Interactions of MinK and e-NOS Gene Polymorphisms Appear to Be Inconsistent Predictors of Atrial Fibrillation Propensity, but Long Alleles of ESR1 Promoter TA Repeat May Be a Promising Marker

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ABSTRACT

Interactions of MinK and e-NOS Gene Polymorphisms Appear to Be Inconsistent Predictors of Atrial Fibrillation Propensity, but Long Alleles of ESR1 Promoter TA Repeat May Be a Promising Marker. We analyzed minK, e-NOS and ESR1 gene polymorphisms in 40 patients with atrial fibrillation (AF) without major structural heart disease compared to 35 healthy controls. A missense polymorphism in the minK gene with A/G substitution at nucleotide 112 causing serine (S) to glycine (G) change, 786 T/C polymorphism in the 5' flanking region of e-NOS gene and TA polymorphism in the regulatory region of estrogen receptor ESR1 gene with long (>19 TA repeats) and short alleles were examined. Only a slight increase in minK G allele frequency, but with marked excess in AG/TT combination of minK and e-NOS polymorphisms was found in the AF group. The interpretation remains tentative due to small groups precluding statistical significance of differences, possible lab flaws and inconsistencies with earlier data. However, ESR1 long allele homozygotes were strikingly more frequent in the AF than in control group, reaching statistical significance surprisingly in males (p<0.02). Functional activity of estrogen receptors may be more critical in males than in females with abundance of circulating estrogen. Contrasting the intricate complexity of genetic polymorphisms influencing cardiac rhythm with our modest research, we would limit the conclusion to the plea for further research of ESR1 role in AF.

Key words: atrial fibrillation, gene polymorphisms, minK gene, e-NOS gene, ESR1 gene

Introduction

The genetics of atrial fibrillation (AF) has evoked considerable interest since R. Brugada identified one of the loci for familial AF ten years ago¹. It appeared that AF, a paramount arrhythmia, may have genetic determinants. The modes of inheritance include simple monogenic and complex polygenic traits². Genetic polymorphisms are less conspicuous, but more common cause of AF than rare monogenic inheritance with ion channel or structural protein alterations². Among many polymorphisms investigated², we confined our research to three of them: minK, eNOS and estrogen receptor á genes.

Human minK protein is the β-subunit of I_K, potassium channel enhancing atrial repolarization²¹. A missense polymorphism in the minK gene has been reported to facilitate AF. It comprises A/G substitution at nucleotide 112 of the minK gene, causing serine to glycine change in the 38 aminoacidic position of the minK protein³.

Deficiency in nitric oxygen (NO) may promote AF³. NO through cGMP stimulates antiarrhythmic calcium channel modulation and inhibits transient outward potassium current (Ito1) restoring plateau phase of AF remodelled action potential. Low NO levels increase Ca²⁺

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current $I_{Ca,L}$ and reduce muscarinic K$^+$ current $I_{Ca,I}$. NO levels are regulated by nitric oxide synthases (NOS), endothelial NOS (eNOS) being essential. NOS are primate regulators of fundamental cardiac functions, including cardiac autonomic balance and L-type calcium channel function, pivotal for sinus rhythm$^{3,4}$. Inducible NOS enhance superoxide production and electrical remodelling in AF. Some eNOS polymorphisms may reduce plasma NO levels: guanine to thymine substitution at nucleotide 894 in exon 7 (894 G>T polymorphism), the C allele of the 786 T>C polymorphism in the 5′ flanking region and a 27 bp variable tandem repeats polymorphism in intron 4 (eNOS 4a4b)$^9$.

While the role of minK and eNOS polymorphisms in AF has been already studied, estrogen gene receptor polymorphisms have been linked to ventricular arrhythmias only$^5$. Women of childbearing age have faster resting heart rates and longer QTc intervals than men with distinctive appearance of final ECG complex and propensity for drug and bradycardia induced polymorphic ventricular tachycardia$^5$. Gender related differences of repolarisation caused by estrogens, gestagens and androgens with receptors in cardiomyocytes and fibroblasts are inferred. The data on electrophysiological effects of sex steroids are scarce. They might modulate proarrhythmic response through $I_NK$ and $I_{Ca,L}$ currents$^8$. Estrogen (17β-estradiol) regulates expression of many genes through estrogen receptors α (gene ESR1) and β (ESR2)$^6$. ESR1 polymorphisms alter expression of many genes, including those of eNOS and inducible NOS$^6$. Estrogen stimulates NO production in vessels by increasing the expression of NOS and NO release from endothelium. It also influences the L-type Ca$^{2+}$ channel gene expression$^6$. Receptor mediated estrogen electrophysiological effects can be conceived independently from more investigated vascular and myocardial effects. Of the polymorphisms identified in the ESR1 gene, the c.454-397T>C (also known as IVS1-397 T/C, rs2234693, and the PvuII restriction site) and c.454-351 A>G (also known as IVS1-351 A/G, rs9340799, and the XbaI restriction site) and those of eNOS and inducible NOS$^6$. Estrogen polymorphisms alter expression of many genes, including those of eNOS and inducible NOS$^6$. Estrogen polymorphisms have been studied in limited populations$^3,8,9$, while ESR1 polymorphisms have not been associated with AF yet.

### Patients and Methods

We considered the research of minK, eNOS and ESR1 gene polymorphisms in patients with AF without gross structural cardiac abnormality worthwhile. Those patients were selected to avoid smothering of inheritance by dominant exogenous factors. Electrical instability caused by certain polymorphisms or their combinations may render atria prone to fibrillation. MinK and eNOS polymorphisms have been studied in limited populations$^3,8,9$, while ESR1 polymorphisms have not been associated with AF yet.

## Results

The frequency of minK G allele in patients with AF was 32.50%, hardly any higher then in controls with
cade2. It can be discerned if the exogenous factors are to AF has been increasingly recognized in the last de-

ception of confounding risk factors. Genetic propensity

However, the primary causes remain obscured in a co-

Discussion

Eight males with AF had SL and six SS genotype. Seven control

males had SL and four SS genotype. 31.43%. Only AA homozygotes and AG heterozygotes, but not GG homozygotes were found.

Surprisingly, T allele of e-NOS gene was more frequent in the group of AF patients than in controls (72.50 vs. 67.14%), opposite to C allele incriminated earlier as possibly arrhythmogenic. The difference was insignificant, but AG/TT combination of minK and e-NOS alleles was not far from reaching the statistical significance (Table 1). Astoundingly, not a single case of CC homozygosity was found.

Long allele of ESR1 gene was more frequent in AF than in control group (52.50 vs. 42.86%), but the difference was insignificant. The homozygotes for long allele (LL) were twice as frequent in AF as in control group. In spite of the striking difference, the level of statistical significance has not been reached yet, due to the small number of examinees. In the group of 40 patients with AF, only 6 were women with the first occurrence AF in childbearing age, too few for further differentiation.

Surprisingly, statistical significance for p<0.02 (Fisher’s exact test) was reached in males. Nine out of 23 males with AF and none of eleven male controls were LL homozygotes (Figure 1). Logistic regression analysis revealed male gender and LL allele as the most significant risk factors for AF (p=0.013).

The research of genetic polymorphisms in AF is an exceedingly arduous task. The effects of a single polymorphism are presumably weak. Its phenotypic expression may vary in a hardly decipherable manner and appear through intricate interactions of cellular biology. Synergistic polymorphisms may reach phenotypic expression in combinations only. There is a myriad of putative polymorphisms and interactions of their expressions. Albeit weak separately, they may be significant in constellation. Detecting weak factors in a complex system is difficult. It may be compared to a mosaic consisting of tiny and seemingly trifling pieces, which put together in a very specific way form an elaborate image.

This figurative concept may explain two standpoints: 1/ the interpretation of small studies should be very cautious since they represent only a small part of an elaborate picture and 2/ inconsistencies between studies are not necessarily contradictions. They may point to different aspects of the same problem. We believe that small studies may be helpful in finding useful clues for the forthcoming research, even without clear-cut answers.

In our small group, the influence of min-K and e-NOS polymorphisms on AF was not obvious. The main limitation was insufficient number of examinees. Group of double size could have yielded more reliable results. Selecting the patients without major structural heart disease and obscuring of genetic causes by exogenous factors alleviates the problem.

There are only a few reports on minK polymorphisms and AF. The association unravelled by a Chinese research was confirmed by two European studies in a modest number of patients. The data on e-NOS gene polymorphisms in association with AF are even fewer. The lack of data may be due to limited availability of molecular diagnostics.

We were not able to confirm prior data. The trend of increased frequency of minK G allele among the patients with AF was the same, but far less convincing and statistically insignificant. In previous studies, two of them with unselected AF patients, and one with lone AF, the differences were driven primarily by GG homozygotes. Astoundingly, we have found none of them. The only probable explanation is a flaw in laboratory technique with insufficient sensitivity in differentiating AG and GG genotypes. In this case, some GG genotypes were erroneously recognized as AG ones, underestimating the frequency of G allele. The probability that GG genotypes were missing entirely is diminutive, considering the expected allele proportions in Hardy Weinberg’s equilibrium.

Negative association of AF with e-NOS C allele and reverse one with T allele are in discordance with prior data. Unexpected results are statistically insignificant and may be dismissed as a chance finding in a small sample. They are still noteworthy. Although C allele may predispose to AF in synergy with minK G allele by reduction of NO synthesis, the concept has been not investigated sufficiently. The intricate pathways of e-NOS influence on atrial arrhythmogenesis are largely speculative. Unexpected results are possible as a part of yet unknown
mechanisms. Thus, the possibility of TT genotype predisposing to AF in certain conditions more than CD genotype should not be disregarded.

The problem of missing CC genotype in our study could be commented similarly as the difficulty with minK GG genotype. The phenotypic differences of CC and CT genotypes are not necessarily limited to the quantity of effects. Assumptions on dominant, co-dominant and recessive modes of inheritance, valid for monogenic traits, are usually oversimplifications if applied to complex systems of polygenic inheritance. We should refrain from speculating on riddles with too many unknowns.

Coupling the polymorphisms of different genes in a statistical analysis may help discovering synergistic pathways\(^6\), but playing with numbers to attain statistical significance has to be avoided. Coupling of minK and e-NOS polymorphisms had been already tried\(^3\), but further combinations with ESR1 polymorphisms seemed inappropriate in our small group. Putative interactions are manifold.

Pursuing advancement in gene polymorphism research in AF a step beyond the concepts already tried, we opted for publication. The prevalence of lone AF is also markedly higher in males\(^12\). Furthermore, estrogen effects are not limited to women in childbearing age, but are exerted later on and in males.

Long alleles of ESR1 gene have been reported to increase risk of coronary artery disease\(^5\). To our knowledge, there are no reports on predisposing to AF. In our study, the frequency of long alleles was modestly increased in AF patients compared with controls, but the proportion of LL homozygotes was doubled. The trend was remarkable, even if not expected to stay at this level in further research (the impact of a single polymorphism is presumably weak). The data are stimulating further research, especially in younger women where the effects of estrogens are supposedly most pronounced.

From the other point of view, the ESR1 polymorphism may be even more important in postmenopausal women and men: with less estrogen available, functional quality of receptors may be decisive. This is a tentative explanation for unexpected result that LL genotype is more relevant for AF in males than in females. We are well aware of the limitations. The groups are small and not well age-matched, but the results are still challenging.

In comparison with already published data\(^3\), the frequencies of minK G allele and e-NOS C-allele in our study are at the lower end of range. This may reflect the underestimation due to the faulty assessment of GG and CC homozygotes. With a wide range of frequencies in studies from different populations, the question arises if some populations are more prone to AF than the others.

Publication of our research may be disputed as premature. The results would be more reliable with more patients. Double-checking may solve the problems of missing minK GG and e-NOS CC homozygotes. However, further research depends on affordability of genetic testing which is at present time uncertain. Therefore, we opted for publication.

In conclusion, we tried to contribute modestly to the solution of intricate puzzle of AF genetic background. In view of minK and e-NOS gene polymorphisms, we created more questions than answers. However, some inclining that ESR1 gene long alleles may predispose to AF, especially in males, has been found. The results are inspiring further research.

**REFERENCES**


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INTERAKCIJE POLIMORFIZAMA MINK I E-NOS GENA SU DVOJBENI PREDSKAZATELJI FIBRILACIJE ATRIJA, ALI DUGI ESR1 ALELI S TA PONAVLJANJEM U PROMOTORSKOM DIJELU MOGLI BI BITI OBEĆAVAJĆI BILJEG

SAŽETAK

Analizirali smo polimorfizme minK, e-NOS and ESR1 gena u 40 pacijenata s atrijskom fibrilacijom (AF), a bez veće strukturne bolesti srca, u usporedbi s 35 zdravih ispitanika kontrolne skupine. Ispitivani su misens polimorfizam minK gena s A/G supstitucijom u 112. nukleotidu uzrokujući zamjenu serina (S) glicinom (G), 786 T/C polymorfizam u 5' pobočnoj regiji e-NOS gena i TA polimorfizam u regulatornoj regiji ESR1 gena za estrogenski receptor s dugim (≥19 TA ponavljanja) i kratkim alelima. U AF skupini nađen je tek lagan porast frekvencije minK G alela, ali s izrazitim viškom AG/TT kombinacije minK i e-NOS polimorfizama. Tumačenje je zasad dvojbeno zbog malih skupina bez dosizanja statističke značajnosti razlika, mogućih laboratorijskih pogrešaka i neslaganja s dosadašnjim podacima. Homozigoti za duge alele ESR1 gena bili su ipak upadljivo češći u AF, nego u kontrolnoj skupini, začudo dosižući statastičku značajnost razlike u muškaraca (p<0.02). Funkcionalna aktivnost estrogenskih receptora mogla bi biti presudnija u muškaraca nego u žena s obiljem cirkulirajućih estrogena. Suprostavljajući zamršenu složenost genskih polimorfizama koji utječu na srčani ritam skromnosti našeg istraživanja, ograničili bismo zaključak na apel za nastavak istraživanja uloge ESR1 gena u atrijskoj fibrilaciji.