Single and multilocus analyses for the identification of at risk genotypes in cardiovascular disease

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Introduction

Parental history of coronary heart disease (CHD) has long been recognized as a risk factor for CHD. Death from coronary heart disease is influenced by genetic factors both in women and men [1].
Several epidemiological studies have described a number of underlying risk factors for cardiovascular disease (diabetes mellitus, hypercholesterolemia, plasma lipids, hypertension) which are as well under a moderate degree of genetic control [2][3][4]. In searching for susceptibility genetic factors associated to coronary artery disease (CAD) we determined the genotypes for 35 candidate genes (63 polymorphisms) in a sample of 757 individuals with angiographically documented coronary artery disease (CAD+, cases), and 320 individuals with angiographically documented normal coronary arteries (CAD-, controls).
It is very hard to discover true combinations of multiple factors contributing to the disease. Recent publications show a growing number of genes being studied and correlated with phenotypic variations. The difficulties in treating the increasing amount of available data indicate the need for new tools able to retrive the relevant information. We propose the implementation of the classification tree procedure joined to backward elimination as an explorative tool to screen for genetic factors that may be associated to the CAD phenotype.

Method

Classification and Regression Trees (CART) have been described by Breiman et al. 1984 [5]. It is a group of recursive partitioning methods that are reminiscent of cluster analysis. The tree-based recursive partitioning was joined to a backward elimination to investigate combinations of genes that may contribute to the risk of disease. The procedure reminds the classical stepwise variable selection. After the tree construction the polymorphism at the root node was removed from list of polymorphisms and then a new tree was built. The method proceeded through successive polymorphism elimination steps. The gene variations selected by CART joint to backward elimination underwent standard association analysis.

Single locus analysis

No significant departure from Hardy-Weinberg equilibrium was observed. Two gene polymorphisms showing a strong linkage disequilibrium (APOC3 -641C>A and APOC3 -455T>C) were found to be associated to CAD (OR 2.1 p: 0.0001; OR 2.0 p: 0.0005, respectively).

Searching for 2 loci interactions

A method that combines classification tree-based recursive partitioning with backward elimination suggested two possible 2 loci combinations that the are associated to an increased risk of disease:
APOC3 641 A/A + ANP 2238 T/C,C/C (OR: 4.41 CI: 1.57 – 17.13 p=0.00184) and APOC3 641 A/A + APOC3 1110 C/C (OR: 3.88 CI: 1.59 – 9.45 p=0.002824).

Conclusion

The results need to be confirmed by independent observations. A detailed investigation with simulated models is required to better understand the features of this procedure and to set a context to analyse and measure the performance of CART in association studies in order to ensure maximum benefit.
The results indicate that multiplex genotyping can detect susceptibility polymorphisms and may help in finding possible gene-gene interactions associated to disease. Our results emphasize the need to account for complex multilocus influences.

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