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RESEARCH ARTICLE

Role of OAS-1 and MxA Gene Polymorphisms in Susceptibility and Treatment of HCV Patients

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Abstract

Background: Response to IFN therapy in HCV infected patients differs among individuals, suggesting a role of host genetic factors. IFN stimulates the expression of a number of enzymes with antiviral activities, including OAS-1 gene and MxA gene. The SNP of OAS1 gene at exon 7-SAS and MxA SNP at nt-88 in the promoter region were found to affect the expression of both OAS-1 protein and MxA protein, respectively.In this study, relation between these SNPs with susceptibility to HCV infection and responsiveness of Egyptian HCV patients to PEG-IFN and ribavirin treatment along with other host-related factors was assessed. **Methods:** The MxA nt-88 SNP and the OAS-1 7-SAS SNP was genotyped by RFLP analysis in 120 interferon treatment-naïve Egyptian patients who were treated with PEG-IFN and ribavirin and 40 healthy control volunteers.Correlations of SNP genotypes with response to interferon and clinical status of patients were statistically analyzed. Also correlation of these SNPs and susceptibility to HCV infection was assessed. **Results:** OAS-1GG, MxAGT genotypes appeared to be risk factor for protection against chronic HCV infection. On the other hand, OAS-1AA genotype and OAS-1A allele appeared to be risk factor for chronic infection with HCV. In addition,OAS-1AA genotype appeared to be risk factor for IFN non responsive. On the other hand, OAS-1AG, MxATT genotypes and MxA T allele appeared to be protective against NR. **Conclusions**: Subsequently, it was concluded that OAS-1 and MxA genetic polymorphisms might be considered as biological markers to identify susceptibility and treatment response to HCV infection.

INTRODUCTION

Around one hundred thirty and one hundred fifty million people are infected with hepatitis C virus (HCV) and higher than 350,000 people deceased every year from hepatitis C-related liver diseases. One of the highest prevalence of HCV infection is in Egypt where it represents a major public health problem (WHO 2011). The primary goal of antiviral therapy is sustained virologic response (SVR) for the eradication of HCV and therefore improving liver histology and patient survival (Niederau et al., 1998). However, by current standard therapy, rates of SVR are only about 54–63% and are still unsatisfactory (Hadziyannis et al., 2004). Current standard-of-care therapy for HCV patients consists of PEG-IFN plus ribavirin (Pearlman and Sjorgen, 2010).

A number of viral and host factors such as viral genotype, viral load, gender and stage of fibrosis have been identified as predictors of treatment response. However, different studies suggest that genetically determined inter-individual differences in immune response may also have an impact on treatment response (Welzel et al., 2009). For that, IFN-induced genes as OAS-1 and MxA genes are studied.

The OAS genes encode the aniviral protein 2',5'-oligoadenylate synthetase enzyme (2'5'AS) which degrades viral and cellular RNA and inhibits protein synthesis and viral replication and thus can affect all viruses including HCV (Kristiansen et al., 2010).

OAS is suspected to be involved in HCV infection as serum of patients under IFN-α therapy had an elevated levels of OAS activity, and the levels correlated with the success of treating HCV infection by PEG-IFN-α (Mihm et al., 2009; Shindo et al., 2008). Also, OAS1 has previously shown to be important for flavivirus susceptibility (Lim et al., 2009; Scherbik et al., 2007).

The 7-SAS SNP of OAS-1 gene showed a strong association with enzyme activity, where the AG sequence is required for normal splicing at this site (Bonnevie et al., 2005). In the current study this SNP was examined for its association with HCV treatment response.

Mx proteins are interferon-induced GTPases where MxA protein possesses an antiviral activity (Samuel, 2001).Myxovirus resistance protein A (MxA) protein quantification is used as a marker of biological activity to monitor the clinical efficiency of IFN therapy, as it is specifically induced in a dose-dependent manner by type I IFNs (Gilliet al. , 2002; Ferna´ndez et al. , 2004).

It was proposed that MxA inhibited HCV because MxA protein is known to inhibit the replication of various other RNA viruses (Haller et al., 1998) and a SNP in the MxA gene promoter has been reported to correlate with the response of hepatitis C patients to IFN-α treatment (Hijikata et al., 2000). In most of the IFN-treated hepatitis C patients, high levels of MxA protein were found in contrast to minimal levels before treatment (Gilli et al., 2002; Meier et al., 2000). These reports suggested a role for the MxA protein in eliminating HCV. Ferna´ndez et al. (1999) reported that the MxA protein levels were greater in virological responders than in non-responders (NR).

In this study a number of host and viral factors have been identified that influence treatment outcomes but mainly the effect of genetic polymorphism of OAS-1 gene and MxA gene and their relation with other factors was studied.

1. Materials and Methods

1.1. Subjects:

The study was carried out on 120 patients with proven chronic hepatitis C who received pegylated IFN + ribavirin treatment and 40 healthy controls (negative for HCV Ab, HCV RNA and HBsAg) were enrolled in this study. Subjects were all Egyptians and informed consent was obtained from each subject before collecting blood samples. The present study complies with the ethical principles and guidelines for the participation of human subjects adopted by the "Research Ethics Committee", Faculty of Pharmacy, Mansoura University and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. All HCV patients were positive for both anti-HCV antibody and serum HCV RNA. Viral RNA quantifications were performed before treatment. IFN treatment and clinical follow-ups were performed at Mansoura University Hospital, Mansoura, Al-Dakahlya, Egypt. Prior to the start of the IFN therapy none of the patients had evidence of metabolic liver diseases, alcohol-induced, drug-induced or autoimmune hepatitis, thyroid hypo- or hyperfunction, or active schistosomiasis infection. A pretreatment liver biopsy was carried out for histopathological examination and assessment of the stages of fibrosis according to the Metavir Score (Bedossa and Poynard, 1996)

Patients were treated with PEG-IFN and ribavirin combination therapy for 48 weeks. They received PEG IFNa-2a, taken once a week s.c., in addition to oral ribavirin taken daily in a dose of

1000–1200 mg (according to body weight). Patients were followed for at least 6 months after completion of therapy. Sustained virological responders (SVR; n = 60) were defined as patients who tested negative for HCV RNA for at least 6 months after end of therapy. All other patients, who did not show SVR, were considered non-responders (NR; n = 60).

1.2. **Extraction of DNA:**

Blood samples from all subjects were collected. The genomic DNA from 200 µl blood samples was isolated using Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit 2012 (#K078 50 Prep) supplied by Thermo Fisher Scientific Inc. according to the protocols outlined in the manual.

1.3. **Primer design and PCR conditions for OAS-1 gene and MxA gene**.

A pair of primers that amplified the SNP region of OAS-1 gene and provided 203 bp PCR fragments that cover exon 7 AG splice-acceptor sites was designed according to El Awady et al. (2011). Also another pair of primers that amplified the SNP region and provided 351 bp PCR fragments that cover the biallelic G/T polymorphism in the promoter region of MxA at position –88 from the transcription start site was designed according to Knapp et al. (2003). The PCR reaction of each gene was carried out separately. The PCR protocol and thermal cycling program for both genes was optimized as follows: PCR amplification was carried out in 25 µL, containing 20–100 ng DNA for OAS-1 gene and 40–200 ng DNA for MxA gene, 500 nM of each primer, 0.2 mM dNTP's (Thermo Scientific), and 1.25 U Pfu DNA polymerase using 1X buffer with magnesium (Thermo Scientific). Thermal cycling in an PCR thermocycler machine FPROGO2D, Tchne LTD, Oxford Cambridge, U.K. included denaturation at 95°C for 5 min followed by 35 cycles each of denaturation at 95°C for 30 s; annealing at 60°C for 30 s for OAS-1 gene and 58°C for 30 s for MxA gene; and extension at 72°C for 1 min. Cycling was followed by a final extension step at 72°C for 7 min. Primer sequences for OAS-1 gene were as follows OAS1 5′-TGCAATGCAGGAAGACTCC-3′ as a forward primer and OAS2 5′-TGCAGGTCCAGTCCTCTTCT-3′ as a reverse primer and for MxA gene were as follows MxA1 5′-TGAAGACCCCCAATTACCAA-3′ as a forward primer and MxA2 5′-CTCTCGTTCGCCTCTTTCAC-3′ as a reverse primer.

1.4. **Determination of exon7 SAS-SNP in human OAS1 gene and the G/T polymorphism in the human MxA gene at nt–88 from the transcription start site**

A restriction fragment length polymorphism (RFLP) analysis was constructed for detection of AG polymorphism at exon 7 SAS of the OAS1 gene and GT polymorphism at nt-88 of the promoter region of human MxA gene. A restriction endonuclease, AluI, which recognizes the sequence of the SNP of OAS-1 gene was used according to a El Awady et al. (2011) where the enzyme recognition site is 5'...A G↑C T...3' ,3'...T C↓G A...5'. Also, a restriction endonuclease, HhaI, which recognizes the sequence of the SNP of MxA was used according to Knapp et al. (2003) where the enzyme recognition site is 5'...GC G↓C…3', 3'...C↑G CG...5'. Both digestions were performed on 5 µL of the PCR product for at least 1 h in a total volume of 15 µL with 0.5U of AluI (Thermo Scientific) or 0.5U of HhaI (Thermo Scientific) according to the manufacturer's recommendations. Then, the digested products were run on a 4% of agarose gel stained with ethidium bromide and observed under UV transillumination.

2. Statistical Analysis

The statistical analysis of data was done by using excel program (Microsoft Office 2013) and SPSS (statistical package for social science) program (SPSS, Inc, Chicago, IL) version 20. Qualitative data were presented as frequency and percentage. Chi square and Fisher's exact tests were used to compare groups. Quantitative data were presented as mean and standard deviation or median and range. The quantitative data were examined by Kolmogrov Smirnov test for normality. For comparison between groups; student t-test, ANOVA, Mann-whitneyand Kruskal Wallis tests (for non parametric data) were used.

The distribution of alleles and genotypes in the studied groups was tested for fitting to the Hardy-Weinberg equilibrium through comparing the observed and expected frequencies of genetic variants using the $\chi 2$ test.

The associations between HCV susceptibility to infection and to NR versus genotypes and alleles were estimated by computing the ORs and their 95% CIs. Logistic regression was used for prediction of risk factors of HCV susceptibility to infection and to NR.

Applying Hardy Weinberg equation, revealed that OAS-1 and MxA genotypes in both cases and control subjects were independent (i.e., they are in HW equilibrium (HWE)). There is no evidence to reject the assumption of HWE in the sample ($p>0.001$ for each).

3. RESULTS

4.1 Cohort description

To explore whether any of the clinical, pathological, biochemical or virological parameters is associated with specific response pattern, a comparison between control group and HCV patients including sustained virological responders (SVR) and non-responders (NR) was outlined in table (1). Among a total of 120 chronic hepatitis C virus (HCV) patients, the univariate analysis of all factors showed that there were no significant differences in age, sex in all groups. Mean age of HCV patients was 39.4 years (SD=8.9). They comprised of 74 males (61.7%) and 46 females (38.3%). Lower BMI was significant in control group compared to HCV group.

No significant difference regarding hematologic laboratory findings was observed.

ALT, AST showed significant increase in HCV patients versus controls. AFP was significantly higher in non responders when compared to responders. Otherwise, no significant differences were found in clinical chemistry data between cases and controls, as well as between responders and non responders.

Fifty five percent of HCV cases had positive IHA for Schistosomiasis. Non responders had higher incidence of positivity than responders, however, it did not reach significant differences.

Splenomegaly had significantly higher frequency in NR than SVR (p=0.011).

Median HCV viral load in all studied patients was $4.9X10^5$. Non responders had significantly higher viral load when compared to responders (p=0.001).

Advanced fibrosis stages were significantly associated with NR (p=0.015), whereas, increased activity was significantly associated with SVR (p=0.028).

4.2 Restriction fragment length polymorphism (RFLP) analysis of OAS-1 at exon 7 SAS

The biallelic A/G polymorphism at exon 7 SAS of OAS-1 gene was genotyped by RFLP using the enzyme AluI to digest the 203 bp PCR fragment. The presence of AA genotype was indicated by complete digestion into 150 bp and 53 bp. While GG genotype was indicated by a retained intact 203 bp PCR fragment. In cases of heterozygosity (AG), three fragments of 203 bp, 150 bp and 53 bp appeared on agarose gel electrophoresis as shown in figure 1.

4.3 Restriction fragment length polymorphism (RFLP) analysis of MxA gene at position -88

The biallelic G/T polymorphism in the promoter region of MxA at position –88 from the transcription start site was genotyped by RFLP using the enzyme HhaI to digest the PCR fragment of 351 bp. The presence of the G allele was indicated by digestion into fragments of 261, 51, 23 and 16 bp, and the presence of the T allele into fragments of 312, 23 and 16 bp. In cases of heterozygosity (GT), two fragments of 312 bp and 261 bp appeared on agarose gel electrophoresis as shown in figure 2.

4.4 Hardy-Weinberg equilibrium

This sample of individuals was selected randomly from unrelated population in Dakahlia Governorate in lower Delta, Egypt. Applying Hardy Weinberg equation, revealed that OAS-1 and MxA genotypes in both cases and control subjects were independent (i.e., they are in HW equilibrium (HWE)). There is no evidence to reject the assumption of HWE in the sample (For OAS-1 P-Control= 0.0671, P-HCV=0.0912 and for MxA P-Control=0.3527, P-HCV=0.0580).

4.5 Frequency of OAS-1 SNP at exon 7 SAS and MxA SNP at nt-88 in a sample of Egyptian population

To study the association of OAS-1 and MxA genetic polymorphisms with chronic HCV infection, the frequency of the tested SNP in both control and infected groups was compared. The results are indicated in table (2). OAS-1GG, MxAGT genotypes appeared to be risk factor for protection against chronic HCV infection (p= 0.016, 0.031 respectively). OAS-1AA genotype and OAS-1A allele appeared to be risk factor for chronic infection with HCV (p= 0.012, 0.004 respectively). Otherwise, no significant differences in distribution of OAS-1, MxA genotypes and alleles regarding susceptibility to chronic infection with HCV were found (Table 2).

4.6 Multiple regression analysis for prediction of susceptibility to chronic HCV infection:

Applying age, gender, ALT, AST, OAS-1 and MxA genotypes as predictors for susceptibility to HCV infection, in univariate logistic regression analysis; revealed that higher BMI and ALT concentration were associated with susceptibility to HCV infection. OAS-1 AG and GG genotypes were protective against susceptibility to HCV infection. However, in multivariate analysis, applying variables that were significant in univariate analysis, only higher ALT, AST and OAS-1 AG genotype were considered independent predictors for susceptibility to HCV infection (Table 3).

4.7 Comparison of OAS-1 genotypes and MxA genotypes between SVR and NR patients

The frequency of the tested SNP in both SVR and NR groups was compared as indicated in table (4). OAS-1AA genotype appeared to be risk factor for NR. OAS-1AG, MxATT genotypes and MxA T allele appeared to be protective against NR. Otherwise, no significant differences in distribution of OAS-1, MxA genotypes and alleles regarding response to therapy were found.

4.8 Multiple regression analysis for the prediction of non-responsein chronic HCV patients treated with interferon combination therapy

Applying age, gender, BMI, hemoglobin concentration, viral load, ALT, fibrosis, OAS-1 and MxA genotypes as predictors for susceptibility to non response, in univariate logestic regression analysis; revealed that higher viral load and more fibrosis showed significant association with NR. While OAS-1AG and MxATT genotypes were predictors for protection against NR. In multivariate analysis, applying those factors that were significant in univariate analysis, higher viral load and more fibrosis were independent bad prognostic factors for NR, while OAS-1AG and MxATT genotypes were independent good prognostic factor for protection against NR (Table 5).

4.9 Influence of different OAS-1 genotypes and MxA genotypes on liver fibrosis and activity stages in HCV patients

Regarding histological parameters, patients having OAS-1AA genotype showed significantly lower fibrosis and activity when compared to AG and GG genotypes (p=0.009, 0.0001 respectively). However, no significant differences between Mx1 genotypes regarding fibrosis and activity in all studied patients (Table 6)

5 Discussion

Several SNPs in IFN inducible genes resulted in altered gene expression or function were found to be associated with different IFN treatment responses (Suzuki et al., 2004; Matsushita et al., 1998; Hijikata et al., 2000; Knapp et al., 2003, El Awady et al. 2011). Among these are the OAS genes and MxA gene.

OAS genes encode the antiviral enzyme 2'5'OAS. Significant association between OAS-1 SNP and OAS enzyme activity was detected at multiple markers. The strongest was at the A/G SNP at the exon 7 SAS of the OAS1 gene. At this SNP, allele G retains the splice site and generates the p46 enzyme isoform with high enzyme activity, whereas allele A ablates the splice site results in splicing at alternate sites and generates a dual-function isoforms with low enzyme activity (Bonnevie et al., 2005).

Myxovirus resistance A (MxA) gene expression is considered as a marker of biological activity to monitor the clinical efficiency of treatment with IFN against viral infections (Ferna´ndez et al., 2004). A unique property of human MxA is its comparatively wide antiviral spectrum against a wide range of RNA viruses **(**Chieux et al., 2001; Gordien et al., 2001) and even some DNA viruses (Netherton et al., 2009). As the levels of the MxA expression during IFN therapy differ between individuals, genomic factors are suspected to be the cause. The SNP at nt-88 and the SNP at nt-123 of the promoter region of MxA gene were found to be most likely associated with the levels of IFN-induced expressionof the MxA protein, and thus further with the response of the hepatitis C patients to the IFN therapy (Knapp et al., 2003; Hijikata et al., 2000, 2001; Suzuki et al., 2004; Ferna´ndez et al., 2004; Gilli et al., 2002).

The correlation of different patient characteristics with susceptibility for chronic HCV infection was studied. Higher BMI, ALT and AST were significantly associated with higher susceptibility for chronic infection. Recent cohort studies have shown higher BMI to be related to an increased risk of hepatic steatosis and fibrosis. Moreover, obesity is associated with the progression of chronic HCV liver disease, with a diminished response to antiviral therapy (Lo Iacono et al., 2007; Delgado-Borrego et al., 2010).

The effect of different OAS-1 and MxA genotypes on the susceptibility to chronic HCV infection was studied. OAS-1GG, MxAGT genotypes appeared to be risk factor for protection against chronic HCV infection. On the other hand, OAS-1AA genotype and OAS-1A allele appeared to be risk factor for chronic infection with HCV. However, in multivariate analysis, applying variables that were significant in univariate analysis, only higher ALT and OAS-1AG genotype were considered independent predictors for susceptibility to HCV infection. These results are in parallel with earlier studies by Zhao et al. (2013) and El Awady et al. (2011). They found that the A allele at 7-SAS SNP of OAS-1 gene was significantly higher among chronic HCV infected patients than among control. In addition, an earlier study by Knapp et al. (2003) found that both TT and GT genotypes at the nt-88 of MxA gene were associated with self-limiting infection indicating that the T allele conferred a protective effect with respect to viral clearance. This is in parallel with the current study results.

Different patient characteristics and its correlation with treatment outcome were studied. Splenomegaly, higher AFP, higher viral load and advanced fibrosis were significantly associated with non-response (p=0.011, p=0.001, p=0.015 respectively). On the other hand, increased activity was significantly associated with SVR (p=0.028).

Also, the correlation of different OAS-1 and MxA genotypes and treatment response of chronic HCV patients were studied. OAS-1AA genotype appeared to be risk factor for NR. On the other hand, OAS-1AG, MxATT genotypes and MxA T allele appeared to be protective against NR. In multivariate analysis, applying those factors that were significant in univariate analysis, higher viral load and more

fibrosis were independent bad prognostic factors for NR, while OAS-1 AG and MxA TT genotypes were independent good prognostic factor for protection against NR. These findings indicate that OAS1 and MxA play a role in treatment response of HCV infection to interferon. These results are in agreement with an earlier study by El Awady et al. (2011) where Egyptian patients harboring G allele at 7-SAS of OAS-1 gene tend to achieve sustained response to interferon therapy. Moreover, these results are in parallel with earlier studies where T-allele was related with higher MxA expression and subsequently with response to treatment (Hijikata et al., 2000). Hijikata et al. (2000) and Suzuki et al. (2004) suggested that the SNP of the MxA promoter at nt –88 can be used as an independent predictor of IFN responsiveness in patients with chronic hepatitis C where MxA-T-positive patients might respond more efficiently than MxA-T-negative patients. Furthermore, in a study by Knapp et al. (2003), the GT genotype was found more frequently in initial response or sustained response patients to interferon therapy. However, in a study by Mohamad et al. (2011) on 42 Egyptian patients MxA nt-88 SNP was not significantly correlated to achieving SVR after IFN-alpha and ribavirin therapy which could be explained by small number size of their study.

Progression of HCV infection differs among individuals, indicating a possibility of participation of host genetic factors. Liver disease progression has been reported to be associated with different SNPs in previous studies (Li et al. 2009). In the current study, OAS-1AA genotype was significantly associated with lower fibrosis and activity when compared to AG and GG genotypes. This is a controversy as OAS-1AA was associated with NR. This is in contrast to a previous study by El Awady et al. (2011) where they found that the hepatic fibrosis score was higher in AA and GA genotypes at exon 7-SAS. On the other hand, they found no significant association between different OAS genotypes and liver activity. No significant differences between MxA genotypes regarding fibrosis and activity were found.

Conclusion:

In conclusion, the SNP of OAS-1 at the exon 7-SAS and MxA SNP at nt-88 of the promoter region might represent an added value as promising markers for prediction of susceptibility and treatment response of chronic HCV infection.

Statement: This study has not been published elsewhere and it has not been submitted simultaneously for publication elsewhere.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Bedossa P. and Poynard T (1996). An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatolog,24: 289–93.

Bonnevie-Nielsen V, Field LL, Lu S, Zheng DJ, Li M, Martensen PM, Nielsen TB, Beck-Nielsen H, Lau YL, Pociot F (2005). Variation in antiviral 2',5'-oligoadenylate synthetase (2'5'AS) enzyme activity is controlled by a single-nucleotide polymorphism at a spliceacceptor site in the OAS1 gene. Am J Hum Genet,76:623–633

Chieux V, Chehadeh W, Harvey J, Haller O, Wattre P, Hober D (2001). Inhibition of coxsackievirus B4 replication in stably transfected cells expressing human MxA protein. Virology,283:84–92.

Delgado-Borrego A, Healey D, Negre B, Christofi M, Sabharwal S, Ludwig DA et al (2010). Influence of body mass index on outcome of pediatric chronic hepatitis C virus infection. J Pediatr Gastroenterol Nutr ; 51: 191–197.

El Awady MK, Anany MA, Esmat G, Zayed N, Tabll AA, Helmy A, El Zayady AR, Abdalla MS, Sharada HM, El Raziky M, El Akel W, Abdalla S, Bader El Din NG(2011). Single nucleotide polymorphism at exon 7 splice acceptor site of OAS1 gene determines response of hepatitis C virus patients to interferon therapy. Journal of Gastroenterology and Hepatology,26 843–850

Ferna´ndez-Arca´s N, Blanco A, Gaita´n MJ, Nyqvist M, Alonso A, Reyes-Engel A (2004). Differential transcriptional expresión of the polymorphic myxovirus resistance protein A in response to interferonalpha treatment. Pharmacogenetics,14:189–193

Fernàndez M., Quiroga J. A., Martín J., Herrero M., Pardo M., Horisberger M. A. & Carren V (1999). In vivo and in vitro induction of MxA protein in peripheral blood mononuclear cells from patients chronically infected with hepatitis C virus. Journal of Infectious Diseases,180:262-267.

Gilli F, Sala A, Bancone C, Salacone P, Gallo M, Gaia E, Bertolotto A (2002). Evaluation of IFN-α bioavailability by MxA mRNA in HCV patients. J Immunol Meth,262:187–190.

Gordien E, Rosmorduc O, Peltekian C, Garreau F, Brechot C, Kremsdorf D (2001). Inhibition of hepatitis B virus replication by the interferon-inducible MxA protein. J Virol ,75:2684–2691.

Hadziyannis SJ, Sette Jr H, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H Jr, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A,Ackrill AM; PEGASYS International Study Group(2004). Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med,140:346–355.

Haller O, Frese M, Kochs G (1998). Mx proteins: mediators of innate resistance to RNA viruses. Rev Sci Tech,17: 220–230.

Hijikata M, Mishiro S, Miyamoto C, Furuichi Y, Hashimoto M, Ohta Y (2001). Genetic polymorphism of the MxA gene promoter and interferon responsiveness of hepatitis C patients: revisited by analysing two SNP sites (-123 and–88) in vivo and in vitro. Intervirology,44: 379–382.

Hijikata M, Ohta Y & Mishiro S (2000). Identification of a single nucleotide polymorphism in the MxA gene promoter (GT at nt 88) correlated with the response of hepatitis C patients to interferon. Intervirology,43:124-127.

Knapp S, Yee LJ, Frodsham AJ, Hennig BJ, Hellier S, Zhang L, Wright M, Chiaramonte M, Graves M, Thomas HC, Hill AV, Thursz MR(2003). Polymorphisms in interferon-induced genes and the outcome of hepatitis C virus infection: roles of Mx1 , OAS-1 and PKR. Genes Immunity,4: 411-419.

Kristiansen H, Scherer CA, McVean M, Iadonato SP, Vends S, Thavachelvam K, Steffensen TB, Horan KA, Kuri T, Weber F, Paludan SR, Hartmann R(2010). Extracellular 2'-5' Oligoadenylate Synthetase Stimulates RNase L-Independent Antiviral Activity: a Novel Mechanism of Virus-Induced Innate Immunity. Journal of Virology,84:11898–11904

Li CZ, Kato N, Chang JH, Muroyama R, Shao RX,Dharel N, Sermsathanasawadi R, Kawabe T, Omata M(2009). Polymorphism of OAS-1 determines liver fibrosis progression in hepatitis C by reduced ability to inhibit viral replication. Liver Int,29:1413–1421

Lim JK, Lisco A, McDermott DH, Huynh L, Ward JM, Johnson B, Johnson H, Pape J, Foster GA, Krysztof D, Follmann D, Stramer SL, Margolis LB, Murphy PM(2009). Genetic variation in OAS1 is a risk factor for initial infection with West Nile virus in man. PLoS Pathog,5:e1000321 doi:10.1371/journal.ppat.1000321

Lo Iacono O, Venezia G, Petta S, Mineo C, De Lisi S, Di Marco V et al (2007). The impact of insulin resistance, serum adipocytokines and visceral obesity on steatosis and fibrosis in patients with chronic hepatitis C. Aliment Pharmacol Ther ; 25: 1181–1191.

Matsushita M, Hijikata M, Matsushita M, Ohta Y, Mishiro S (1998). Association of mannose-binding lectin gene haplotype LXPA and LYPB with interferon-resistant hepatitis C virus infection in Japanese patients. J. Hepatol.,29: 695–700.

Meier V, Mihm S, Ramadori G (2000). MxA gene expression in peripheral blood mononuclear cells from patients infected chronically with hepatitis C virus treated with interferon-α. J Med Virol,62:318–26.

Mihm, U., Ackermann O., Welsch C., Herrmann E, Hofmann WP., Grigorian N, Welker MW, Lengauer T, Zeuzem S, Sarrazin C (2009). Clinical relevance of the 2'-5'-oligoadenylate synthetase/RNase L system for treatment response in chronic hepatitis C. J. Hepatol.,50:49–58.

Mohamed MS, Sabet SF, Moustafa MM, Salama MS (2011). Correlation between a single nucleotide polymorphism (G/T at nt –88) in the Mx1 gene promoter and the response to interferon therapy for hepatitis C virus in Egyptian patients. African Journal of Biotechnology,Vol. 10(62) 13376-82

Netherton CL, Simpson J, Haller O, Wileman TE, Takamatsu HH, Monaghan P, Taylor G (2009). Inhibition of a large double-stranded DNA virus by MxA protein. J Virol,83:2310-20.

Niederau C, Lange S, Heintges T,,Erhardt A, Buschkamp M, Hürter D, Nawrocki M, Kruska L, Hensel F, Petry W, Häussinger D(1998). Prognosis of chronic hepatitis C: results of a large prospective cohort study. Hepatology,28: 1687–1695.

Pearlman BL and Sjogren MH (2010). Treatment Options for HCV Nonresponders and Relapse Patients. Gastroenterol. Hepatol.,6: 1-12.

Ronni T, Melen K, Malygin A, Julkunen I (1993). Control of IFN inducible MxA gene expression in human cells. J Immunol,150: 1715–26.

Samuel CE (2001). Antiviral actions of interferons. Clin Microbiol Rev,14: 778–809.

Scherbik SV, Kluetzman K, Perelygin AA, Brinton MA (2007). Knock-in of the Oas1b(r) allele into a flavivirus-induced disease susceptible mouse generates the resistant phenotype. Virology,368: 232–237.

Shindo M., Hamada K, Morikawa T, Harano Y, Nakajima T, Okuno T (2008). In vivo interferon system assessed by 2'-5' oligoadenylate synthetase activity in chronic hepatitis C virus patients treated with pegylated interferon and ribavirin. Hepatol. Res.,38:1213–1220.

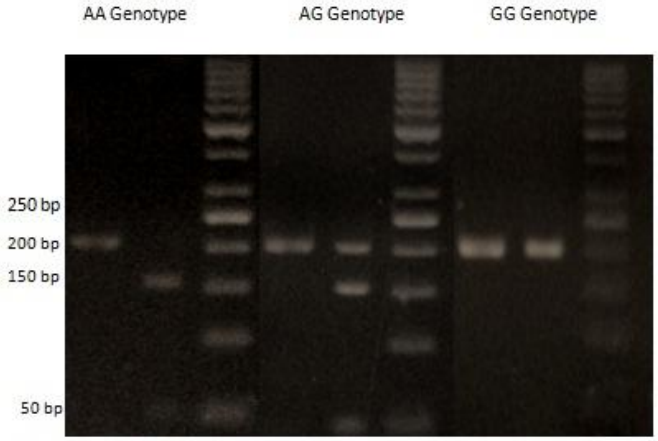
Suzuki F, Arase Y, Suzuki Y, Tsubota A, Akuta N., Hosaka T, Someya T, Kobayashi M, Saitoh S, Ikeda K, Kobayashi M, Matsuda M, Takagi K, Satoh J, Kumada H(2004). Single nucleotide polymorphism of the Mx1 gene promoter influences the response to interferon monotherapy in patients with hepatitis C viral infection. J. Viral Hepatitis,11: 271-276.

Welzel TM, Morgan TR, Bonkovsky HL,Naishadham D, Pfeiffer RM, Wright EC, Hutchinson AA, Crenshaw AT, Bashirova A, Carrington M, Dotrang M, Sterling RK, \Lindsay KL, Fontana RJ, Lee WM, Di Bisceglie AM, Ghany MG, Gretch DR, Chanock SJ, Chung RT, O'Brien TR; HALT-C Trial Group (2009). Variants in interferon-alpha pathway genes and response to pegylated interferon-Alpha2a plus ribavirin for treatment of chronic hepatitis C virus infection in the hepatitis C antiviral long-term treatment against cirrhosis trial. Hepatology,49:1847–1858.

WHO, Factsheet No 164, June 2011. http://goo.gl/5m3sY, accessed 10.01.2012.Updated July 2013 , http://www.who.int/mediacentre/ factsheets/fs164/en/).

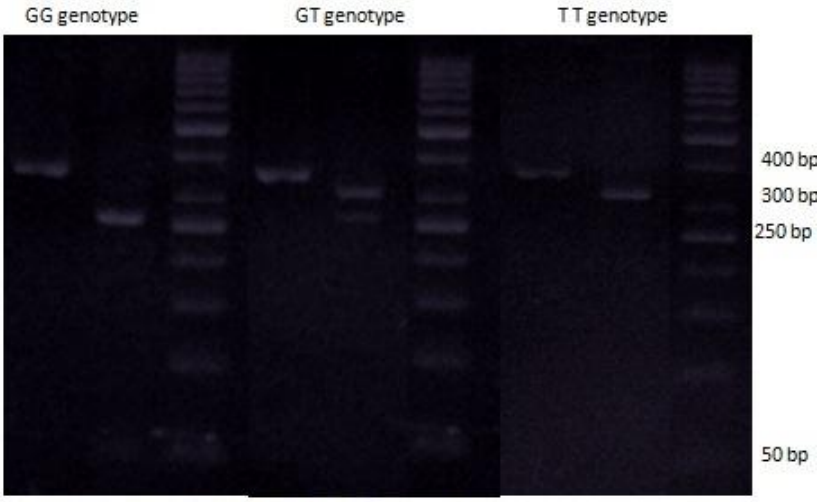
Zhao Y, Kang H, Ji Y, Che X (2013). Evaluate the relationship between polymorphisms of OAS1 gene and susceptibility to chronic hepatitis C with high resolution melting analysis. Clin Exp Med 2013,13:171–176

Fig. (1): Agarose gel electrophoresis of OAS-1 gene restriction by AluI enzyme



AA genotype → complete digestion into two fragments of 150 bp and 53 bp.
GG genotype → absence of AluI site a retained intact 203 bp PCR
heterozygosity (AG) → three fragments of 203 bp, 150 bp and 53 bp

Fig. (2): Agarose gel electrophoresis of MxA gene restriction by HhaI enzyme



G allele: cut into fragments of 261, 51, 23 and 16 bp
T allele : cut into fragments of 312, 23 and 16 bp.
GT : 312 + 261 bp

Table (1).Demographic, anthropometric data distribution, laboratory findings in all studied groups

		Control (n=40)			HCV Patients									P^1	P^2
					Total (n=120)			SVR (n=60)			NR (n=60)				
		Median	Range		Median	Range		Median	Range		Median	Range			
Age (years); mean ± SD		36.90	6.921		39.39	8.925		40.62	8.421		38.07	9.336		0.0715	0.132
Gender	Males; N (%)	26	65		74	61.7		34	56.7		40	66.7		0.706	0.260
	Females; N (%)	14	35		46	38.3		26	43.3		20	33.3			
BMI (kg/m2)		26	24	29	28.7	21	40.8	29.29	22	41	28.4	21	33	0.001	0.134
Total leucocytic count ($X10^9$/L)		6.6	4.5	9.1	6.45	2.9	9.8	6.25	2.9	9.8	6.55	3	8.8	0.384	0.860
Hemoglobin concentration (g/dL)		13.75	12.6	15	13.9	11	16	13.75	11.6	16	13.9	11	15.6	0.316	0.504
Platelet count ($X10^9$/L)		225.5	156	380	208.5	102	450	221	136	450	203.5	102	300	0.132	0.121
INR		1.07	0.9	1.1	1.03	0.8	1.33	1	0.8	1.09	1.05	1	1.33	0.065	0.276
Albumin (g/dL)		4.6	3.5	5	4.4	3.4	5.1	4.5	3.9	5	4.35	3.4	5.1	0.171	0.269
ALT (U/mL)		29.5	23	36	62	24	249	72.6	25	249	54.5	24	137	<0.001	0.953
AST (U/mL)		27.5	23	31	56.3	21	121	62.3	21	119	53	22	121	<0.001	0.188
Bilirubin (mg/dL)		0.85	0.6	1.15	0.8	0.4	1.5	0.8	0.4	1.5	0.9	0.5	1.4	0.246	0.078
ALP (U/mL)		83	76	115	86	56	250	96	65	250	85	56	190	0.577	0.371
Creatinin (mg/dL)		0.8	0.6	1.2	0.8	0.6	1.2	0.8	0.7	1.2	0.8	0.6	1.2	0.857	0.983
Glucose (mg/dL)		94.75	82	110	95	60	129	95	85	125	92.5	60	129	0.468	0.136
AFP (ng/mL)		2.6	0.9	6.8	4	0.8	28.5	2.4	0.9	8.6	5	0.8	28.5	0.119	0.003
TSH (uIU/mL)		1.5	0.9	2.9	1.5	0.4	4.9	1.45	0.4	4.9	1.5	0.7	4.7	0.785	0.281
Positive IHA (N,%)		-	-	-	66	55		28	46.7		38	63.3		-	0.067
Splenomegaly (N,%)		-	-	-	18	15		4	6.7		14	23.3		-	0.011
HCV viral load		-	-	-	$4.9x10^5$	$4.0x10^3$	$1.9x10^6$	$3.6x10^5$	$4.1x10^4$	$1.95x10^6$	$5.8x10^5$	$4.0x103$	$1.5x10^6$	-	0.001
METAVIR Fibrosis; N (%)	F1, F2	-	-	-	108	90		58	96.7		50	83.3		-	0.015
	F3, F4	-	-	-	12	10		2	3.3		10	16.7		-	
METAVIR Activity; N (%)	A1	-	-	-	60	50		24	40.0		36	60.0		-	0.028
	A2, A3	-	-	-	60	50		36	60.0		24	40.0		-	

The univariate analysis of clinical and virological factors was compared in all groups.P-value <0.05 are considered statistically significant. Data are represented as median and range or mean and SD or number and percentage.

P1, comparison between total HCV patients versus control subjects; p2, comparison between responders and non responders. SVR, sustained virologicalresponder; NR, non-responder.

Table (2). Distribution of *OAS-1*and *MxA*(alleles and genotypes) in HCV patients and healthy control subjects.

	Genotype and alleles	Control (n=40)		HCV Patients (n=120)		OR	95% CI		*P*
		N	%	N	%				
OAS-1	***AA***	4	10	40	33.3	4.5	1.497	13.525	0.004
	AG	25	62.5	66	55.0	0.733	0.352	1.528	0.407
	GG	11	27.5	14	11.7	0.348	0.1430	0.8480	0.016
	A allele	33	41.25	146	60.8	2.212	1.322	3.703	0.002
	G allele	47	58.75	94	39.2				
MxA	***GG***	23	57.5	87	65.0	1.949	0.926	4.101	0.076
	GT	16	40	27	22.5	0.436	0.203	0.935	0.031
	TT	1	2.5	6	5.0	2.053	0.240	17.586	0.681
	G allele	62	77.5	201	83.75	1.496	0.799	2.801	0.206
	T allele	18	22.5	39	16.25				

P-value <0.05 are considered statistically significant.

Table (3). Regression analysis for prediction susceptibility to HCV infection.

		Univariate				Multivariate			
		P	**OR**	**95% CI**		***P***	**OR**	**95% CI**	
Age (years)		0.155	1.177	0.997	1.389	-	-	-	-
Gender (females versus males)		0.706	1.154	0.547	2.436	-	-	-	-
ALT (U/L)		<0.001	1.210	1.114	1.314	<0.001	1.180	1.080	1.289
AST (U/L)		<0.001	1.147	1.056	1.174	<0.001	1.110	1.025	1.012
OAS-1	**AA**	Reference	1	-	-	-	-	-	-
	AG	0.439	0.020	0.264	0.086	0.015	0.143	0.030	0.689
	GG	0.954	0.002	0.127	0.035	0.887	0.767	0.345	0.876
MxA	**GG**	Reference	1	-	-	-	-	-	-
	GT	0.140	0.554	0.253	1.214	-	-	-	-
	TT	0.622	1.724	0.198	14.998	-	-	-	-

Table (4). Distribution of *OAS-1*and *MxA*(alleles and genotypes) in HCV responders and non responders groups.

	Genotype and alleles	SVR (n=60)		NR (n=60)		OR	95% CI		*P*
		N	%	N	%				
OAS-1	***AA***	14	23.3	26	43.3	2.513	1.144	5.517	0.020
	AG	40	66.7	26	43.3	0.382	0.182	0.802	0.010
	GG	6	10	8	13.3	1.385	0.450	4.265	0.570
	A allele	68	56.7	78	65	0.704	0.418	1.185	0.186
	G allele	52	43.3	42	35				
MxA	***GG***	40	66.7	47	78.3	1.808	0.800	4.087	0.152
	GT	14	23.3	13	21.7	0.909	0.386	2.142	0.827
	TT	6	10	0	0	0.474	0.390	0.575	0.012
	G allele	94	78.3	107	89.2	0.439	0.214	0.903	0.023
	T allele	26	21.7	13	10.8				

P-value <0.05 are considered statistically significant.SVR, sustained virologicalresponders; NR, non-responder.

Table (5). Regression analysis for prediction of non response.

		Univariate			Multivariate		
		P	**OR**	**95% CI**	***P***	**OR**	**95% CI**
Age (years)		0.134	0.968	0.927-1.010	-	-	-
Gender (females versus males)		0.261	0.654	0.312-1.372	-	-	-
BMI (kg/m^2)		0.091	0.914	0.823-1.014	-	-	-
Hemoglobin concentration (g/dL)		0.550	1.114	0.782-1.587	-	-	-
Viral load (IU/mL)		0.012	2.392	1.935-6.120	0.004	1.850	1.014-4.154
ALT (U/L)		0.425	0.996	0.986-1.006	-	-	-
Fibrosis (F3,4 vs F1,2)		0.028	1.800	1.213-7.728	0.006	1.973	1.030-5.566
OAS-1	**AA**	Reference	1	-	-	-	-
	AG	0.012	0.350	0.155-0.792	<0.001	0.140	0.051-0.385
	GG	0.601	0.718	0.207-2.486	0.156	0.247	0.059-1.036
MxA	**GG**	Reference	1	-			
	GT	0.829	0.962	0.676-1.369	0.716	0.835	0.315-2.213
	TT	0.019	0.649	0.287-0.847	0.049	0.376	0.251-0.987

P-value <0.05 are considered statistically significant.

Table (6). Effect of different OAS-1 genotypes and MxA genotypes on liver activity and liver fibrosis of HCV patients.

	Genotype and alleles	F1,F2 (n=108)		F3,F4 (n=12)		OR	95% CI		*P*
		N	%	N	%				
OAS-1	*AA (40)*	40	37.04	0	0	14.78	0.85	256.38	0.009
	AG (66)	58	53.70	8	66.67	0.58	0.16	2.04	0.382
	GG (14)	10	9.26	4	33.33	0.20	0.052	0.799	0.014
	A allele	138	63.89	8	33.33	3.54	1.45	8.64	0.003
	G allele	78	36.11	16	66.67				
MxA	*GG (87)*	77	71.29	10	83.33	0.49	0.10	2.39	0.376
	GT (27)	25	23.15	2	16.67	1.51	0.31	7.33	0.609
	TT (6)	6	5.56	0	0	1.59	0.08	29.86	0.402
	G allele	179	82.87	22	91.67	0.44	0.09	1.95	0.268
	T allele	37	17.13	2	8.33				
	Genotype and alleles	**A1 (n=60)**		**A2,A3 (n=60)**		**OR**	**95% CI**		***P***
		N	**%**	**N**	**%**				
OAS-1	*AA (40)*	30	50	10	16.67	5.00	2.14	11.66	0.0001
	AG (66)	24	40	42	70	0.29	0.13	0.61	0.0009
	GG (14)	6	10	8	13.33	0.72	0.23	2.22	0.5659
	A allele	84	70	62	51.67	2.18	1.29	3.71	0.0036
	G allele	36	30	58	48.33				
MxA	*GG (87)*	42	70	45	75	0.78	0.35	1.74	0.5397
	GT (27)	16	26.67	11	18.33	1.62	0.68	3.86	0.2744
	TT (6)	2	3.33	4	6.67	0.48	0.09	2.74	0.4022
	G allele	100	83.33	101	84.17	0.94	0.47	1.87	0.8611
	T allele	20	16.67	19	15.83				

P-value <0.05 are considered statistically significant.