Mansoura Journal of Chemistry Vol 40 (2) Dec., 2013.

LINKAGE DISEQUILIBRIUM OF TLR2 POLYMORPHISMS IN THE OUTCOME OF LIVER TRANSPLANTATION FOR CHRONIC HEPATITIS C VIRUS

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(Received: 17 / 7 / 2013)

ABSTRACT

Background &Aims: Liver transplantation activates the innate immune system by toll-like receptors (TLRs), potentially leading to allograft rejection and graft failure. So this study was to evaluate the possible association of two single nucleotide polymorphisms (SNPs); Arg753Gly and Arg677 Trp in TLR2 gene with the incidence of acute graft rejection in liver transplant recipients for hepatitis C virus (HCV)-related cirrhosis. **Methods:** This study included 114 adult patients who underwent Adult liver transplantation for cirrhosis due to HCV infection. Two SNPs of the TLR2 gene were genotyped using PCR-RFLP and sequencing analysis. Allelic carriages for each SNP were compared among patients with and without acute rejection within the first 3 months post transplantation. **Results:** Mutant alleles (A allele of Arg753Gln and T allele of Arg677Trp) were significantly associated with acute cellular rejection and showed a trend toward a higher rate of graft rejection [mutant A allele of Arg753Gln OR 3.51, 95% CI 1.27– 9.686, P =0.015] and OR of mutant T allele of Arg677Trp was 3.6 with 95% CI 1.519-8.530 and *P* = 0.0036)]. Also both mutant alleles show a significant low level of Serum TLR2 protein compared to wild alleles of both SNPs (A allele *P*= 0.001 and mutant T allele *P*= 0.02) **Conclusion:** This study suggests an important role for TLR2 SNPs Arg753Gly and Arg677Trp in acute rejection in liver transplant patients for HCV-related cirrhosis. Moreover, these SNPs may have impact on TLR2 gene expression.

Keywords: Innate immunity, Toll like receptors, acute liver rejection.

INTRODUCTION

Liver transplant can be life-saving for patients who have a shortened long-term survival due to a variety of liver diseases **(Gores et al., 2010)**. With the advances in immunosuppression, surgical techniques, organ preservation and improvements in patient management, LT has become the gold standard in treatment of advanced chronic liver disease and fulminant hepatic failure **(Tome et al., 2008)**. Currently, end-stage liver failure due to chronic HCV is the leading indication for LT **(Verna & Brown, 2006)**. Unfortunately, acute rejection occurs in about 45%-65% of LT patients and usually within the first few weeks after transplantation **(Williams et al, 1996).**

In solid organ transplantation, the role played by adaptive immune response in allograft rejection is well established, However, the contribution of the innate immune system to allograft rejection, in particular Toll-like receptors family, remains less clear **(Eksteen & Neuberger, 2008).**

TLRs are a class of PRRs that play a key role in microbial recognition and induction of the immune response. It was discovered at the end of 20^{th} century and shown to induce expression of genes involved in inflammatory responses. It comprises a highly conserved family of 13 known cell surface and intracellular proteins that bind to pathogen associated molecular patterns **(Apetoh et al., 2007**). They recognize specific molecular patterns derived from microbial components (exogenous ligands) and endogenous ligands released by damaged cells, initiating the innate and subsequently adaptive immune response **(Kumar et al., 2009).**

Emerging evidence suggested that TLRs and regulatory T cells may act in concert to control immune responses. As TLRs are generally activated in response to foreign antigens and hence enhance host immunity **(Pasare & Medzhitov, 2003).** In solid organ transplantation, there is increasing evidence that TLRs activation are involved in the innate immune recognition of an allograft, playing a critical role in allograft rejection **(Alegre et al., 2008).**

TLR2 has a special place among the 10 members of the human TLR family **(Zhang et al., 2010).** It is encoded by the gene bearing the same name; humanTLR2 gene which is located on chromosome 4q32, it is composed of 3 exons; the complete open-reading frame is located on exon III **(Haehnel et al., 2002).**

Because of role of TLRs at the interface between host and environment and furthermore as key molecules for both innate and adaptive immunity, genetic variations within these genes could have a major impact on host defense **(Turvey & Hawn, 2006)**. A great variety of polymorphism exists in TLR2 coding region, and more than 175 single nucleotide polymorphisms have been reported for the gene (**Kleinnijenhuis et al., 2011)**.

Arg677Trp is a non synonymous SNP in TLR2 gene, it consists in a C/T substitution at nucleotide 2029 from the start codon of TLR2 predicted to replace arginine (Arg) with tryptophan (Trp) at position 677 (Arg677Trp) **(Medzhitov et al 1998).** Another functional TLR2 variant consists of a G/A substitution at nucleotide 2257 from the start codon of TLR2 predicted to replace arginine with glutamine (Gln) at position 753 (Arg753Gln) **(Lorenz et al., 2000).** Both SNPs have been shown to lead to defective intracellular signaling and impaired cytokine secretion in response to their ligands and an increased predisposition to microbial sepsis **(Schroder and Schumann, 2005).**

Because TLR2 has been described to mediate the innate immune responses to HCV **(Dolganiuc et al., 2004**) it is possible that incidence of acute rejection after liver transplantation for HCV infected patients may be influenced by these functional polymorphisms in TLR2 gene.

The aim of this work was to study the potential associations between TLR2 gene polymorphisms (TLR2 Arg753Gln and TLR2Arg677Trp) and the risk of liver allograft rejection. Moreover, the role of such polymorphisms in the expression of TLR-2 gene was evaluated by estimating serum level of TLR-2 protein among HCV infected liver transplant patients.

METHODS

Study design and population

The study population consisted of 114 adult patients who performed liver transplantation for chronic HCV between February 2010 and April 2012 at Gastroenterology Surgery Center, Faculty of Medicine, Mansoura University. None of the patients had either concomitant alcoholic liver disease or co-infection with hepatitis B virus, human immunodeficiency virus or cytomegalovirus or any other immunologic or genetic diseases. Clinical data were retrospectively obtained from medical records and from electronic database prospectively maintained. All participants provided written informed consent. The study protocol was approved by research ethical committee of Faculty of Medicine, Mansoura University

Initial post-transplant immunosuppression regimens were based on calcineurin inhibitors and corticosteroids with or without mycophenolate mofeti, azathioprine, and/or anti-interleukin-2 receptor antibodies. Acute rejection episodes were histologically proven according to Banff Rejection Activity Index (RAI) Criteria. **(Banff schema for grading liver allograft rejection, 1997)**

Analysis of TLR Polymorphisms:

Genomic DNA preparation

Genomic DNA from liver recipients was isolated from EDTA anti-coagulated peripheral white blood cells using a membrane –based extraction kit (Qiagen, Hilden,Germany) according to the method recommended by the manufacturer. DNA concentration was determined using nanodrop 2000 spectrophotometer.

Detection of TLR polymorphism

All patients were screened for 2 SNPs in TLR2 gene (Arg753Gln, rs5743708), and (Arg677Trp, rs 121917864). These 2 SNPs were genotyped by the method previously reported by **Schroder et al, (2003),** using PCR-based restriction fragment length polymorphism (PCR-RFLP) technique. The amplification was carried out on a thermal cycler Techne Tc 312 *(Applied Biosystems, UK)* using PCR master mix *(Fermentas International INC, Canada)* containing approximately 2x ready master mix, in a 25µl reaction mixture containing 15 µl green taq master mix, 20

pmol of forward primer 5'-GCCTACTGGGTGGAGAACCT-3' and, 20 pmol of reverse primers 5'-GGCCACTCCAG GTAGGTCTT-3', and 50 ng genomic DNA. Thermal cycling conditions were: initial denaturation at 95˚C for 5 minutes, followed by 35 cycles at 94˚C for 30 seconds, 62˚C for 30 seconds, and 72˚C for 1 minute, with a final extension at 72˚C for 5 minutes. After confirmation of successful PCR amplification using 1.5% ethidium promid stained agarose gel electrophoresis, a 3 µl of PCR product (341 bp) was digested with 1 u *AciI (New England Biolabs, Hertfordshire, UK)* in a total volume of 10 µl and incubated at 37˚C for 1 hours. The digested products were then electrophoresed on 3 % agarose gel to identify digestion patterns characteristics of wild-type and mutant TLR2 Figure 1 (E).

Genotyping was carried out using gel documentation system as follow: TLR2 Arg753Gly *AciI* digestion yields 228, 75, and 38 bp fragments for the presence of the G allele and 266- and 75-bp fragments for the presence of the A allele while for TLR2 Arg 677Trp yields 228, 75, and 38bp fragments for the presence of the C allele and 303 and 38bp fragments for the presence of the T allele. All patients were genotyped twice in two independent sets

To confirm the obtained results of PCR-RFLP, sequencing of PCR ampilicon was performed on randomly selected patients from each group with different genotypes. Sequencing was performed using an automated capillary genetic analyzer (*ABI Prism® BigDyeTerminator version 3.1 Cycle Sequencing Kit, Applied Biosystems,Foster City, CA*) according to manufacturer's standard protocol. Before sequencing the PCR products were purified using QIAquick PCR Purification Kit (*Qiagen, Hilden, Germany*). Figure 1 (A,B,C,D).

Serum sTLR2 measurement

Serum TLR2 levels were measured by sandwich ELISA (*BlueGene Biotech, Shanghai, China*) according to manufacturer's standard protocol, with an assay sensitivity for serum TLR2 of 0.1ng/mL using Tecan-sunrise Absorbance Reader. (*Tecan, Austaria, GmbH- Magellan software).*

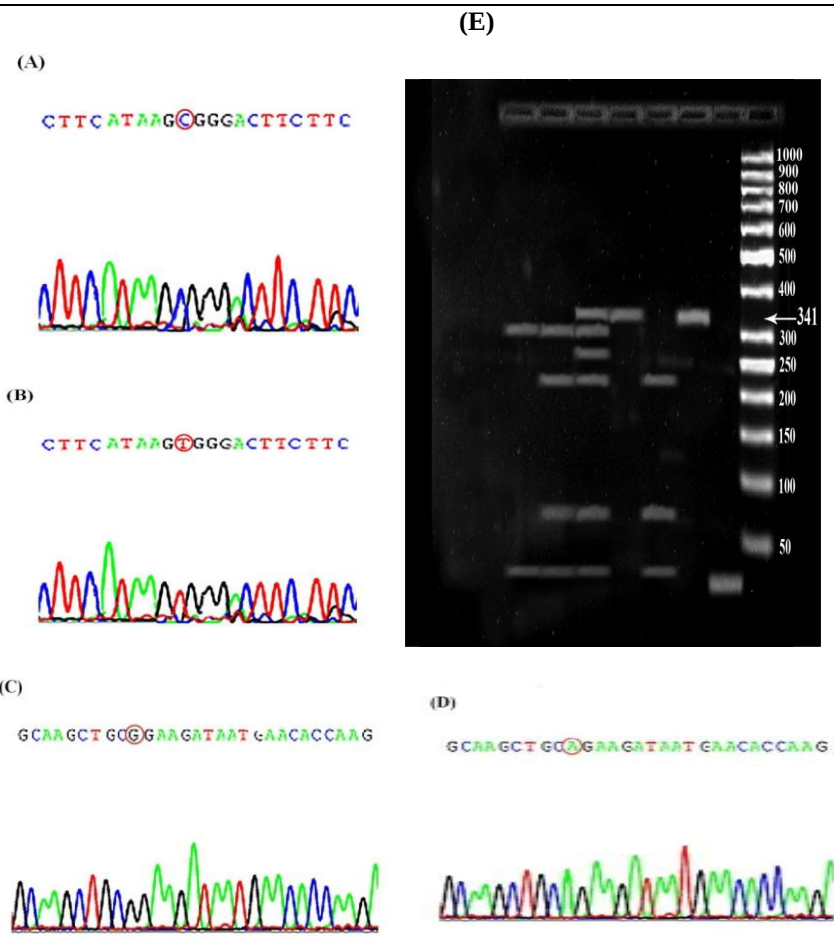

Fig. (1): Genotyping and sequencing analysis of *tlr2* gene Arg677Trp And Arg753Gly polymorphism in liver transplanted patients.:The circled area demonstrates C nucleotide in the wild type Arg677Trp (A) was substituted with T nucleotide in mutant one (B) and G nucleotide in the wild type Arg753Gly (C) was substituted with A nucleotide in mutant one (D). Figure E shows representative results of samples tested for TLR2 Arg753Gly and Arg677Trp polymorphism with PCR-RFLP. 1: DNA 50 bp ladder marker, 2: negative control, 3: PCR ampilicon spanning both polymorphism without digestion (341 bp), 4-8 digestion products of *AciI* for different genotypes; 4:wild type TLR2 (C/C, G/G), 5: homozygous mutant for Arg677Trp and Arg753Gly(T/T, A/A), 6: Heterozygous for both SNPs (C/T, G/A), 7: Heterozygous for Arg677Trp only (C/T,G/G) and 8: homozygous mutant for Arg677Trp only (T/T,G/G).

Statistical analysis

Results for continuous variables are reported using descriptive statistics such as means, standard deviation (SD). Means of the groups were compared by t-test. The statistical significances of differences in frequencies of variants between groups were tested using chi-square (v2) test. SNPs were tested for Hardy–Weinberg equilibrium and their genotypic and allelic disease association analysis was performed using the DeFinetti program. Odds ratios (ORs) with 95% confidence intervals (CI) were calculated as a measure of the association of the TLRs alleles with acute allograft rejection. Data were analyzed using SPSS software (version 17.0.0; SPSS Inc., Chicago, Illinois,USA), and a P-value less than 0.05 was considered to be significant.

RESULTS

Demographic characteristics

From 114 patients underwent liver transplantation for chronic hepatitis C were recruited in this current study, 56 patients (49.12%) shown at least one episode of acute cellular rejection (Rejectors), 58 patients (50.88%) shown no rejection episode for at least 3 months post transplantation (Non rejectors). The mean (± SD) age of patient was 45 ± 9.24 years, one hundred and one (88.6%) were male and thirteen (11.4%) were female. The clinical characteristics and demographics data of patients are given in Table 1. The two groups of patients did not display significant deviation of demographic characteristics (age and gender). Furthermore, no significant differences in, Child- Pugh score MELD score, underlying etiology or other laboratory data were found.

Table (1): Demographic and clinical characteristics of studied groups:

	Rejectors N (%) 56(49.12)	**Non Rejectors N (%) 58(50.88)**	***P* value**
Age (SD)	50.57 (8.37)	47.25 (10.48)	0.08
Gender			
Male	50.00(89.29)	51.00(87.93)	
Female	6.00(10.71)	7.00(12.07)	0.69
MELD Score (SD)	16.2(3.25)	15.1(4.02)	0.29
Child-Pugh score			
A	8(17.02)	7(13.21)	
B	21(44.68)	26(49.06)	0.41
C	18(38.30)	20(37.73)	
Etiology			
HCV alone	37(78.72)	40(75.47)	
HCV and HCC	10(21.28)	13(24.53)	0.52
Laboratory findings (SD)			
ALT (mg/dl)	38.64(36.64)	38.36(22.96)	0.9
AST(mg/dl)	75.02(61.83)	65.85(28.96)	0.3
Albumin(g/dl)	3.05(0.54)	3.21(0.61)	0.17
T.Bilirubin(mg/dl)	3.49(3.65)	3.48(3.24)	0.9
INR	1.65(0.57)	1.60(0.54)	0.6
S.Creatinin(mg/dl)	0.73(0.31)	0.77(0.22)	0.3
Donor			
Related	34(64.15)	26(49.06)	0.14
Unrelated	13(24.53)	27.00(50.94)	

P significant if < 0.05

Genotype distribution and allele frequencies of TLR2polymorphisms

All patients were tested for TLR2 Arg753Gln and Arg 677Trp polymorphisms. Distribution of genotypes and alleles for the TLR2 Arg753Gln and Arg 677Trp polymorphisms in all studied groups were summarized in Table 2. Distribution of Arg753Gln and Arg 677Trp genotypes were in agreement with Hardy–Weinberg equilibrium in both groups.

56 patients (49.12%) had at least one episode of acute graft rejection within the first 3 months post transplantation. Among them 24 (42.86%) had at least one allele that had TLR2 Arg677trp polymorphism (T allele); 17 patients (30.36%) were heterozygous, and 7 (12.5%) were homozygous for this polymorphism. On the other hand, from these 56 patients 14 (25%) posses at least one allele of TLR2Arg753Gly polymorphism (A allele); 11 patients (19.64%) were heterozygous while only 3 (5.36%) are homozygous as presented in Table (2).

Table (2): Genotype distribution and allele frequencies of TLR2Arg677Trp and Arg753Gly polymorphisms of studied groups**.**

	Arg677Trp (C/T)		**Arg753Gly (G/A)**	
	Rejectors No (%) 56 (49.12)	**Non rejectors No (%) 58(50.88)**	**Rejectors No (%) 56 (49.12)**	**Non rejectors No (%) 58(50.88)**
Wild type	32(57.14)	48(82.87)	42(75)	52(89.65)
Heterozygote	17(30.36)	9(13.26)	11(19.64)	5(8.62)
Homozygote mutant	7(12.5)	1(1.72)	3(5.36)	1(1.72)
Allele frequency				
	C:81(72.32)	C:105(90.52)	G:95(84.82)	G:109(93.97)
	T:31(27.68)	T:11(9.48)	A:17(15.18)	A:7(6.03)
PIC	53.20%	15.63%	22.43%	10.69%
PD	56.67%	29.07%	39.60%	18.84%
Homozygosity	69.64%	84.48%	80.35%	91.37%
Heterozygosity	30.35%	15.51%	19.64%	8.62%
Hardy-Weinberg				
x^2	3.272	0.535	3.597	3.336
P	0.070	0.464	0.057	0.068

PIC: Polymorphic information content
PD: Power of discrimination
X^2: Chi square
P: significant if <0.05 (HWE is in equilibrium if P is insignificant*)*

Mutant T allele of TLR2 Arg677Trp was found to be significantly associated with increased risk of acute rejection when compared with C allele, (OR 3.6, 95% CI 1.52-8.53, *P* =0.0036). For TLR2 Arg753Gly the mutant A allele was also significantly associated with risk of acute rejection when compared with the wild-type A allele [odds ratio (OR) 3.51, 95% confidence interval (95% CI) 1.27– 9.69, P =0.015] Table (3).

Table (3): Association between TLR2 Arg677Trp and Arg753Gly and the development of acute rejection.

	OR	**95% CI**		**Statistical values**
		lower	**higher**	X^2
TLR2 Arg677Trp				
Carriage of T allele	3.6	1.52	8.53	0.0036
TLR2 Arg753Gln				
Carriage of A allele	3.51	1.27	9.69	0.015

OR: Odd ratio.
95% CI: 95% confidence interval of odd ratio
P: significant if < 0.05

Haplotype analysis

Because the number of carriage of TA haplotype was less than 6, so we are unable to perform statistical analysis. Instead we combined haplotypes TA, TG and CA that contain mutant risky allele [A and/or T] and compared them collectively to patients with CG haplotype that did not contain any risky allele. OR was 3.4, 95% CI of OR (1.22-9.44) and *P* value = 0.0188 suggesting that theses haplotypes may be more risky than CG haplotype in developing acute allograft rejection. Table (4)**.**

Table (4): Haplotype analysis of TLR2 SNPS with Liver rejection.

	TG	**TA**	**CA**	**CG**	**OR**	**95 % CI**	**P**
Non rejectors	5	0	1	48			
Rejectors	11	5	1	40	3.4	1.22 – 9.44	0.0188

OR: Odd ratio.
95% CI: 95% confidence interval of odd ratio
P significant if < 0.05

After genotyping of all patients, we assess whether these SNPs were reflected on serum TLR2 protein level. No significant difference was found between rejectors and non rejectors groups. But when comparing mutant alleles with wild alleles a decrease in serum TLR2 in A allele compared to G allele and in T allele compared to C allele in both rejectors and non rejectors was observed, however these decrease was significant only in rejectors group, P value for A allele: 0.001 and for T allele: 0.02. Same decrease observed al the level of genotypes suggesting that these 2 SNPs may have impact on TLR2 expression Table (5)

Table (5): Serum TLR2 (ng/ml) in studied groups in different Arg753Gl and Arg677Trp alleles and genotypes.

	Rejectors (N= 56)		**Non rejectors (N= 58)**	
	Median	**Range**	**Median**	**Range**
All patients	3.23	0.72-7.51	2.80	0.24-7.14
P1	0.13			
Arg753Gln				
G allele	3	0.74-7.51	3.1	0.24-7.14
A allele	1.7	0.53-3.2	2.3	0.8-3.14
P2	0.001*		0.07	
GG genotype	5.1	3.5-7.4	5.4	3.7-6.9
GA genotype	2.61	2.12-3.32	1.4	1.3-1.6
AA genotype	1.03	0.71-1.3	1.11	1.11
P3	0.04*		0.03*	
P4	0.01*		0.02*	
P5	0.4		0.4	
Arg677Trp				
C allele	2.9	0.72-7.51	3.1	0.24-7.14
T allele	2.01	0.53-7.51	2.4	0.89-3.35
P2	0.02*		0.05	
CC genotype	5.5	3.3-8.8	7.1	3.6-8.5
CT genotype	3.5	2.3-5.1	4.9	3.9-5.9
TT genotype	1.29	0.6-1.99	3	3-3
P3	<0.001*		0.008*	
P4	<0.001*		0.04*	
P5	<0.001*		0.4	

*P*1 comparison between rejectors and non rejectors
*P*2 comparison between alleles
*P*3 comparison between wild & heterozygote genotypes
*P*4 comparison between wild & homozygote mutant genotypes
*P*5 comparison between heterozygote and homozygote mutant genotypes

DISCUSSION

Although liver transplant (LT) has become a valuable therapeutic choice for patients with end-stage liver disease **(Samuel et al., 2011),** acute rejection remains rather frequent. Several studies have reported the role played by innate immunity in solid organ allograft immune recognition. TLRs, the most recent discovered class of innate immunity, have emerged as a critical element for allograft rejection response (**Deng et al., 2007)**.

This study demonstrates clinical evidence that support the potential role of TLR2 Arg753Gln and Arg677Trp polymorphism in pathobiology of acute cellular rejection after LT for chronic HCV cirrhosis. First we observed that recipients experienced ACR episode had a higher Arg763Gln and Arg677Trp frequency compared to non rejectors, second we found that recipients with single nucleotide polymorphism in at least one allele had a significant higher risk for developing ACR after transplantation. Lastly we demonstrated a significant lower level of serum TLR2 protein in association with mutant alleles (A allele of Arg753Gln and T allele of Arg677Trp) in rejectors group.

Our results are in harmony with **(Kijpittayarit et al., 2007**), they study TLR2 Arg753Gln among 92 liver transplanted patients with CMV infection, they found that 12 patients (13%) had at least 1 allele that had the TLR2 Arg753Gln polymorphism; 7 patients (8%) were heterozygous, and 5 (5%) were homozygous for this polymorphism. And with another study conducted by **Eid and his colleagues in 2007**; they reported that homozygous TLR2 Arg753Gln was associated with increased allograft failure and mortality in chronic HCV liver transplanted recipients.

To our knowledge this is the first study of Arg677Trp conducted in Egyptians. Moreover it is first study of Arg677Trp in liver transplantation. Among all patients recruited in this study 34 patients (29.8%) demonstrate Arg677Trp SNP, of them 26 patients (22.81%) carry CT genotype and 8 patients (7.2%) carry TT genotype. This finding is consistent with finding of other researcher that demonstrate a remarkable association of Arg677Trp SNP with mycobacterial diseases,

which was detected in 22% of lepromatous leprosy patients in a Korean population **(Tae-Jin & Gue-Tae., 2001).**

Increased risk of ACR in patients carry T allele of Arg677Trp and/or A allele of Arg753Gln may be explained by the fact that, this mutation may affect the association of TLR2 homodimers and/or TLR2/TLR1 heterodimers that have been involved in responses to several ligands including DAMPs that released as a consequencs of ischemia and post surgical trauma **(Krutzik et al., 2003)**. This SNP may increase receptor dimerization with more subsequent MyD88 binding and more activation of signaling cascade. This signaling cascade leads to NF-κB activation which induces the secretion of proinflammatory cytokines and interferons which found to be important in inducting and regulating the adaptive immune response **(Iwasal& Medzhitove, 2004)** that may participate in graft rejection.

Intracellularly, TLR signaling pathway can be regulated by cytoplasmic molecules, such as MyD88s, IRAK-M, TOLLIP, and by activation of the PI3K/Akt pathway, among others **(Candiaa et al, 2012).** Extracellularly, soluble TLR had been detected in serum **(LeBouder et al. 2003).** It behaves as a decoy receptor that counteracts receptor activation., it has been shown to block inflammatory responses driven byTLR2 activation, through negative regulatory effects, by acting as a decoy receptor, and by disrupting the close proximity between the coreceptor (CD14) and the receptor (TLR2) that is crucial to highly efficient signaling **(Raby et al. 2009).**

This can explain that TLR2 protein in this study was observed to be significantly decreased in mutant alleles A and T of Arg753Gln and Arg677Trp respectively in ACR patients compared to non ACR patients. This is in consistent with our conclusion that TLR2 is a protective against rejection and its polymorphic allele were associated with increased risk of rejection , and being low in ACR group compared to non ACR group add another explanation for that risk as its negative regulation on TLR2 signaling that modulate immune response involved in rejection is reduced.

In summary, this study identifies TLR2 Arg753Gln and Arg677Trp polymorphisms as novel immunologic markers that could be useful to identify a subpopulation of patients with a higher risk of rejection after liver transplantation for HCV-related cirrhosis. Thus in the future it could be possible to tailor immunosuppressive regimes based on the patient's pre-transplant innate immune phenotype, allowing the sparing of high-dose immunosuppressants in patients who are at low risk of developing rejection or even develop immunosuppression protocols that incorporate TLR2-signaling inhibitors. Nevertheless, these findings must be prospectively validated in other cohorts of patients as well as in patients after liver transplantation for other etiologies than HCV.

REFERENCES

Alegre ML, Leemans J, Le Moine A, et al **(2008)**: The multiple facets of toll-like receptors in transplantation biology. Transplantation 86:1.

Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C and Criollo A et al **(2007).**Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med*.13:1050–1059.

Banff schema for grading liver allograft rejection **(1997*).*** An international consensus document. *Hepatolo.*25: 658–663.

Candiaa E, Diaz-Jiméneza D, Langjahra P, N̊uneza L, de la Fuentea M and Farfan N et al **(2012)**. Increased production of soluble TLR2 by lamina propria mononuclear cells from ulcerative colitis patients. *Immuno*. 217: 634– 642.

Deng J, Geng L, Qian Y, Li H, Wang Y and Xie H et al **(2007).** The Role of Toll-Like Receptors 2 and 4 in Acute Allograft Rejection After Liver Transplantation. Trans*pla Proceed*.39:3222–3224.

Dolganiuc A, Oak S, Kodys K, et al., **(2004)**. Hepatitis C core and nonstructural 3 proteins trigger toll-like receptor 2-mediated pathways and inflammatory activation. *Gastroenterology*; 127: 1513.

Eid A, Brown R, Paya C, Razonable R **(2007).** Association between toll-like receptor polymorphisms and the outcome of liver transplantation for chronic hepatitis C virus. *Transplanta*.84:511–516.

Eksteen B and Neuberger J **(2008).** Mechanisms of disease: the evolving understanding of liver allograft rejection. *Nat Clin Pract Gastroenterol Hepatol.* 5: 209–219.

Gores G, Heimbach J and Rosen C **(2010)**. Liver transplantation for nonhepatocellular carcinoma malignancies. *Liver Transpl*.16:22–25.

Haehnel V, Schwarzfischer L, Fenton M and Rehli M **(2002).** Transcriptiona regulation of the human toll-like receptor 2 gene in monocytes and macrophages. *J Immunol.*168:5629–5637.

Iwasak A and Medzhitov R **(2004).** Toll like receptor control of adaptive immune responses. *Nat immunol.* 5:987-995.

Kijpittayarit S, Albert J, Robert A, Brown V and Razonable R **(2007).** Relationship between Toll-Like Receptor 2 Polymorphism and Cytomegalovirus Disease after Liver Transplantation. *Clini Infect Dise*.44:1315–1320.

Kleinnijenhuis J, Oosting M, Joosten L, Netea M, and Van Crevel R **(2011)** .Innate Immune Recognition of Mycobacterium tuberculosis. *Clin dev immune,* Article ID :405310.

Krutzik, S, Ochoa M, Sieling P, Uematsu S, Legaspi A and Liu P et al **(2003).** Activation and regulation of Toll-like receptors 2 and 1 in human leprosy. *Nat. Med*.9:525– 532.

Kumar H, Kawai T, Akira S **(2009):** Toll-like receptors and innate immunity. Biochem Biophys Res Commun 388:621.

LeBouder E, Rey-Nores J, Rushmere N, Grigorov M and Lawn S et al **(2003).** Soluble forms of Toll-like receptor (TLR)2 capable of modulating TLR2 signaling are present in human plasma and breast milk. *J Immunol*, 171: 6680–6689.

Lorenz E, Mira J, Cornish K, Arbour N and Schwartz D **(2000)**. A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. *Infect Immun*.68:6398-6401.

Medzhitov R, Preston-Hurlburt P, Kopp A. Chen S, Ghosh, S and Janeway C **(1998).** MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* 2:253–258.

Pasare C and Medzhitov R **(2003).** Toll pathway-dependent blockade of CD4+CD25 +T cell-mediated suppression by dendritic cells. *Sci*.299.

Raby A, Le Bouder E, Colmont C, Davies J and Richards P et al **(2009).** Soluble TLR2 reduces inflammation without compromising bacterial clearance by disrupting TLR2 triggering. *J Immunol*.183:506–517.

Samuel D, Colombo M, El-Serag H, Sobesky R and Heaton N **(2011).** Toward optimizing the indications for orthotopic liver transplantation in hepatocellular carcinoma. *Liver Transpl*.17(2):6-13.

Schro◌der N and Schumann R **(2005).** Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease. *Lancet Infect Dis*.5:156–164.

Schr◌oder N, Hermann C, Hamann L, G◌bel U, Hartung T and Schumann R **(2003)**. High frequency of polymorphism Arg753Gln of the Toll-like receptor-2 gene detected by a novel allele-specific PCR. *J Mol Med*.81:368–372.

Tae-Jin K and Gue-Tae C **(2001).** Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. *Immunol Med Microbiol*.31:53-58.

Tome S, Wells J, Said A and Lucey M. **(2008).** Quality of life after liver transplantation. A systematic review. *J Hepatol*.48:567-577.

Turvey S and Hawn T **(2006)**. Towards subtlety: understanding the role of Toll-like receptor signaling in susceptibility to human infections. *Clin Immunol*.120:1–9.

Verna E and Brown R **(2006)**. Hepatitis C virus and liver transplantation. *Clin Liver Dis*.10:919.

Williams R, Neuhaus P, Bismuth H, McMaster P, Pichlmayr R, calne R, Oyyo G, Groth C **(1996)**. *Two-year data from the eurpopean multicenter tacrolimus (FK506) liver study*. Transp Int.*9:144-150.*

Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T and Junger W et al **(2010).** Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nat*.464:104–107.

التباين الجيني لمستقبلات اشباه تول في نتائج زراعه الكبد في حالات الالتهاب الكبدي سي المزمن

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يُعَدُّ تَلَيُّفَ الْكَبِدِ الناجِمِ عن الإصابَةِ بِفيروس الإلْتِهَابِ الكبِدِيِّ الوَبَائِيِّ المُزْمِنِ سي مِنْ أَشْهَرِ أَسْبَابِ زِراعَةِ الكَبِدِ في مِصْر ، وعلى الرَّغْمِ منْ تَحَسُّنِ نتائِجِ هذِهِ العَمَلِيَّةِ خِلالَ السّنَوَاتِ الخَمْسِ الماضِيَةِ إلا أنّ العَديدَ مِنَ المُضَاعَفَاتِ يُمْكِنُ أَنْ تحْدُث، مِنْ ضِمْنِها الرّفْضُ الحَادُّ لِلْكَبِدِ المَزْروعِ والّذِي لا يَزَالُ يُشَكِّلُ تَحَدِّيًا كبِيرًا في عَمَلِيّاتِ زِراعةِ الكَبِدْ.

الهَدَفَ مِنْ هذِهِ الدِّراسَةِ هُوَ التَّحَقُّقُ مِمّا إذا كانَ التباينُ الجِينيُّ في الجِينِ المُحَدِّدِ لِمُسْتَقْبِلاتِ أَشْبَاهِ تول 2 (أرجنين 753 جلايسين ، أرجنين 677تربتوفان) يُمْكِنُ أنْ يُسْهِمَ في خَطَرِ الرَّفْضِ الحَادِّ لِلْكَبِدِ المَزْرُوعِ، وقد شَمَلَتْ هذِهِ الدِّراسَةُ 114 مَرِيضًا خَضَعُوا لِعَمَلِيَّةِ زِرَاعَةِ كَبِدٍ نَتِيجَةً للإلْتِهَابِ الْكَبِدِيِّ الْوَبَائِيِّ المُزْمِنِ سي.

اظهرت نتائج هذه الدراسه:وُجود فُرُوقٍ ذات دَلالَةٍ إحْصَائِيَّةٍ تُبَيِّنُ أنّ التّبَايُنَ الجِينِيَّ لِمُسْتَقْبِلَاتِ أَشْبَاهِ تول 2 (أرجنين753 جلايسين) قَدْ يُؤَدِّي إلَى ارْتِفَاعِ خَطرِ الرّفْضِ الحادِّ لِلْكَبِدِ 3.5 ضِعْفًا مُقَارنةً بِالْمَرضَى غَيْرِ الحَامِلِينَ لِهَذا التَبايُن وكذلك وُجُودُ فُرُوقٍ ذَاتُ دَلالَةٍ إحْصَائِيَّةٍ تُبَيِّنُ أنّ التَّبَايُنَ الْجِينيّ لِمُسْتَقْبِلَاتِ أَشْبَاهِ تول 2 (أرجنين677 تربتوفان) قَدْ يُؤَدِّي إلَى ارْتِفاعِ خَطَرِ الرّفْضِ الحادِّ لِلْكَبِدِ 3.6 ضِعْفًا مُقَارَنَةً بِالْمَرْضَى غَيْرِ الحَامِلِينَ لِهَذَا التَّبَايُنِ.و على هذا فانه مِنَ المُمْكِنِ اسْتِخْدَامُ نَتَائِجِ هذِهِ الدِّرَاسَةِ كَعَلاماتٍ مَنَاعِيَّةٍ لِتَحْدِيدِ الْمَرْضَى الَّذِينَ قَدْ يُعَانُونَ مِنْ خَطَرِ الرَّفْضِ الْخَلَوِيِّ الْحَادِّ بَعْدَ زِراعةِ الْكَبِد.