Letters to the Editor


Molecular analysis of connexin 40 in the familial form of atrial fibrillation

Atrial fibrillation (AF) is the most common sustained cardiac rhythm disturbance. Sixteen percent of the male population that are affected with AF suffer from the familial form (FAF)\(^1\) for which the molecular basis is still unknown. Cardiac conduction disturbances and block are due either to changes of ionic channel properties (essentially sodium channels, \(I_{Na}\), myocardial architecture or intercellular coupling through gap junctions (composed of connexins, Cxs)\(^2\). Several subtypes of sodium channel proteins are expressed in the heart\(^3\) but their respective physiological roles appear to be indistinguishable and a heterogeneous distribution has not been shown in this tissue.

In contrast, Cxs distribution patterns are specific for each region of the heart\(^4\). The presence of Cx40, one of the three protein subtypes which form cardiac gap junctions, is mostly restricted to the conduction system and atria. Cx40 presents important changes in expression level and distribution during atrial fibrillation\(^5,6\). In addition, Cx40 knock-out mice showed an increased susceptibility to atrial tachyarrhythmias\(^7,8\). These data suggest Cx40 as a logical candidate gene for the familial atrial fibrillation syndrome.

Family study

Two French–Canadian families were identified in which atrial fibrillation appears to be segregating as an autosomal dominant disease with a high penetrance. The first family consisted of 11 living members spanning three generations, of which four members present clinical criteria for FAF. One patient refused to participate in the study for ethical reasons. The second family included 13 living members spanning four generations, of which three members were affected.

Clinical evaluation

Evaluation of the subjects was performed on the basis of the clinical criteria associated with this disease. Subjects that presented the same phenotype but caused by other problems such as hypertension, valvular disease, thyroid disease, etc. were excluded from this study. Both families were identified and referred to the Department of Electrophysiology at Laval Hospital. Studies were carried out according to ethical guidelines of Laval Hospital and after obtaining informed consent from the subjects.

Preparation of genomic DNA from whole blood of subjects and isolation of connexin 40

Blood was collected from each family member and genomic DNA was extracted using the QIAamp DNA Blood Midi kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. The Cx40 promoter and coding regions were amplified from genomic DNA using the polymerase chain reaction (PCR). For the Cx40 coding region, genomic DNA (100 ng) was mixed with oligonucleotides primers (1 \(\mu\)M final) corresponding to 5’S- GAAGTTTTGGCATCTGTTCCCT G-3’ (sense primer -33) and 5’S- CCCACTTGGTCTCTGCCCTCC-3’ (antisense primer 1110) of the human Cx40 gene (Genbank accession number U03486). For the Cx40 promoter region, the genomic DNA (100 ng) was mixed with oligonucleotide primers (1 \(\mu\)M final) corresponding to 5’S-AATCCCAAAAAAGGC TTTGTTAAT-3’ (antisense primer Cx40E-1) and 5’S-CTGCTTCTTTTCCT CCTCC-3’ (antisense primer Cx40E-1) of the human Cx40 gene (Genbank accession number NT 004434:1). Amplification was carried out with Pfu Turbo and Perfect Match (Stratagene, La Jolla, CA) according to the manufacturer’s protocol.

Briefly, samples were denatured at 94 °C for 5 min and then cycled by denaturing at 94 °C for 1 min, annealing at 65 °C for 1 min, and extending at 72 °C for 2 min (30 cycles). A final 10 min extension period was added. Both reaction products (1200 bp and 540 bp) were visualized by ethidium bromide staining and were gel-purified by electrophoresis through a 1% agarose gel. The PCR products were then purified by excising the band and using a QIAquick PCR purification kit according to the manufacturer’s directions (Qiagen, Valencia, CA). The Cx40 gene of each patient was sequenced using fluorescent dideoxy terminator sequencing by the automated sequencing facilities at Laval University (Ste-Foy, Canada). For each subject, both strands of DNA were completely sequenced. Sequence comparisons between the wild type and the affected subjects Cx40 gene were performed using the GCG software package (Madison, WI). Validation of the PCR amplification was carried out using a mock amplification procedure. Comparison of Cx40 gene sequence from patients affected with FAF with wild type Cx40 gene did not reveal any mutations within the entire coding region. Results from this study thus do not support a role for Cx40 in FAF.

Although the Cx40 gene appears not to be involved in FAF for these two French–Canadian families, we cannot exclude its implication in other cases of FAF. The present study will help to focus on new genetic targets for investigation of FAF, which affects a significant proportion of patients with AF.

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References

IgA of nasal mucosa decreases and training, leptin and inhibin B are kind of depression after intensive disease, which supports the specific inflammatory changes including increased phagocytes, inflammatory reactions, decrease of NK cells, and increase of TNF-alpha, IL-6, IL-10 or IL-1ra (IL-1 receptor antagonist) in plasma[3]. The IgA of nasal mucosa decreases and antigen presentation to macrophages is reduced[4]. The overall impression is that the immune system shows some kind of depression after intensive physical exercise. In case of over-training, leptin and inhibit B are reduced and may be of diagnostic value[5]. Some therapeutic interventions in the acute phase of the over-training syndrome are carbohydrates, glutamin or vitamins, although definite recommendations cannot be made.

Obviously, the neurohumoral and immunological changes which are discussed by the authors as prognostic factors in patients with coronary heart disease are markedly similar to those parameters used in sports medicine as a diagnostic tool in the over-training syndrome, which occurs in normally well trained athletes with a great deal of compensation capability. If the physician in charge of the athlete does not react adequately, a decrease in performance is the consequence and somatic sequelae cannot be excluded, if the athlete does not discontinue over-training. We therefore ask the question, is over-training in well compensated athletes comparable to inflammation processes in coronary heart patients with lack of compensation? Encouraged by the excellent papers presented we have begun to compare data of both entities. Possibly the over-training syndrome may be a model which will help in our understanding of the long-term prognostic factors of coronary heart disease.

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References

Effects of oral sotalol administration before electrical cardioversion of persistent atrial fibrillation

I read with attention the article by Frick et al.[1], that compared, in a randomized controlled trial, the effect of magnesium alone or as an adjuvant to sotalol in the cardioversion of atrial fibrillation and subsequent maintenance of sinus rhythm. I observed that their data can help to elucidate some clinical situations in which the efficacy of oral sotalol has not yet been demonstrated. One is its ability in the pharmacological conversion of atrial fibrillation and another is its effect on the success of electrical cardioversion. Sotalol has been largely used for the maintenance of sinus rhythm in patients with atrial fibrillation[2–5]. However, the scientific documentation in support of an effect on cardioversion to sinus rhythm is weak. While several studies failed to demonstrate efficacy with sotalol[6–11], only one randomized controlled study reported a higher cardioversion rate with intravenous sotalol when compared to digoxin[12]. The difference between sotalol’s ability in the maintenance of sinus rhythm and the absence of efficacy in cardioversion to sinus rhythm has been attributed to ‘reverse rate dependence'[13]. Some studies have demonstrated that a previous administration of

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