dysfunction.⁹ Stühlinger et al⁷ now show that increased cultured endothelial cell elaboration of ADMA by homocysteine and its precursor L-methionine is associated with a dose-dependent impairment of the activity of endothelial dimethylarginine dimethylaminohydrolase (DDAH), the enzyme that degrades ADMA. Homocysteine directly inhibited DDAH activity in a cell-free system by targeting a critical sulfhydryl group on this enzyme. Thus, one could envision that the balance of cardiovascular health and disease could well be determined by the ability of an intact NO synthase system to overcome environmental, dietary, and even genetic factors. In patients with altered enzymatic defense systems, elevated homocysteine, oxidized lipoproteins, inflammation, and other vasotoxins may dominate even the most potent defense mechanisms.

These studies raise a number of issues. Do we need to add to our list of established cardiovascular risk factors to accommodate new findings and associations? Is there a final common pathway for all risk factors or perhaps even a unified factor theory into which all potential risks can be grouped? And, as always, should we consider NO at the core of this universality? Finally, should we change our focus altogether and speak not of risk factors but of genetic predisposition, extent of biochemical aberration, and degree of physical damage?

If Risk Factors Are in our Past, Are Causal Agents in our Future?

It is difficult, seeing that there is no such accuracy in the Art, to hit always on what is most expedient, and yet many cases occur in medicine which would require this accuracy, as we shall explain. But on that account, I say, we ought not to reject the ancient Art, as if it were not, and had not been properly founded, because it did not attain accuracy in all things, but rather, since it is capable of reaching to the greatest exactitude by reasoning, to receive it and admire its discoveries, made from a state of great ignorance, and as having been well and properly made, and not from chance.

—Hippocrates¹

Robert Koch isolated *Vibrio cholera* and *Mycobacterium tuberculosis* using not only sound microbiological techniques but also rigorous epidemiological formulations. Koch noted that a specific microorganism can be identified with disease if it is present in all cases of disease and absent in health, if it can be isolated from diseased animal and grown in pure culture, and if the fresh microorganism, when inoculated into

a healthy laboratory animal, caused the same disease seen in the original animal and could be reisolated in pure culture from the experimental infection.¹⁰ Use of these postulates, however, was deemed problematic for chronic diseases or exposures that may have predated disease onset by years, been further triggered or exacerbated by other factors, and in situations where there was a limited conceptualization of putative causative factors and imperfect knowledge prevented precise association of causality to expected risk.¹¹ These are the very issues that confront the investigation of the root causes of cardiovascular diseases. Thus, when it became increasingly evident that tobacco abuse was potentially harmful, a more refined set of principles was used in the 1964 US Department of Health, Education, and Welfare study on the association of tobacco and health risks.¹² In these criteria, validation arose from the strength of association between factor and disease, the presence of a dose-response, lack of temporal ambiguity between exposure and disease, the fact that elimination or modification of the putative cause or host response would eliminate or reduce the disease, and the biological plausibility of the hypothesis.^{11,13} In short, it was suggested that association requires both statistical and scientific validation.

In the 12th century Maimonides offered virtually the same criteria for distinguishing between legitimate medical treatment and magic, but required either that valid procedures be based on scientific understanding, or that empirical evidence show that the proposed cure works, even if the mechanism is not understood.¹⁴ Can we today accept Maimonides' *Guide of the Perplexed* and validate associations based on one of these criteria alone? Would we reject an excellent correlation between factor and disease or the eradication of disease with reduction in risk if there was not biological plausibility? And is it similarly the case that for the most plausible of stories, one might forego a coherent and well-documented association? These issues are far from academic and have produced great pain and suffering, as well as frustration and disappointment. In modern times, the study of epidemics (Hippocrates' term for diseases whose incidence exceeds expectation) has helped us clean our water, reduce environmental damage and occupational toxin exposure, and likely has contributed to the continued decline in cardiovascular mortality and morbidity. Yet, the need for both biological and epidemiological support has also prevented many from embracing sound scientific data that, early on, linked tobacco abuse with disease, produced erroneous notions of the prevention of infectious diseases, and promoted useless and cruel interventions in the name of therapy.

Hippocrates taught that environment and lifestyle are related to disease occurrence, but he was vexed by association, noting that "... if one would compare the diet of sick persons with that of persons in health, he will find it not more injurious than that of healthy persons."¹¹ From the onset of the age of reason in the 16th century and the introduction of the scientific method, physicians and scientists have attempted to apply laws of physics to laws of mortality. Although early scientists often failed to establish the correlation of dictates from the one realm to the other, the results of these endeavors were profound. In 1747, James Lind supplemented the diets

of 12 British sailors at sea who were sick with the symptoms of scurvy with various foods, including citrus fruits. Lind rediscovered what French explorers had reported in the late 1600s, after Native Americans taught them that a brew of pine bark prevented this illness. In neither case was biological plausibility demonstrated or statistical association achieved, and yet a devastating disease was eliminated. Neither the Native Americans nor the French knew that pine bark contains proanthocyanidin complexes, which reduce collagen degradation, and the British were unaware that citrus fruits contain vitamins essential for collagen synthesis. Of Lind's dozen experimental subjects, only 2 were provided with citrus fruits, and it was their remarkable recovery that changed the entire manner in which the British Navy was sent to sea.¹⁵ Limes and lime juice accompanied the men and, to this day, British sailors are known as "Limeys."

From 1848 to 1854, John Snow, a founding member of the London Epidemiological Society, tracked the incidence and geographical distribution of cholera in London. He determined that homes supplied by one water company had an 8.4-fold higher incidence of cholera than homes whose water was drawn from elsewhere along the Thames.¹⁶ From these findings, and the Board Street Pump cholera outbreak, Snow postulated that there existed a "cholera poison" transmitted through water.¹⁷ Within 2 years of Snow's report and 16 years before Robert Koch isolated *Vibrio cholera*, all London water companies were required to filter their domestic water. Metropolitan regulation of a public resource was imposed, although there was not then a direct causal scientific explanation, only an association that today we would deem important, but one whose supporting data we would not find statistically significant.

Some would view these remarkable success stories and the repeated association of hyperhomocyst(e)inemia with coronary, cerebral, and peripheral vascular disease and simply advocate for increased folic acid intake for all.¹⁸ Indeed, this intervention of negligible cost and insignificant side effect is already partially in place; many foods are fortified with folate to prevent congenital neural tube defects. What happens, though, when we fail to continue to pursue causality, the linkage of biological significance or scientific plausibility with epidemiologically or statistically significant association? In the absence of the connection of these 2 elements, we place ourselves at the mercy of those who would hold fast against scenarios supported by one without the other, allow the public to assume that disease strikes because some are lucky and others not or, worse, enable others to impose ineffective therapies. When the intricacies of associating actions, exposures, or one disease with another is coupled with public anxiety, skepticism, and lack of comprehension, painfully devastating effects can ensue. Great surgeons like Samuel Gross challenged the adherents of Joseph Lister to identify causal agents transmitted through *unsterile* techniques.¹⁹ Until such time as they could be found, he and others insisted on operating with family in attendance, to a full crowd, in street clothes, and without gloves, thus contributing to in-hospital mortality rates after surgery and childbirth on the order of 50%.

Lest we think this a 19th century quirk, Karl Pearson, a statistician and mathematician of great renown, challenged the idea that tuberculosis could be contained by quarantine, and the power of his conviction and the strength of his name led to the propagation of this contagious disease.²⁰ What is not understood is subject to ridicule. Public opinion scoffed at Edward Jenner and William Heberden as they tried to introduce smallpox vaccination, and the use of vaccinations was delayed and is still resisted by some. *Risk* refers to the hazard one is exposed to by virtue of engaging in some activity and is, as such, an engaging probabilistic event. In medicine, risk becomes the likelihood that people without a disease will acquire the disease through contact with factors thought to increase disease risk. In an odd way, this notion can even convey expectation of benefit for assumption of the risk. Many, no doubt, enjoy cigarettes, donuts, and eating to excess. Daring movie actors flick cigarette ash on the Surgeon General's report while driving fast cars and chasing evil, wine, and women, and the incidence of tobacco abuse among the teenage population continues to rise. Finally and perhaps most insidiously are the actions of those like Egas Muniz, whose desire to help people with difficult problems clouded the interpretation of flawed data and the recognition of flawed interventions, thus providing Muniz with a Nobel prize and countless others with lobotomies.²¹ Thus, like Stühlinger et al.,⁷ physicians have the obligation to strengthen associations, seek causality, and provide mechanistic insight into epidemiological observations. Perhaps then, we can speak in an informed and scientific, rather than alarmist, tones and reduce association from an assumption of risk to embracement of mechanism.

Multiple Cardiac Risk Factors and the Unified Field Theory of Risk Factors

The causes and principles of different things are in a sense different, but in a sense, if one speaks universally and analogically, they are the same for all.

—Aristotle²²

When Hopkins and Williams²³ evaluated and categorized the 246 cardiac risk factors identifiable 20 years ago, they grouped factors into 4 major categories on the basis of their putative mechanism of action. Indeed, risk factors are clustered in syndromes or associations, for example, of glucose intolerance and dyslipidemia. Risk factors were seen as initiating atherogenesis, promoting lipoprotein accumulation in the arterial wall, potentiating thrombosis, or precipitating acute vascular instability or insufficiency through generation of ischemia or arrhythmia. Many of these events can be controlled by the NO pathway, and it has even been shown that plasma ADMA levels are elevated in patients with hypertension, tobacco exposure, diabetes mellitus, and hypercholesterolemia.²⁴ Is it possible then that all of the cardiac risk factors are simply a manifestation of one pathway of cardiovascular injury and, in this case, one that terminates in NO synthase?

As appealing as this thought may be, it is unlikely and dangerous. First, as we have seen time and again in cardio-

vascular biology, although one molecular biological pathway may predominate in driving health, system redundancy and the multiple stimuli of disease create a complex series of bypass pathways that can continue to drive disease, even when what is deemed primary is blocked or obliterated. Second, although the presence of a common pathway removes factor analysis from the ethereal realm of risk to the *terra firma* of causality, at the end of the day, we may have rejected one level of uncertainty in assigning causality from association for another. Just as we cannot be certain that an epidemiological risk factor associated with a specific disease is causal for that disease, we cannot be certain that biological events in cell culture or molecular or enzymatic findings in genetically modified mice, laboratory monkeys, or even clinical trials are causal. The causal web of connection between individual and organ, organ and cell, and cell and enzyme is complex indeed, and arguments that try to infer pathophysiological mechanisms from selected enzymatic responses are potentially perilous.

Surrogate Markers of Risk or More Direct Measures of Disease

And it appears to me that one ought also to know what diseases arise in man from the powers, and what from the structures. What do I mean by this? By powers, I mean intense and strong juices; and by structures, whatever conformations there are in man.

—Hippocrates¹

Tobacco is a mixture of toxins; hypertension is both a response to a variety of stimuli and a parameter whose values often vacillate widely in time and place; glucose and cholesterol are essential metabolites whose serum levels reflect complex biochemical regulation. All of these risk factors are then, by nature, imprecise and nonspecific. They are stochastic measures of what will happen to normal people who fall into particular measures of these parameters. The daring may be willing to accept these risks, citing friend and foe who live well beyond or for far lesser times than anticipated by risk alone. Such concerns may well become moot if we can simultaneously identify patients at risk by linking phenotype with genotype, gene expression with protein elaboration, and environmental exposures with the biochemical consequences and direct anatomic aberrations they induce. This kind of characterization may well replace a family history of arterial disease as a rough estimate of genotype, serum cholesterol as an indirect measure of the health of lipoprotein metabolism, serum glucose as a crude determinant of the ravages of diabetes mellitus, blood pressure measurement as a marker of long-standing endogenous exposure to altered flow, and tobacco abuse as a maker of long-standing exposure to exogenous toxins. Rather than identifying patients on the basis of their serum cholesterol, we will have a direct measures of their LDL receptor number, internalization rate, macrophage content in the blood vessel wall, metalloproteinase activity, etc. Serum glucose will similarly give way to insulin receptor metabolism, oxidative state, and glycated burden. Blood pressure will remain an important measure, but

so will direct determination of wall thickness in various vascular beds, arterial compliance, and biochemical regulators of hemodynamics. Genotype and even proteotyping is rapidly becoming a reality with microchip technology, and the advances in imaging and computational sciences may make gene clustering a routine tool in risk factoring.

If we begin to think in these terms, we may be able to not only predict risk, but to also alter prognosis, follow disease course, and choose from a range of potential therapies. The approach of Stühlinger et al⁷ is the approach of the future, and the day may come soon when we speak not of a plasma cholesterol or homocysteine levels, but of measures of specific enzymes like ADMA or DDAH. For now, I am avoiding donuts and cigarettes, eating leafy vegetables and cereal fortified with folic acid, and still searching for the causes of disease and the role of NO.

“What you do not smell is iokaine powder, odorless, tasteless, dissolves instantly in liquid, and is among the more deadly poisons known to man.”

—The Dread Pirate Roberts²⁵

“Iokaine? Why not use its generic name, nitric oxide, like everyone else? I have never seen, smelled, or detected NO, and NO is never responsible for any of my experimental results, but I do know that NO is of the most potent compounds and critical to every disease known to man.”

—The Dread Postdoc Robert
(Robert Pirate, EdD, oral communication, 2001,
Edelman Laboratory)

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