Resveratrol is a natural substance produced by plants in response to stress or infection. This chemical is a small molecule that neutralizes toxic metabolic processes of the body. It is present in the skins of red grapes, and therefore in red wine. Resveratrol has attracted attention in the last two decades for its many purported health-promoting benefits. In particular, because resveratrol may have an ability to slow down tumor growth or even prevent cancer (which is called chemo-prevention). Additionally, resveratrol could slow down the development of atherosclerosis and heart disease.

Curcumin is another natural product that currently generates a lot of interest for its chemopreventive and cardio-protective activities. This yellow die is a dietary polyphenol present in a spice called turmeric, which is derived from powdered rhizomes of the plant *Curcuma longa*. Scientific data suggest strong anti-cancer effects of curcumin, especially on gastrointestinal tumors. Curcumin can also slow down the proliferation of cancer cell lines cultured in the laboratory, in part by inducing apoptosis in them.

Resveratrol, curcumin, and similar phenolic phytochemicals became very popular recently as ingredients of vitamin formulas, dietary supplements, superfoods, feed additives, or even as food preservatives and technological enhancers. However, the exact mechanism of their action on human / animal cells, and bioavailability are still uncertain. Therefore, there is a great interest in identifying biomarkers of cellular responses to curcumin and resveratrol. Monitoring the effects of antioxidants on tissues will let us verify if and how they are useful in prevention / therapy of diseases, and safe as food or feed additives.

One of the most promising strategies to identify such biomarkers is the analysis of gene expression changes induced by curcumin and resveratrol using techniques of functional genomics. Many transcriptomic datasets were published for curcumin or resveratrol, including data for different tissue- and cell-types in rodents, pigs, non-human primates, and human. The resulting datasets were deposited in special bioinformatics databases where they are available for a re-analysis. Thus, anyone can download these datasets and re-analyse the data. Such a research strategy is called a meta-analysis. A meta-analysis may be used to test research hypotheses that could not be tackled using the datasets in isolation; but progress is possible if the datasets are analysed together. Moreover, one can use novel and customized statistical tools that are more appropriate than standard tools previously used.

Such a meta-analysis is proposed in this project. The first goal is to identify genes that could be biomarkers of the response to curcumin or resveratrol in diverse cell types. Such biomarkers could be used to monitor human clinical trials (especially if their expression in target cells is correlated with that in circulating white blood cells that can be easily isolated from a sample of blood).

The second goal is to develop and characterize a pair of novel statistical tests. The first is a test of hormesis for gene expression data. Hormesis is a common phenomenon observed when the cell is positively stimulated at low does of a chemical but high doses are toxic. Such hormetic responses probably take place in the case of resveratrol. Low doses are likely protective against mutations and inflammation, while high concentrations kill cancer cells. This hypothesis might be tested using resveratrol gene expression datasets already available in databases.

The second statistical method is a special type of procedure to estimate the significance of correlation called a permutation test. Such permutation tests consist of enumerating all possible arrangements of data and re-calculating the statistics for each. The universality and exactness of such tests were already appreciated by the first applied statisticians such as the famous English statistician Ronald Fisher. However, in Fisher's time, permutation tests were too tedious to carry our in practice. Today, this is possible thanks to computers. But, though widely used in genomics, the use of permutations tests is unsystematic and haphazard. I want to partially remedy this problem through a systematic examination of the performance of permutation tests applied to gene pair-wise expression correlations in microarray data. Next, I want to apply such a test to curcumin and resveratrol datasets. (But the test and software developed will be universal, easily applied to other problems.)

Both the biomarkers and statistical procedures will be tested on our own gene expression datasets obtained from cells cultured *in vitro*. We will also test the hormesis hypothesis and identify signalling pathways involved. In summary, this grant application combines the development of statistical, mathematical and analytical methods and their application to transcriptomics of resveratrol, curcumin and similar natural antioxidants.