Journal of Clinical & Cellular Immunology - Open Access

Research Article
OPEN ACCESS Freely available online
doi:10.4172/2155-9899.1000101

A Polymorphism at Position +874 in The IFN-γ Gene is Associated with Susceptibility for Dilated Cardiomyopathy

Erika Lindberg[1], Bert Andersson[1], Robert Eggertsen[2], Ernst Nyström[2] and Yvonne Magnusson[1,3]*

[1]Department of Molecular and Clinical Medicine, University of Gothenburg, Sweden
[2]Department of Primary Health Care, Institute of Medicine, University of Gothenburg, Sweden
[3]Department of Clinical Chemistry, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Sweden

Abstract

The etiology of idiopathic dilated cardiomyopathy (DCM) is largely unknown. One hypothesis is that immunological factors are responsible for disease development. Single nucleotide polymorphisms (SNP) at position +874 of the IFN-γ gene and at position -168 of the MHC2TA gene have previously been shown to associate with inflammatory and autoimmune disorders. We analysed their possible influence on susceptibility and prognosis in patients with DCM. Genotypes were determined by real-time PCR in 442 patients and 425 controls. Genotype frequencies were found significantly different between patients and controls for IFN-γ (p=0.029), but not for MHC2TA (p=0.26). In a logistic regression analysis, the high producing TT genotype of IFN-γ was signficantly more common in patients than controls (OR 1.55 [1.12-2.15], p=0.009). None of the polymorphisms had an influence on long-term outcome, 10-years mortality with a HR of 0.94 (p=0.74) and 0.85 (p=0.30), IFN-γ and MHC2TA, respectively. A 50% higher risk to develop DCM for individuals with the TT genotype of the IFN-γ gene is a new finding and emphasize the role of IFN-γ in DCM pathology.

Keywords: Genetics; Single nucleotide polymorphisms; Autoimmunity; Cytokines; Cardiomyopathy

Abbreviations: DCM: Dilated Cardiomyopathy; HR: Hazard Ratios; MHC II: Major Histocompatibility Complex II; SNP: Single Nucleotide Polymorphisms; IFN-γ: Interferon-γ; MHC2TA: Major Histocompatibility Complex II Transactivator Gene

Introduction

Although idiopathic dilated cardiomyopathy (DCM) is the third most common cause of heart failure after coronary artery disease and hypertension, the etiology is largely unknown. Various explanations for its etiology have been proposed, mostly with the hypothesis that an initial viral myocarditis develops into a chronic myocardial disease. However, there are no conclusive studies to prove this theory. Proinflammatory cytokines such as TNF-α and IL-6 have long been reported as elevated in plasma of patients with DCM and shown to correlate with heart dysfunction. These cytokines are today considered part of the heart failure syndrome [1,2]. Repetitive coxsackievirus infection has been reported to induce cardiac dilatation in post-myocarditic mice [3]. If the acute viral myocarditis progresses into a chronic inflammation of the myocardium, autoimmune factors might be involved. In fact, this is supported by several studies reporting presence of auto antibodies in patients with DCM [4,5].

IFN-γ was first discovered for its antiviral activity but is today known to modulate almost all parts of the immune response including the expression of the major histocompatibility complex II (MHC II) via the MHC II transactivator CIITA [6-8]. A functional SNP at position +874 of the human IFN-γ gene correlating with differential cytokine production has been reported by Pravica, et al., [9]. A functional SNP has also been reported in the MHC2TA gene, which encodes for CIITA. The SNP at position -168 of the MHC2TA gene was shown to associate with differential expression of MCH2TA and with differential MHC transcription upon IFN-γ stimulation of leukocytes in vivo [10]. In addition, the polymorphism was shown to associate with rheumatoid arthritis, multiple sclerosis and myocardial infarction.

The aim of the present study was to investigate a possible influence of functional polymorphisms of the IFN-γ and MHC2TA genes on susceptibility to, and prognosis of DCM.

Methods

Study subjects

Patients with idiopathic dilated cardiomyopathy (DCM) were recruited from a prospective multicenter study of DCM (n=286), recruited consecutively from 1997 in seven Swedish hospitals. Patients with a medical history and investigations compatible with idiopathic DCM were included (left ventricular dilatation and ejection fraction <0.50). Patients with idiopathic heart failure from a previous cross-sectional epidemiological survey of idiopathic DCM in five counties of western Sweden during 1985-1988 were also included (n=156) [11,12]. The following causes of HF were excluded: ischemic heart disease (ruled out by coronary angiography or a history of myocardial infarction); definite hypertension (blood pressure >170/100 mm Hg or ongoing treatment for hypertension); significant valvular disease; significant systemic infection; excessive alcohol consumption; insulin treated diabetes mellitus; endocrine disorders such as pheochromocytoma, acromegaly, thyroid disease; systemic diseases; cancer treatment including irradiation; tachycardia-induced cardiomyopathy or other primary cardiomyopathy.

The control group consisted of 425 individuals from a community in Western Sweden. They were selected through a random procedure, using the population registry of Göteborg (~ 450 000 inhabitants) and the Mölnlycke community (~14 000 inhabitants) and were invited

***Corresponding author:** Yvonne Magnusson, Associate Professor, Institute of Biomedicine, Sahgrenska Academy, University of Gothenburg, P.O. BOX 419, SE-405 30 Göteborg, Sweden, Tel: +46 31 786 2904; Fax: +46 31 82 98 23; E-mail: yvonne.magnusson@gu.se

Received July 17, 2010; **Accepted** September 15, 2010; **Published** September 15, 2010

Citation: Lindberg E, Andersson B, Eggertsen R, Nyström E, Magnusson Y (2010) A Polymorphism at Position +874 in The IFN-γ Gene is Associated with Susceptibility for Dilated Cardiomyopathy. J Clin Cell Immunol 1:101. doi:10.4172/2155-9899.1000101

by a personal letter or a telephone call for an examination at the Sahlgrenska University Hospital or at the Primary Health Care Centre, Mölnlycke, Sweden. The characteristics of the study groups are given in table 1. The study complies with the Declaration of Helsinki. All patients and controls had given informed consent and the study was approved by the Ethics Committee at Göteborg University.

Genotyping

Genomic DNA was isolated from whole blood using the QIA amp Blood DNA mini kit according to the manufacturer's instructions (Qiagen Inc, Hilden, Germany). Allelic discrimination with TaqMan real-time PCR was used to genotype for polymorphism in the IFN-γ +874T/A and the MHC2TA -168A/G genes. For IFN-γ the following primers and probes were used: forward primer 5´ATT CAG ACA TTC ACA TTC ACA ATT GAT TTT ATT CTT AC 3´and reverse primer 5´ACT GTG CCT TCC TGT AGG GTA TTA TT 3´. Probe 1: 5´MGB-AAT CAA ATC TCA CAC ACA C 3´-FAM and probe 2: 5´MGB-ATC AAA TCA CAC ACA CAC 3´-VIC (rs2430561) [13]. The PCR and end point analysis was performed in a volume of 15 μL containing 60 ng genomic DNA, 300 nM of each primer, 50 nM of each probe 7.5 μL PCR TaqMan Buffer (Applied Biosystems, Branchburg, New Jersey, US). The PCR was accomplished in 40 cycles (50°C, 2 minutes; 95°, 10 minutes; 92°C, 15 seconds and 60°C, 1 minute). For MHC2TA pre-designed primers and probe were used (rs3087456) (Applied Biosystem). The PCR and end point analysis were carried out in a volume of 10 μL, containing 60ng genomic DNA, 0.5 μL Primer-Probe mix and 5 μL PCR TaqMan Buffer and according to cycling conditions as described above.

Statistics analyses

The polymorphism at position +874 of the IFN-γ gene results in TT, TA and AA genotypes and polymorphism at position -164 of the MHC2TA gene results in GG, GA and AA genotypes. Logistic regression analysis uses dichotomised variables; therefore analysed genotypes were divided as follow: TT vs. TA+AA for IFN-γ, and GG vs. GA+AA for MHC2TA. Logistic regression was performed to estimate odds ratios (ORs) with 95% confidence intervals (CI) of genotype frequencies. It was estimated that a total of 425 cases in each group would detect a difference with an OR of 2.0, a significance of $p<0.05$, and a statistical power of 95 %, if the prevalence was 25%. A multivariate Cox proportional hazards model was used to explore associations between genotype and mortality rate. Risk evaluation was expressed as hazard ratios (HR) with 95% CI. The Kaplan-Meier method was used to construct survival curves and differences between curves were evaluated with the log-rank test. Genotype frequencies were compared by use of the chi-square test. Statistical analyses were performed using SPSS statistical software (version 15.0; SPSS, Chicago, Ill). All genotypes were in Hardy-Weinberg equilibrium

Results

Baseline characteristics of patients and controls are presented in (Table 1).

Susceptibility for DCM

The single nucleotide polymorphism at position +874 of the IFN-γ gene and at position -168 of the MHC2TA gene was determined in 442 patients with DCM and 425 control subjects, Table 1. For IFN-γ, the frequency of the T allele was 0.48 and 0.43 in patients and controls, respectively ($p=0.05$). Genotype frequencies were significantly different between patients and controls ($p=0.03$). In a logistic regression analysis there was a significant association between IFN-γ gene polymorphism and DCM (OR 1.544 [1.116-2.135], $p=0.009$), the TT genotype more common in patients than in controls, (Table 2). For MHC2TA, the frequency of the G allele was 0.23 and 0.21, patients and controls, respectively ($p=0.26$). Genotype frequencies did not differ between patients and controls ($p=0.11$). Neither did we found any association between the MHC2TA gene polymorphism and DCM

	Controls n=425[a]	DCM n=442[b]
Gender		
Male	45 %	82 %
Female	55 %	18 %
Age (y)	61 ± 11	53 ± 12 (n=439)
Heart rate (beats/min)	67 ± 11 (n=321)	74 ± 16 (n=351)
Systolic blood pressure (mmHg)	138 ± 18 (n=321)	125 ± 23 (n=364)
EF (%)	Not applicable	31 ± 15 (n=381)
NYHA class (I:II:III:IV)	Not available	69:123:155:15 (n=362)
β-Blockers	8 % (n=321)	66 % (n=365)
ACE inhibitors	Not available	69 % (n=365)

EF: Ejection fraction; ACE: Angiotensin-converting enzyme. [a]n=424 for MHC2TA; [b]n=440 for IFN-γ

Table 1: Baseline characteristics of patients with idiopathic dilated cardiomyopathy (DCM) and control subjects.

Polymorphism	Phenotype	Controls n=425[a] % (n)	DCM n=442[b] % (n)	OR (95 % CI)	p-value
IFN-γ					
AA	Low	31 (134)	29 (127)	1.0	
TA	Intermediate	51 (215)	46 (202)		
TT	High	18 (76)	25 (111)	1.54 [1.12-2.14]	0.009
MHC2TA					
GG	Low	7 (28)	6 (25)	1.0	
GA	Intermediate	28 (119)	34 (150)		
AA	High	65 (277)	60 (267)	1.25 [0.95-1.64]	0.101

For calculation of OR, heterozygotes were coded as AA of IFN-γ and GG of MHC2TA, respectively. [a]n=424 for MHC2TA; [b]n=440 for IFN-γ

Table 2: Genotype frequency in patients with idiopathic dilated cardiomyopathy (DCM) and control subjects.

(OR 1.252 [0.954-1.643], p=0.101). The GG+GA genotypes were slightly more common in patients than controls, (Table 2).

Including both polymorphisms in a stepwise logistic regression analysis the association with DCM was stronger for IFN-γ than MHC2TA with OR values that remained almost identical to the univariate analysis, OR 1.543 [1.116 -2.135], p=0,009; OR 1.252 [0.953-1.644], p= 0,107, respectively (data not shown).

Severity of DCM

Long-term outcome: The 10-years total mortality risk was ~50% but did not differ between genotypes for neither of the polymorphisms with a hazard risk of 0.94 [0.66 – 1.35], p=0.74 for IFN-γ, and 0.85 [0.62 – 1.16], p=0.30 for MHC2TA (Figure 1 and Figure 2).

Cardiac function: We also analyzed whether cardiac function (median left ventricular EF) or functional class (NYHA I-II versus III-IV) were dependent on genotype among patients with DCM. However there were no significant differences in genotype frequencies between groups (data not shown).

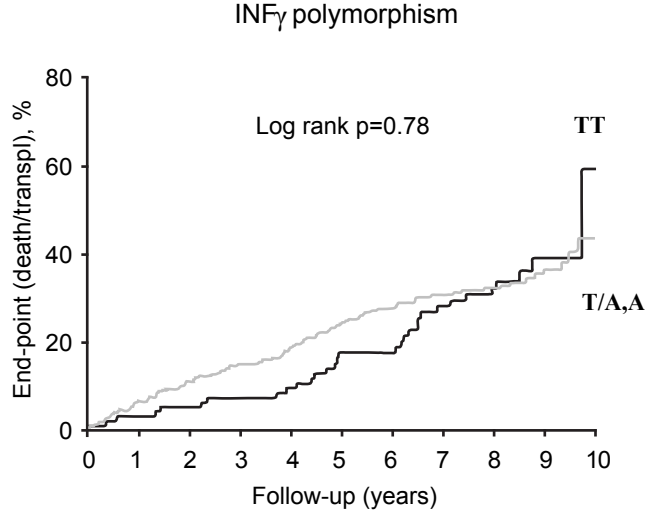

Figure 1: Ten year total survival according to polymorphisms in the IFN-γ gene in patients with DCM.

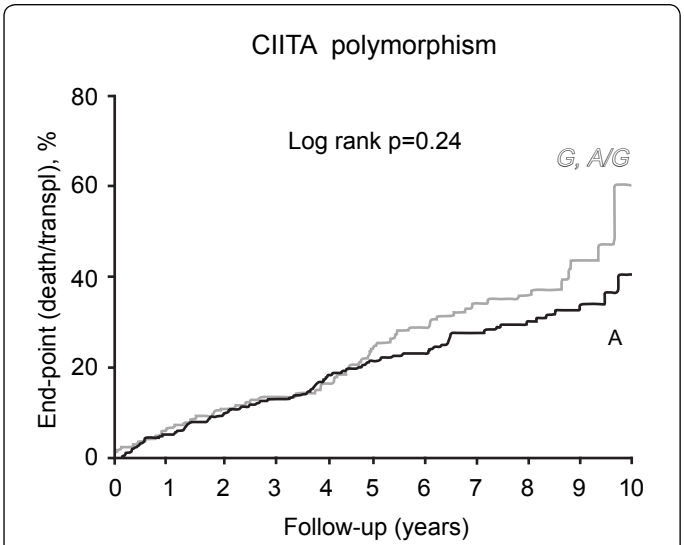

Figure 2: Ten year total survival according to polymorphisms in the MHC2TA gene in 425 patients with DCM.

Discussion

The finding of this study is an association between the high producing IFN-γ TT genotype and an increased risk to develop DCM. IFN-γ is an important cytokine regulating a number of immunological pathways including activation of macrophages, Th1 development, the MHC II expression, cell proliferation and apoptosis [7]. Individuals carrying the TT genotype had 50% increased risk to develop DCM compared with a carriers. This is an interesting finding which might explain the autoimmune features in a subgroup of patients with DCM.

IFN-γ is shown to both accelerate and prevent disease progression [14]. In experimental autoimmune myocarditis, IFN-γ deficiencies enhance acute inflammation and promote chronic progression into heart failure and DCM [15-17]. In patients with DCM intravenous immunoglobulin treatment, shown to block the IFN-γ gene, is associated with improve cardiac function and increased plasma levels of IL-10 [18]. We recently reported lower plasma levels of IL-10 in DCM compared with ischemic cardiomyopathy [19]. Together this suggests an imbalance between IL-10 and IFN-γ in DCM.

IFN-γ is a strong inducer of MHC II expression on antigen presenting cells [20]. In our study, the allele and genotype frequencies of the MHC2TA -168A/G polymorphism was in the same range as reported by Swanberg, but did not associate with the susceptibility to DCM [10]. Other studies have failed to confirm the association between the MHC2TA polymorphism and RA, SLE, Wegener's granulomatosis and inflammatory bowel disease [21-23].

In the present study neither of the polymorphisms, IFN-γ or MHC2TA, had any significant influence on long-term outcome of patients with DCM. This is in contrast to a study on patients with type 2 diabetes and a previous MI where the low expressing variants of MHC2TA was associated with increased cardiovascular mortality [24]. DCM prognosis is, however, influenced by many other factors, like heart failure treatment, age, and co-morbidity, which probably are more important to survival.

We recently reported that patients with DCM display a significantly lower number of IFN-γ producing $CD4^+$ T cells upon stimulation in vitro compared with healthy controls [25]. Whether a high producing genotype and a reduce number of functionally active INF-γ producing T cells are additive disease components needs to be further studied.

We did not measure auto-antibodies, plasma IFN-γ or plasma BNP. Among DCM patients, there is a well-known gender difference with dominance of male subjects. The control population was originally chosen to investigate equal number of genders. However, there were no gender or age differences with regard to genotypes in controls or in patients. A family history of autoimmunity was reported by several patients, but was not consecutively recorded.

In conclusion, IFN-γ is an important regulatory cytokine where the amount and the duration must be delicately balanced for optimal host wellness. Our observation that individuals with the TT genotype of the IFN-γ gene have a 50% higher risk to develop DCM is a new finding and emphasize the role of IFN-γ in DCM pathology.

Acknowledgement

We thank Anna-Karin Petersson for assistance with genotype analysis. We also thank doctors Ingrid Kockum and Hans Nissbranth for control DNA of the investigated polymorphisms in the MHC2TA and IFN-γ genes. This work was supported by the Swedish Heart Lung Foundation, the Swedish Research Council and the Federal Government under the LUA/ALF agreement.

References

1. Levine B, Kalman J, Mayer L, Fillit HM, Packer M (1990) Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med 323: 236-241.
2. Mann DL (2002) Inflammatory mediators and the failing heart: past, present, and the foreseeable future. Circ Res 91: 988-998.
3. Nakamura H, Yamamoto T, Yamamura T, Nakao F, Umemoto S, et al. (1999) Repetitive coxsackievirus infection induces cardiac dilatation in post-myocarditic mice. Jpn Circ J 63: 794-802.
4. Magnusson Y, Marullo S, Hoyer S, Waagstein F, Andersson B, et al. (1990) Mapping of a functional autoimmune epitope on the beta 1-adrenergic receptor in patients with idiopathic dilated cardiomyopathy. J Clin Invest 86: 1658-1663.
5. Caforio AL, Mahon NJ, Tona F, McKenna WJ (2002) Circulating cardiac autoantibodies in dilated cardiomyopathy and myocarditis: pathogenetic and clinical significance. Eur J Heart Fail 4: 411-417.
6. Wheelock EF (1965) Interferon-like virus-inhibitor induced in human leukocytes by phytohemagglutinin. Science 149: 310-311.
7. Boehm U, Klamp T, Groot M, Howard JC (1997) Cellular responses to interferon-gamma. Annu Rev Immunol 15: 749-795.
8. Morris AC, Beresford GW, Mooney MR, Boss JM (2002) Kinetics of a gamma interferon response: expression and assembly of CIITA promoter IV and inhibition by methylation. Mol Cell Biol 22: 4781-4791.
9. Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV (2000) A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. Hum Immuno l 61: 863-866.
10. Swanberg M, Lidman O, Padyukov L, Eriksson P, Akesson E, et al. (2005) MHC2TA is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction. Nat Genet 37: 486-494.
11. Andersson B, Caidahl K, Waagstein F (1995) Idiopathic dilated cardiomyopathy among Swedish patients with congestive heart failure. Eur Heart J 16: 53-60.
12. Magnusson Y, Levin MC, Eggertsen R, Nystrom E, Mobini R, et al. (2005) Ser49Gly of beta1-adrenergic receptor is associated with effective beta-blocker dose in dilated cardiomyopathy. Clin Pharmacol Ther 78: 221-231.
13. Rad R, Dossumbekova A, Neu B, Lang R, Bauer S, et al. (2004) Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during Helicobacter pylori infection. Gut 53: 1082-1089.
14. Rosloniec EF, Latham K, Guedez YB (2002) Paradoxical roles of IFN-gamma in models of Th1-mediated autoimmunity. Arthritis Res 4: 333-336.
15. Afanasyeva M, Wang Y, Kaya Z, Stafford EA, Dohmen KM, et al. (2001) Interleukin-12 receptor/STAT4 signaling is required for the development of autoimmune myocarditis in mice by an interferon-gamma-independent pathway. Circulation 104: 3145-3151.
16. Afanasyeva M, Georgakopoulos D, Rose NR (2004) Autoimmune myocarditis: cellular mediators of cardiac dysfunction. Autoimmun Rev 3: 476-486.
17. Eriksson U, Kurrer MO, Sebald W, Brombacher F, Kopf M (2001) Dual role of the IL-12/IFN-gamma axis in the development of autoimmune myocarditis: induction by IL-12 and protection by IFN-gamma. J Immunol 167: 5464-5469.
18. Gullestad L, Aass H, Fjeld JG, Wikeby L, Andreassen AK, et al. (2001) Immunomodulating therapy with intravenous immunoglobulin in patients with chronic heart failure. Circulation 103: 220-225.
19. Lindberg E, Magnusson Y, Karason K, Andersson B (2008) Lower levels of the host protective IL-10 in DCM--a feature of autoimmune pathogenesis? Autoimmunity 41: 478-483.
20. Ramsdell F, Fowlkes BJ (1990) Clonal deletion versus clonal anergy: the role of the thymus in inducing self tolerance. Science 248: 1342-1348.
21. Koizumi K, Okamoto H, Iikuni N, Nakamura T, Kawamoto M, et al. (2005) Single nucleotide polymorphisms in the gene encoding the major histocompatibility complex class II transactivator (CIITA) in systemic lupus erythematosus. Ann Rheum Dis 64: 947-950.
22. Akkad DA, Jagiello P, Szyld P, Goedde R, Wieczorek S, et al. (2006) Promoter polymorphism rs3087456 in the MHC class II transactivator gene is not associated with susceptibility for selected autoimmune diseases in German patient groups. Int J Immunogenet 33: 59-61.
23. Orozco G, Robledo G, Linga Reddy MV, Garcia A, Pascual-Salcedo D, et al. (2006) Study of the role of a functional polymorphism of MHC2TA in rheumatoid arthritis in three ethnically different populations. Rheumatology (Oxford) 45: 1442-1444.
24. Lindholm E, Melander O, Almgren P, Berglund G, Agardh CD, et al. (2006) Polymorphism in the MHC2TA gene is associated with features of the metabolic syndrome and cardiovascular mortality. PLoS One 1: e64.
25. Lindberg E, Andersson B, Hornquist EH, Magnusson Y (2010) Impaired activation of IFN-gamma+CD4+ T cells in peripheral blood of patients with dilated cardiomyopathy. Cell Immunol 263: 224-229.