Considering Genotype in the Selection of Study Populations May Alter Outcomes in Clinical Trials and Meta-Analyses: A Re-Examination of the HOPE-Studies

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Introduction

The HOPE and HOPE-TOO Studies

In 1993 the Heart Outcomes Prevention Evaluation Study (HOPE-study) began.[1] Approximately 9,000 men and women at high risk for cardiovascular disease, among them diabetics, were followed over a period of four and half years. They took 400 IU vitamin E daily or placebo. The results demonstrated that in the vitamin E group, 750 undesirable events had taken place, such as myocardial infarction, stroke and death. Approximately the same results were seen in the placebo group. The researchers concluded that in patients at high risk for cardiovascular events, treatment with vitamin E for a mean of 4.5 years had no apparent effect on cardiovascular outcomes.

The HOPE-study was immediately followed up by the HOPE-The Ongoing Outcomes (HOPE-TOO) Study.[2] The study population was reduced to approximately half of the initial HOPE study population but the total intervention period was prolonged to 11 years. In these high risk patients long-term vitamin E supplementation did not prevent major cardiovascular events. Compared to placebo, patients in the vitamin E group might even have an increased risk for heart failure.

Previous Studies with Vitamin E

The outcomes of the HOPE and HOPE-TOO Studies were contrary to prior findings. Previous large epidemiologic studies in the 1990s had shown that a high consumption of vitamin E significantly reduced the risk of cardiovascular disease.[3,4] In the Health Professional Follow-up Study of 40,000 men, 40 to 75 years of age, were followed over four years.[3] As compared with men who did not take vitamin E supplements, men who took at least 100 IU per day for at least two years had a multivariate relative risk of coronary disease of 0.63 (95% CI, 0.47 - 0.84). A similar study, the Nurses Health Study, was done among 90,000 women, 34 to 59 years of age, over eight years. Women who took vitamin E supplements for more than two years had a relative risk of major coronary disease of 0.59 (95% CI, 0.38 - 0.91).[4] In 1996, the results of the Cambridge Heart Antioxidant Study (CHAOS) in patients with coronary atherosclerosis was published in *The Lancet*. The median duration in this randomized controlled trial was 510 days. It was concluded that in these high risk patients vitamin E supplements (800 IU to 400 IU) substantively reduced the rate of non-fatal MI, with beneficial effects apparent after one year of treatment.[5] The results of these studies convinced many American cardiologists to take antioxidant supplements on a daily basis, especially vitamin E.[6]

Up to now no explanation has been found for the contradictory results between these previous studies and the two HOPE-studies.

Nutrigenomics

A boost to all genetic research has come from the Human Genome Project, launched in 1990 and completed in

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2003. This project supplied a considerable amount of knowledge regarding the impact of genetic failures and polymorphisms on the etiology of illnesses. These phenomena possibly could throw a new light on the contradictory outcomes of the vitamin E studies. This is where nutrigenomics comes in: the application of genomics to human nutrition, especially the relationship between nutrition and health.

The Israeli researcher, Dr. Andrew Levy, hypothesized that one reason why vitamin E may have failed to demonstrate benefit in the HOPE-studies was due to inappropriate patient selection.[7] In a review, he provided supporting evidence that a selection taking into account genetic differences in the study population may give another view in both HOPE-studies on the effect of vitamin E supplementation. These results would be in accordance with the body of evidence regarding the protective role of vitamin E for cardiovascular disease in high risk patients, as has been shown in preclinical research, animal studies and epidemiological studies. It is plausible that the protective role of vitamin E can be attributed to the reduction of this vitamin of oxidative stress as contributor to vascular complications.

Haptoglobin and Haptoglobin Genotype

Levy has conducted research on haptoglobin (Hp) and the haptoglobin genotype. Hp is an abundant plasma glycoprotein which binds free haemoglobin, released from red blood cells in haemolysis. Hp exerts an antioxidant function by its ability to bind to haemoglobin. Free iron acts as an oxidant and causes damage to tissues, for example to blood vessels. When Hp takes up haemoglobin, iron is bound and cannot act as a destructive oxidant.

In men a polymorphism has been found for the Hp-gene. The two alleles are called Hp-1 and Hp-2. Research has shown that iron is more tightly bound when the Hp-glycoprotein is genetically encoded by the Hp-1 allele. Originating from Hp-2 allele iron is more available and may exert detrimental oxidative damage. This has been shown in a cell culture, in transgenic mice, in vitro and in men. Moreover, in contrast to Hp-2-Hb complex, the Hp-1-Hb complex increases anti-inflammatory agents like interleukine 10 (IL-10).

Polymorphism as an Explanation of the Paradox

It has been estimated that in the Western population the homozygote Hp 1-1 genotype exists in 16% of the population, the Hp 2-2 genotype in 36% and the heterozygote genotype (Hp 1-2) in 48%.[7]

In the last five years, existing study populations have been looked over, in total 20,000 individuals. It has been established that diabetes patients with the Hp-2 allele have an increased risk of cardiovascular disease. In diabetic persons with the Hp-1 allele no increased risk for cardiovascular disease was observed. The main studies which were involved in this analysis were the Strong Heart Study,[8] the Munich Stent Study[9] and the Rambam myocardial infarction outcomes in diabetes study.[10]

On basis of these outcomes the Hp-gene seems to be a significant predictor for cardiovascular disease in patients with diabetes. But this is not yet evidence that the Hp-1 gene is the cause for the reduced risk. It is possible that another gene monitors the Hp gene and so exerts a lower risk. For this reason Levy carried out an experiment with two groups of mice.[9] – one group of ordinary mice and the other group of mice with a incorporated Hp-2 allele (mice themselves exclusively have the Hp-1 gene). It appeared that all diabetes complications occurred only in the mice with the introduced Hp-2 allele. From this and the previous studies, Levy concluded that antioxidative substances could be effective in patients with the Hp-2 allele only.

Re-Examination of HOPE

Levy got the opportunity to examine the data of the entire HOPE cohort, including the genotypes Hp-1 and Hp-2 of this cohort. He and his team found that in Hp-2-2 group with diabetes vitamin E reduced cardiovascular death (RR 0.45; 95% CI 0.23–0.90) and non-fatal myocardial infarction (RR 0.57, 95% CI 0.33–0.97).[11] No benefit from vitamin E was observed in Hp-1-1 or Hp-2–1 individuals with diabetes.

These outcomes lead to the initiation of a four year double blinded clinical trial involving over 1500 Hp 2-2 DM individuals. Natural source vitamin E supplementation is compared to placebo in order to try to validate the findings presented above for Hp 2-2 diabetes patients in the HOPE study. The results are not yet available.

Discussion

Levy postulates that if this prospective study validates the HOPE findings then the Hp genotype may serve as an appropriate test to identify those diabetic individuals in whom antioxidant therapy with vitamin E should be administered.

In a general perspective, another phenomenon should be taken into consideration. On the basis of the clinical trials with antioxidant supplementation, the current scientific consensus seems to be that antioxidant supplementation does not provide protection from cardiovascular disease, cancer and total mortality. However, genotype differentiation may be an overlooked variable in clinical studies, which may be determining the outcomes as has been shown by Levy for the Hp genotype in diabetic patients for cardiovascular disease. If so, other clinical trials should be re-examined as has been done by Levy with the HOPE studies. This also sheds new light on how meta-analyses are performed. Taking into account the recent meta-analysis of Bjelakovic,[12] the main criticism on this study was the diversity of the populations of the selected original studies. Additionally, the outcome of such a meta-analysis may now be disputable because a possible major variable, the genotype, was not considered. A re-evaluation of studies with antioxidant supplementation, taking into account the genotype, should be taken into consideration. This also applies to the design of future studies. This approach is in full accordance with the concept of Williams on biochemical individuality.[13]

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