Q705K variant in *NLRP3* gene confers protection against myocardial infarction in female individuals

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Abstract. Inflammation is a multifaceted process that underlies the pathophysiology of acute myocardial infarction (MI). Variations in the inflammasome-related *NLRP3* gene have been associated with risk for a number of different inflammatory diseases. Therefore, Q705K polymorphism in *NLRP3* gene likely confers susceptibility to risk for MI. A First-ever myocardial Infarction study in Ac-county (FIA) cohort comprising 555 MI patients and 1,016 controls was used to genotype rs35829419 in the *NLRP3* gene by TaqMan single-nucleotide polymorphism assay. C-reactive protein (CRP) was measured in the study participants by ELISA. The results showed no significant association between the variant rs35829419 and MI. However, the minor A allele of the rs35829419 polymorphism conferred a protective effect against the risk of developing MI in females. The minor A allele of rs35829419 polymorphism was also associated with increased CRP levels in males. Results of the study suggested a gender-specific deregulation of *NLRP3* gene mediated by rs35829419 polymorphism that confers protection against MI in females but has no effect on MI susceptibility in males. However, the rs35829419 polymorphism was associated with increased CRP levels among the male subjects, thereby demonstrating the possible effect of the Q705K polymorphism in elevating the basal active state of innate immune response.

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Abbreviations: MI, myocardial infarction; SNP, single nucleotide polymorphism; IL, interleukin; CRP, C-reactive protein; NLRP, NLR family, containing pyrin domain; CARD, caspase recruitment domain; NLR, NOD-like receptor or NOD and LRR containing; FIA, first-ever myocardial infarction study in Ac-county

Key words: myocardial infarction, Q705K polymorphism, *NLRP3*, inflammasome, rs35829419, cytokine

Introduction

Coronary artery disease, including myocardial infarction (MI), is known to be a leading cause of mortality worldwide. The individual's genetic constitution as well as environmental factors are crucial in the pathological progression of the disease (1). Among the known etiological factors, inflammation is a major contributor for the progression of atherosclerosis and the subsequent rupture of the unstable plaque, resulting in MI (2). Defense mechanisms against inflammation serve as a defense tool of innate immune response against both exogenous pathogens and endogenous sterile injury. However, the uncontrolled inflammatory response often suppresses the repair mechanism, thereby leading to the clinical manifestation of the inflammatory disease characterized by elevated levels of inflammatory markers, such as interleukin (IL)-1β, IL-18, interferon γ, tumor necrosis factor-α and C-reactive protein (CRP). The recent finding regarding the NLRP3-induced production of the proinflammatory cytokine IL-1β in individuals with a mutation in the *NLRP3* gene has placed much focus on the NLRP3 inflammasome (3,4). The NLRP3 inflammasome, an intracellular macromolecular complex, which consists of the NLRP3 scaffold, the adaptor protein apoptosis speck-like protein contaning CARD and caspase-1, processes the immature proinflammatory cytokine IL-1β to its active form and renders the recognition of pathogen and danger-derived molecules in the cytosol (5).

Genetic variants in the genes encoding for proteins of the NLRP3 inflammasome have been studied in association with various inflammatory diseases (6-11). Germline alterations in the *NLRP3* gene encoding the NLRP3 protein have been associated with susceptibility to different inflammatory disorders such as hereditary periodic fever syndromes including familial cold urticaria, Muckle-Wells syndrome and neonatal-onset multisystem inflammatory disease (also known as chronic infantile neurologic cutaneous and arthropathy syndrome) (4,11-13) leading to the constitutive activation of NLRP3 and subsequent overproduction of IL-1β (3). Several recent studies have also revealed the association of the Q705K polymorphism in the NLRP3 gene (rs35829419) with various inflammatory diseases including celiac disease (9), diabetes type-1 (8), abdominal aortic aneurysms (14), Crohn's disease (15) and rheumatoid arthritis (10). Moreover, the combination of the polymorphism in *NLRP3* (Q705K) and

Table I. Overview of allele frequency of Q705K polymorphism in the *NLRP3* gene in patients with myocardial infarction and healthy controls in First-ever myocardial Infarction study in Ac-county (FIA) cohort.

Samples	Minor allele frequency in case	Minor allele frequency in control	Case ratio (AA/AC/CC)	Control ratio (AA/AC/CC)	Odd ratio (95% CI)	P-value
All subjects	0.08	0.09	5/74/412	6/159/746	0.90 (0.68-1.18)	0.46
Male	0.10	0.09	4/62/283	5/114/534	1.06 (0.78-1.44)	0.70
Female	0.04	0.09	1/12/129	1/45/212	0.51 (0.27-0.95)	0.03

Table II. Overview of association between the Q705K polymorphism and C-reactive protein (CRP) level in the First ever myocardial Infarction study in Ac-county (FIA) cohort.

Sample	No. of samples	β^a	SE	P-value
All subjects	1372	0.56	0.40	0.16
Male	993	0.93	1.90	0.05
Female	379	-0.65	-0.92	0.35
Total no. of controls	889	0.88	0.47	0.06
Male	646	1.19	0.58	0.04
Female	243	0.01	0.80	0.98
Total no. of patients	483	-0.05	0.74	0.94
Male	347	0.45	0.87	0.60
Female	136	-1.65	1.44	0.25

β^a indicates the magnitude of effect per allele.

CARD8 (C10X) genes was found to be associated with rheumatoid arthritis (10) and Crohn's disease (15,16).

Considering a possible key role of the NLRP3 inflammasome in promoting myocardial inflammation (17) and atherosclerosis through the production of pro-inflammatory cytokine IL-1β (18), the aim of the present study was to investigate the effect of Q705K polymorphism in *NLRP3* gene and the risk of developing MI in a northern Swedish population.

Materials and methods

Study subjects. DNA from 555 patients with MI according to WHO criteria and from 1,016 referents without MI matched for age, gender, date of sampling and geographical area from the First-ever myocardial Infarction study in Ac-county (FIA) cohort was collected in northern Sweden and was utilized for genotyping of the rs35829419 polymorphism in the *NLRP3* gene. Information regarding the sampling and baseline characteristics of the FIA cohort has been previously described (19). The study has been approved by the Research Ethics Committee of Umeå University and the National Computer Data Inspection Board, and was conducted according to the ethical guidelines of the Declaration of Helsinki. Written consent was obtained from the participants.

Genotyping of the rs35829419 polymorphism and its association with MI. The genotyping of the single-nucleotide polymorphism (SNP) rs35829419 in the *NLRP3* gene was performed for the FIA cohort using TaqMan® SNP genotyping assay according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). The analysis was performed in 7900HT Fast Real-Time PCR system, using Sequence Detection Systems Software version 2.3 for allelic discrimination (Applied Biosystems). The accuracy of the genotyping was confirmed by repeating the polymerase chain reaction (PCR) analysis randomly for 10% of the samples.

Plasma concentration of CRP measurements. ELISA assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA) was used to measure high sensitivity C-reactive protein (hs CRP) in the FIA cohort (20).

Statistical analysis. PLINK 4.0 was used to test the SNP for the Hardy-Weinberg equilibrium (21). The association between rs35829419 polymorphism and MI as well as CRP was analyzed using PLINK 4.0 (21).

Results

Association between the rs35829419 polymorphism and MI. The SNP rs35829419 encoding Q705K in the *NLRP3* gene was tested for Hardy-Weinberg equilibrium and showed no significant deviation in the study group. The investigation on the association of rs35829419 for risk of MI development revealed no overall statistical significant association between the rs35829419 variant and MI at the genotype level. However, gender-specific analysis revealed a significant association between the heterozygous (CA) and homozygous females (CC)

($P_{genotype}$=0.014; OR=0.44; 95% CI, 0.21-0.89), indicating a protective effect against MI in females. In addition, the recessive genetic model (AA + CA vs. CC) also conferred a protective effect against MI in females ($P_{genotype}$=0.019; OR=0.46; 95% CI, 0.23-0.93). Futhermore, the allele frequency for the SNP rs35829419 exhibited a significant difference between MI patients and controls. The minor allele frequency was 0.049 in MI patients compared to 0.091 in MI controls, suggesting a protective effect against MI ($P_{allelic}$=0.033; Table I). No association was evident between the SNP and MI in males.

Association between the rs35829419 polymorphism and CRP levels in serum. The effect of rs35829419 polymorphism on CRP levels in the study group and the controls was investigated. In the cohort, the distribution of CRP levels was significantly higher in cases when compared to the controls (20). However, no difference was observed in CRP levels between males and females in both the cases and controls.

No significant association was observed between the minor allele of rs35829419 and CRP in the overall analysis. Investigation for a possible association of the minor allele of rs35829419 to CRP levels in healthy controls and MI patients demonstrated a moderate association of the minor allele with the increase of CRP levels in the healthy control subjects (P=0.066; Table II). No evidence of SNP association with the CRP levels in MI patients was observed. Gender-specific analysis in the healthy control subjects conferred the association of the minor allele with an increase of CRP levels in males (P=0.042; Table II) but not in females.

Discussion

The present study suggests a gender-specific role of the *NLRP3* gene in regulating inflammatory mediators during the pathophysiology of MI. Specifically, we report that the minor allele rs35829419 (Q705K) polymorphism in the *NLRP3* gene confers protection against MI in females and is associated with a higher CRP level in unaffected males. These results support the gender-specific differences in genetic patterns of the Q705K polymorphism influencing the genotype-phenotype interaction of MI as described in a previous study (15).

The reported association of *NLRP3* polymorphism conferring protection against MI in females is contrasted by the absence of a similar association in males, suggesting a distinct effect of the *NLRP3* gene across genders. The reason for this discrepancy remains unclear. The protective effect of the variant against MI in females may be partly due to the synergistic influence of female hormones and the ability of IL-1β to counteract the negative impact of inflammation. The role of estrogen in modulating the inflammatory events is well documented in the reciprocal inhibitory crosstalk between estrogen receptor and nuclear factor κ light chain enhancer of activated B cells (22). However, the majority of females with MI in our study were postmenopausal, suggesting that the hormonal difference does not attribute to the observed gender difference. Lifestyle factors, including type of food intake and physical activity, between males and females of northern Sweden may contribute to the gender-specific genetic association and MI observed in the present study (23). Furthermore, the physical performance of females decreases with age compared with males (24), thereby possibly predominating the influence of genetic variation in older age. Additionally, sexual dimorphism of a disease may also serve as a contributory factor to genetic changes such as mutation and gene expression patterns prevalent with aging. In a recent study, the X-linked miR-223 was found to suppress NLRP3 expression by binding to the conserved 3'-untranslated region of *NLRP3*, thereby negatively controlling the inflammasome activity (25). Thus, miRNA driven gene regulation may be elucidate this gender-specific response. This genetic advantage of X-linked miRNA in females may therefore contribute to the immunological advantages when facing immune challenges (26).

Moreover, the minor allele showed a distinct pattern of gender-specific association with CRP levels. The minor allele was significantly associated with the higher CRP levels in males in contrast to the affected females. The association of the Q705K polymorphism with upregulated CRP levels in unaffected males suggests that the polymorphism may impart an inflammasome-mediated inflammatoty phenotype to males. The polymorphism Q705K in the *NLRP3* gene is a gain-of-function polymorphism responsible for the abnormal production of IL-1β leading to the deleterious effect of inflammation (18). During inflammation, IL-1β induced the expression of CRP, suggesting a link between the inflammasome and minor allele of the Q705K polymorphism and CRP present in males (27). This finding may explain the association of the minor allele of Q705K polymorphism with the upregulated CRP levels in unaffected males. The absence of a genetic association in the MI population remains to be elucidated but may be due to the possibility that factors unrelated to NLRP3 play a regulatory role on CRP levels.

In conclusion, the Q705K polymorphism in the *NLRP3* gene is protective against MI in females and is associated with an increase in CRP levels in unaffected males. Mechanistic studies should, however, be conducted to determine the gender-specific effect of the Q705K polymorphism. The finding in the present study demonstrates the importance of the Q705K variant in the pathogenesis of inflammatory disease, including MI, across the genders.

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