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Blood Gene Expression Profiling in Liver Transplant Recipients with Hepatitis C and Post Transplant Diabetes Mellitus

Carolyn J. Driscoll, PhD, RN¹, Ann K. Cashion, PhD, RN¹, Donna K. Hathaway, PhD, RN¹, Carol Thompson, PhD, RN¹, Yvette Conley, PhD², Caroline Riely, MD³, Lijing Xu, MS⁴, and Ramin Homayouni, PhD⁴

¹University of Tennessee Health Science Center, College of Nursing

²University of Pittsburgh, School of Nursing

³University of Tennessee Health Science Center, College of Medicine

⁴University of Memphis, Bioinformatics Program

Abstract

Intro—Hepatitis C (HCV) is a risk factor for developing post transplant diabetes (PTDM) after liver transplantation; little is known about the biological mechanisms involved with this risk. This study investigates gene expression differences to provide insight into potential mechanisms.

Methods—Gene expression profiles of blood samples obtained from 6 HCV+ liver transplant recipients were determined using Affymetrix U133 Plus 2.0 microarrays. Differential gene expression was assessed between HCV+ recipients with PTDM (n=3) and no-PTDM (n=3) using GeneSpring 7.3 software package. Welch's t-test was used to identify significant (p<0.05) differences between groups. Gene expression profiles for 6 HCV negative liver transplant recipients (PTDM=3, no-PTDM=3) were used as a blind test set to evaluate a subset of genes to predict PTDM.

Results—Expression levels of 347 genes were significantly different between PTDM and no-PTDM recipients. 74 genes were upregulated; 270 were downregulated in PTDM. Genes were categorized into functional classes: apoptosis (n=69 genes); immune function (n=110); diabetes (n=17); hepatitis c (n=12); liver transplant (n=69). The expression profile of a subset of genes was evaluated for predicting PTDM in 6 HCV negative liver transplant recipients. We accurately predicted the presence or absence of PTDM in 5/6 recipients.

Conclusion—PTDM in HCV+ liver transplant recipients is associated with downregulated expression of a large number of genes. A subset of these genes was useful in predicting PTDM in HCV negative recipients. Most genes were associated with apoptosis and immune function. HCV may act as a primer by affecting a group of genes involved in developing diabetes.

The reported incidence of post transplant diabetes mellitus (PTDM) in liver transplant recipients ranges between 10% and 30%.¹ Hepatitis C (HCV) has been consistently identified as a risk factor for PTDM.^{1, 2} However, little is known about the pathophysiology of

Corresponding Author: Carolyn J. Driscoll PhD, APN, FNP-C 920 Madison Ave Suite 507N Memphis, TN 38163 Phone: 901-448-1941 Fax: 901-448-1762 Email: cdrisco2@utmem.edu.

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PTDM³ and few studies have evaluated the etiology of PTDM in HCV+ liver transplant recipients.

Evaluation of the etiology of diabetes in the general population has identified several different pathogenic processes including autoimmune destruction of the pancreatic β -cells or deficient insulin action⁴ that are reflective of the different influences that may contribute to the development of diabetes. The multifactorial nature of diabetes is influenced by both environmental and genetic factors.^{4, 5} HCV, a highly prevalent environmental risk factor in the liver transplantation population, may further affect genetic risk for the development of PTDM in these individuals.^{1, 2} In addition to increased insulin resistance,³ it has also been hypothesized that HCV may directly damage the pancreatic β -cells.⁴ DNA microarray analysis technology, which provides a global genetic evaluation, has been used to profile gene expression levels in different diseases in the general population, including diabetes,⁵ and may be useful in identifying pathogenic processes and genetic risk factors for PTDM in liver transplant recipients. This study investigates gene expression profiles in blood cells of liver transplant recipients to decipher the biological pathway(s) involved in the development of PTDM.

Patients

Twelve subjects were included in the study: six HCV+ subjects (PTDM+, n=3; PTDM-, n=3) and six HCV- subjects (PTDM+, n=3; PTDM-, n=3). Demographic and clinical data were similar between groups in regard to gender, race, age, body mass index, years since transplant, and immunosuppression. PTDM+ subjects were identified as those who developed new-onset, prolonged hyperglycemia after liver transplantation requiring oral or subcutaneous hypoglycemic medication. HCV+ subjects had a diagnosis of HCV prior to transplantation. Patients who received treatment with pegylated interferon and ribavirin after liver transplantation and no longer had evidence of HCV RNA in the blood were excluded. Enrolled subjects were between 1 and 5 years after liver transplantation. This study was conducted with approval of the institutional review board at the University of Tennessee. All subjects gave written informed consent prior to participating in the study.

Methods

RNA was isolated from peripheral whole blood and specimens were stored at -80°C until RNA isolation was performed using the PAXGeneTM blood RNA kit (Qiagen, Valencia, CA) with the on-column DNase step, followed by globin reduction⁶. RNA was amplified and samples were hybridized to Affymetrix GeneChip[®] Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA).⁷ Absolute gene expression levels were calculated by Affymetrix GCOS software and further evaluated using GeneSpring software version 7.3 (Agilent Technologies). Welch's t-test was used to identify significant ($p<0.05$) differences between groups. Gene function was evaluated using web tools GOTM (<http://bioinfo.vanderbilt.edu/gotm/>) and Chilobot (www.chilobot.net).

Results

Expression levels of 347 genes were significantly different between HCV+ individuals with and without PTDM. Among these transcripts, 74 were up regulated and 270 were down regulated in PTDM. Using several text mining approaches, genes were categorized into the following functional classes: immune function (n=110), apoptosis (n=69 genes), diabetes (n=17), hepatitis c (n=12), and liver transplant (n=69) (Table 1). We next examined whether gene expression profiles could be used to predict PTDM in transplant recipients. Using the gene expression profiles of 6 HCV+ recipients as a training set, a subset of genes were selected

that had the highest predictive power using a Fisher's Exact statistic. Using this gene set, we were able to predict the presence or absence of PTDM in 5 of 6 recipients in the HCV- test set.

Discussion

Gene expression profiles of HCV+ liver transplant recipients demonstrated a large number of genes with down regulated expression. Most of these genes were associated with immune function and apoptosis. Surprisingly, few diabetes related genes were identified in our results. These findings represent the first published report of blood gene expression changes in liver transplant recipients with PTDM.

PTDM is thought to be most similar to type 2 diabetes. In HCV+ patients, proinflammatory cytokines have been implicated as a pathogenic mechanism, supporting the idea that it is similar to type 2 diabetes.⁴ In contrast, studies examining blood of patients with type 1 diabetes⁸ have found down regulation of expression for genes associated with apoptosis, similar to the findings from this study. Despite evidence to support the hypothesis that diabetes associated with HCV + is most similar to type 2 diabetes,⁴ this study suggests a possible autoimmune component to the development of PTDM based on the predominance of genes associated with immune function and apoptosis; thus, raising the possibility that multiple pathogenic mechanisms contribute to the development of PTDM. In addition, HCV may act as a primer by affecting a group of genes involved in developing diabetes.

Interestingly, we also identified a subset of genes whose expression levels were predictive, in 5 out of 6 cases, of PTDM in HCV- recipients after using HCV+ recipients as a training set. The predictive power of this subset of genes can now be tested in a larger cohort of transplant recipients using less costly assays such as quantitative real time polymerase chain reaction methodology. Such gene expression based class prediction may allow earlier detection and management of PTDM to prevent long term complications. Furthermore, this approach may provide the means to identify recipients at risk for PTDM, allowing initiation of early intervention to prevent or delay onset of this post transplant complication.

References

1. Soule JL, Olyaei AJ, Boslaugh TA, et al. Hepatitis C infection increases the risk of new-onset diabetes after transplantation in liver allograft recipients. *Am J Surg* May;2005 189(5):552–557. [PubMed: 15862495]discussion 557
2. Driscoll C, Cashion A, Hathaway DK, et al. Posttransplant diabetes mellitus in liver transplant recipients. *Progress in Transplantation* June;2006 16(2)
3. Delgado-Borrego A, Casson D, Schoenfeld D, et al. Hepatitis C virus is independently associated with increased insulin resistance after liver transplantation. *Transplantation* Mar 15;2004 77(5):703–710. [PubMed: 15021833]
4. Lecube A, Hernandez C, Genesca J, Simo R. Glucose abnormalities in patients with hepatitis C virus infection: Epidemiology and pathogenesis. *Diabetes Care* May;2006 29(5):1140–1149. [PubMed: 16644655]
5. van Tilburg J, van Haeften TW, Pearson P, Wijmenga C. Defining the genetic contribution of type 2 diabetes mellitus. *J Med Genet* Sep;2001 38(9):569–578. [PubMed: 11546824]
6. Globin Reduction Protocol: A method for processing whole blood RNA samples for improved array results. Electronic] http://www.affymetrix.com/support/technical/technotes/blood2_technote.pdf. Accessed June 14, 2006
7. GeneChip ® Human Genome Arrays Data Sheet. electronic] www.affymetrix.com/support/technical/datasheets/human_datasheet.pdf. Accessed June 14, 2006
8. Cashion A, Sabek O, Driscoll C. Correlation of genetic markers of rejection with biopsy findings following human pancreas transplant. *Clinical Transplantation* 2006;20(1):106–112. [PubMed: 16556164]

Table 1
Representative genes for selected functional categories

Immune response		Apoptosis	
Gene Title	Gene Symbol	Gene Title	Gene Symbol
Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 interacting protein	NFATC2IP	Granzyme H	GZMH
CD8a molecule	CD8A	Ubiquitination factor E4B	UBE4B
Interleukin 16	IL16	Family with sequence similarity 40, member B	FAM40B
Prostaglandin D2 receptor	PTGDR	Huntingtin interacting protein 1	HIP1
Nuclear transcription factor, X-box binding 1	NFX1	Transducer of ERBB2, 1	TOB1
PR domain containing 13	PRDM13	Heterogeneous nuclear ribonucleoprotein D	HNRPD
Integrin, alpha L	ITGAL	Fibroblast growth factor 7	FGF7
Tryptophanyl tRNA synthetase 2	WARS2	TRAF2 and NCK interacting kinase	TNIK
Chemokine (C-C motif) ligand 5	CCL5	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 interacting protein	NFATC2IP
Family with sequence similarity 40, member B	FAM40B	Interleukin 16	IL16

This table highlights genes representative of the functional categories determined by text mining approaches.