ONOMY Science

International Journal of Pancreatology & Liver Disorders

Research Article Open Access

Detection by Suppression-Subtractive Hybridization: Genes in the Liver of Patients with Chronic Hepatitis C Virus Infection

Kathryn J, Michele Candiello and Brosens LA

Department of Veterinary & Animal Sciences University of Massachusetts, Amherst, 661 N. Pleasant Street, ISB, rm 427C, Amherst, MA 01003

***Corresponding Author:** Brosens LA, Department of Veterinary & Animal Sciences, University of Massachusetts, Amherst, 661 N. Pleasant Street, ISB, rm 427C, Amherst, MA 01003

Citation: Detection by Suppression-Subtractive Hybridization: Genes in the Liver of Patients with Chronic Hepatitis C Virus Infection. Inte J Panc & Liv Dis. 2019; 1(1): 001-008.

Submitted: 15 June 2019; **Approved:** 21 June 2019; **Published:** 23 June 2019

Abstract

Hepatitis C virus (HCV) infection causes acute and often also chronic liver disease. Worldwide, prevalence of infection is estimated to exceed that of human immunodeficiency virus infection fourfold. Because of the lack of appropriate animal models, knowledge of interactions between virus and host is still limited. Assumptions regarding pathogenesis or the activation status of innate antiviral host responses, for instance, derive mainly from clinical observations and from expression analyses of selected genes. To obtain a more objective insight into virus-host interrelationships, we used suppression-subtractive hybridization to compare gene expression in HCV-infected and non-HCV-infected liver tissues samples. Four differentially expressed genes were found: (i) the gamma interferon (IFN-γ)-inducible chemokine IP-10 gene; (ii) the IFN-α/β-inducible antiviral MxA gene; (iii) the gene encoding IFN-α/β-inducible p44, shown to be associated with ultrastructural cytoplasmic entities within hepatocytes of non-A, non-B hepatitis-infected chimpanzees; and (iv) the gene encoding IFN-α/β/γ-inducible IFI-56K, a protein recently shown to interact with the eukaryotic translation initiation factor eIF-3. Compared to hepatic gene expression in patients with liver diseases unrelated to viral infections, expression in patients with chronic HCV infection was up to 50-fold higher. While in patients with chronic HBV infection IP-10 was slightly activated as well, the IFN-α/β-regulated genes were not. Revealing a dominance of hepatic interferon-regulated processes in chronic HCV infection, data on the enhanced expression of the IFN-γ regulated IP-10 support earlier findings and may explain the composition of the hepatic cellular infiltrate. The data on enhanced expression of IFN-α/β inducible genes might be germane to therapeutic considerations.

INTRODUCTION

Hepatitis C virus (HCV) infection causes acute and often chronic liver disease (8, 25). Worldwide, prevalence of infection is 0.3 to 4.0% (38). Since its molecular cloning, considerable advances have been made in the fields of diagnostics and molecular virology. However, knowledge of virus-host interactions is still limited because of the restricted species specificity of HCV and the resulting lack of an appropriate animal model. Clinical observations argue against a direct cytopathic effect of the virus and favor a destructive mechanism mediated by the host immune system (6, 14, 42).

Experimental approaches for understanding virus-host interactions addressed expression analyses of genes or proteins which are known to be involved in related pathological conditions. Proteins whose expression was reported to be elevated in chronic HCV infection are gamma interferon (IFN-γ) (3, 13, 32, 36), the inducible isoform of nitric oxide synthase (31, 44), and class I and class II major histocompatibility complex molecules (2, 4), which are known to be regulated by IFN-γ, as well as the accessory molecules CD80, CD40, and B7 (4, 34) and intercellular adhesion molecule 1 (2). Highly confirmatory of hepatic cytokine expression, phenotype and specificity analyses of infiltrating cells revealed Th1 lymphocytes as a predominant population. These cells were shown to be mostly antigen nonspecific and of a memory phenotype (3, 23, 33). Thus, it is assumed that in addition to CD8+ lymphocytes and monocytes, preferentially antigen-nonspecific activated Th1 helper lymphocytes are attracted to the liver. By secreting IFN-γ, the latter cells are thought

to activate monocytes/macrophages, thereby initiating a delayed-type hypersensitivity reaction.

Regarding the activation of innate antiviral host responses, for instance, we have no conclusive evidence as to whether HCV is able to stimulate endogenous IFN-α production (5, 9, 22). This study aimed to obtain a more general view of the processes which might be involved in virus-host interrelationships, e.g., to identify genes of enhanced expression in the liver of HCV-infected patients by the generation of a subtracted library. Since HCV is assumed to infect only a minority of liver cells, at least transcripts which may be directly induced by the virus are expected to be of low abundance. We thus chose the method of suppression-subtractive hybridization (SSH) described by Diatchenko and colleagues (11) to compare gene expression in liver tissues from HCV-infected and non-HCV-infected patients. SSH was designed to generate a cDNA library which is enriched in differently expressed sequences and, more importantly, equalized for the number of individual cDNA species, thus allowing the detection of rare transcripts (19).

MATERIALS AND METHODS

Liver tissue

Liver tissue that served as tester material for SSH was obtained directly after explantation from a 63-year-old female patient infected with HCV genotype 1b. Histologic evaluation revealed marked fibrosis, moderate infiltration, lymphoid aggregates, and steatosis with no signs of malignancy. The diagnosis indicated a moderate portal chronic-aggressive hepatitis C with transition to cirrhosis and mild to moderate active lobular hepatitis. For driver material, RNA preparations from three non-HCV-infected liver explants were pooled. Criteria for the selection of driver material were comparability in qualitative and quantitative histologic changes as far as possible, various etiologies of liver disease, and absence of hepatic viral infections. The liver disease of an 18-year-old male patient was diagnosed as cryptogenic cirrhosis with low activity. Histologic evaluation revealed moderate infiltration, marked fibrosis, and lymphoid aggregates without bile duct lesions or steatosis. Explanted liver tissue from a 53-year-old female patient showed marked fibrosis, moderate infiltration, lymphoid aggregates, bile duct lesions, marked intrahepatic cholestasis, and mild steatosis. The diagnosis indicated active primary biliary cirrhosis stage II, locally stage III, with marked intrahepatic cholestasis. A 48-year-old male patient suffered from nonactive alcoholic cirrhosis. Histologically, the tissue showed mild infiltration, locally lymphoid aggregates, and a low degree of siderosis of epithelial and Kupffer cells without steatosis.

Liver biopsy procedures from a total of 65 consecutive outpatients with chronic HCV infection (n = 38) (18 females and 20 males; mean age, 44.6 years; range, 18 to 67 years), chronic hepatitis B virus (HBV) infection (n = 12) (5 females and 7 males; mean age, 35 years; range, 17 to 54 years), or nonviral liver diseases (n = 15) (7 females and 8 males; mean age, 48 years; range, 27 to 63 years) were performed as part of a routine clinical evaluation. HCV infection was diagnosed by the presence of anti-HCV antibodies and HCV-specific RNA within the serum. Liver injury was proven histopathologically and categorized according to established criteria as described elsewhere (10, 30). Chronic HBV infection was diagnosed by the presence in sera of HBV surface and core antigen antibodies of the immunoglobulin G isotype. The diagnosis was confirmed histologically. Sera from all patients were found to be positive for HBV-specific DNA. Serum alanine transaminase activities ranged between 7 and 39 U/Iliter, with one exception of 167 U/liter. Histologic degree of inflammation was scored as absent to mild (n = 3), mild (n = 6), or moderate (n = 3). Histopathological findings for patients with no obvious hepatic viral infection comprised fibrosis (n = 2), primary biliary cirrhosis (n = 1), steatosis (n = 4), crytogenic hypodense liver foci (n = 1), hydropic swelling of hepatocytes (n = 1), cryptogenic cirrhosis (n = 2), primary sclerosing cholangitis (n = 1), Stauffer's syndrome (n = 1), and the absence of any pathological condition (n = 2). Patients with concomitant non-B or non-C viral infections and those with continued alcohol or drug abuse were excluded.

Purification of poly(A)+ mRNA from liver tissue

Total cellular RNA from liver tissue was isolated as described previously (32). Poly(A)+ mRNA was purified from the same source, using Oligotex spin columns (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Generation of a subtracted cDNA library

SSH was performed between the above-described tester and driver liver tissue RNA preparations using a PCR Select cDNA subtraction kit (Clontech, Heidelberg, Germany) essentially according to the manufacturer's manual. In brief, 2-µg aliquots each of poly(A)+ mRNA from the tester and the pooled driver were subjected to cDNA synthesis. Tester and driver cDNAs were digested with RsaI. The tester cDNA was subdivided into two portions, and each was ligated with a different cDNA adapter. In a first hybridization reaction, an excess of driver was added to each sample of tester. The samples were heat denaturated and allowed to anneal.

Cite this article: Detection by Suppression-Subtractive Hybridization: Genes in the Liver of Patients with Chronic Hepatitis C Virus Infection. Inte J Panc & Liv Dis. 2019; 1(1): 001-008.

Because of the second-order kinetics of hybridization, the concentration of high- and low-abundance sequences is equalized among the single-stranded tester molecules. At the same time single-stranded tester molecules are significantly enriched for differentially expressed sequences. During a second hybridization, the two primary hybridization samples are mixed together without denaturation. Only the remaining equalized and subtracted single-stranded tester cDNAs can reassociate forming double-stranded tester molecules with different ends. After filling in the ends with DNA polymerase, the entire population of molecules is subjected to nested PCR with two adapter-specific primer pairs. A targeted clone was used for injection into the blastocysts of C57BL/6J mice and transferred into pseudopregnant female mice as described (27). Chimeric male offspring were bred to C57BL/6J females, and agouti F1 offspring were tested for transmission of the disrupted allele by Southern blot analysis of EcoRI-SalI-digested genomic DNA by using the NcoI fragment probe. Heterozygous matings of the F1 mice were carried out to produce homozygous F2 mutant mice.

Animal Experiments

Mice were fed a standard diet (Harlan Teklad irradiated mouse diet 7912), unless indicated otherwise. Three- and eight-month-old male homozygous and wild-type littermates were killed for routine histologic examination of the liver. In addition, liver specimens were snap frozen for subsequent Northern and Western blot analyses, determination of MAT activity as described under saturating concentrations of the substrates, 5 mM methionine, and 5 mM ATP (28); levels of AdoMet, S-adenosylhomocysteine (AdoHcy), and methylthioadenosine (MTA), as determined by HPLC after the procedure described by Fell et al. (29) and modified by Miller et al. (30); and glutathione (GSH) and oxidized GSH, as described by Reed et al. (31). MAT activity was also determined in the kidney homogenates of these animals. At time of death, serum samples were collected and deproteinized by ultrafiltration, and methionine levels were analyzed by using a Beckman 6300-aa analyzer, following the procedure recommended by the manufacturer for physiological sample analysis.

In one series of experiments, 3-month-old male knockout mice and wild-type littermates were fed the amino acid-defined low methionine (0.17%) choline-free diet, Lombardi choline-deficient (CD) diet (Dyets, Bethlehem, PA), or control diet (0.4% methionine, 0.2% choline, Harlan Teklad) for 6 days. Four groups of animals were established (five mice per group): wild-type and MAT1A knockout mice on 3- and 8-month old MAT1A knockout and wild-type littermates, fed either a CD diet or a control diet for 6 days, or a normal diet for 3 or 8 months, were examined by two pathologists blinded to the animals' identity and treatment. All sections were stained with Masson's trichrome or hematoxylin and eosin. Liver tissues from four knockout and four wild-type animals were examined and compared.

Nucleic Acids Extraction and Northern Hybridization Analysis

RNA was isolated from frozen liver specimens according to the method of Chomczynski and Sacchi (32). RNA concentration was determined spectrophotometrically before use, and integrity was checked by electrophoresis with subsequent ethidium bromide staining. Electrophoresis of RNA and gel blotting were carried out as described (33).

MAT1A and MAT2A cDNA probes were as described (21–23). The probes (cDNA fragments) for betaine homocysteine methyltransfease (BHMT), AdoHcy hydrolase (SAHH), and phosphatidylethanolamine N-methyltransferase 2 (PEMT2) were cloned by reverse transcriptase–PCR (34–36). The probes for glycine N-methyltransferase (GNMT), cystathionine β-synthase (CBS), and α fetoprotein (AFP) were the generous gifts of C. Wagner (Vanderbilt University, Nashville, TN), J. T. Brosnan (Memorial University, Newfoundland, Canada), and J. L. Danan, (Centre de Recherche sur l'Endocrinologie Moleculaire, Meudon, France), respectively.

Northern hybridization analysis was performed on total RNA by using standard procedures, as described (21–23, 33), with specific cDNA probes for MAT1A, MAT2A, GNMT, SAHH, PEMT2, CBS, BHMT, and AFP. All probes were labeled with [32P]dCTP by using a random-primer kit (Primer-It II Kit, Stratagene) or the Megaprime DNA labeling system (Amersham Pharmacia). To ensure equal loading of RNA samples and transfer in each of the lanes, before hybridization, membranes were rinsed with ethidium bromide and photographed, and the same membranes were also rehybridized with 32P-labeled 18S rRNA cDNA probe (22). Autoradiography and densitometry (Gel Documentation System, Scientific Technologies, Carlsbad, CA and NIH image 1.60 software program) were used to quantitate relative RNA. Results of the Northern blot analysis were normalized to 18S rRNA.

Western Blot Analysis

The steady-state levels of liver-specific MAT and proliferating cell nuclear antigen (PCNA) were measured as described (23). Anti-PCNA antibodies were obtained from Zymed.

DNA Methylation Status

Genomic DNA was isolated from livers of MAT1A

knockout and wild-type mice, as described (37). DNA methylation at the CpG site assay was carried out essentially as described

Affymetrix genechip Analysis

The RNA samples were processed as recommended by Affymetrix (Santa Clara, CA). Briefly, after column cleanup of the RNA [Qiagen RNeasy (Chatsworth, CA)], cDNA was synthesized by using an oligo-dT primer attached to a sequence of the T7 promoter region. This cDNA was then used to perform in vitro transcription by using a bacteriophage T7 RNA polymerase promoter and incorporating biotin-labeled nucleotides [Enzo BioArray kit (Enzo Biochem)]. The in vitro transcription reaction product was purified and analyzed via agarose gel electrophoresis to confirm a size distribution ranging from 500 to 1,200 bp. cRNA was fragmented and hybridized at 45°C overnight to an Affymetrix murine U74A array. The arrays were washed, stained, and scanned by using a Fluidics Station 400 and Hewlett–Packard scanner, components of the Affymetrix genechip Instrument System. Finally, the results were analyzed by using genechip analysis suite software (Ver. 3.3, produced by Affymetrix) and the fold change between the hybridization intensity of the MAT1A homozygous deletion and wild-type samples obtained.

Statistical Analysis

Data are given as mean ± SD. For changes in metabolites, methylation, MAT activity, and liver weight, statistical analysis was performed by using the Kruskall–Wallis test (STATWORKS). For changes in mRNA and protein levels, ratios of the respective mRNA or protein to 18S or Coomassie blue staining densitometric values were compared between knockouts and wild-type littermates by Student's t test. Significance was defined as $P < 0.05$.

RESULTS

Generation of MAT1A Knockout Mice

A sequence-replacement gene-targeting vector (Fig. (Fig.11A), designed to interrupt the coding sequences of the murine MAT1A gene in exon 3, was used for electroporation of embryonic stem cells. After homologous recombination, the EcoRI-SalI fragment increased from 7.2 to 9.0 kb, which can be easily differentiated from the wild-type allele on Southern blot analysis (Fig. (Fig.11B). One targeted clone of about 300 was identified by Southern blot analysis, used for injection into the blastocysts of C57BL/6J mice, and transferred into pseudopregnant female mice for generation of chimeric mice. Chimeric males were bred with C57BL/6J females, and the agouti F1 offspring were tested for transmission of the disrupted allele by Southern blot analysis (Fig. (Fig.11B). Heterozygous matings of the F1 mice were carried out to produce homozygous F2 mutant mice. From 271 offspring, 86 wild-type mice, 111 heterozygotes, and 74 knockout homozygotes were identified.

MAT1A Is Not Expressed in MAT1A Knockouts, but MAT2A and AFP Are Induced

Absence of MAT1A expression is confirmed on both Northern (Fig. (Fig.22A) and Western blot analyses (Fig. (Fig.22B). In heterozygotes, the MAT1A mRNA and protein levels are reduced by 50% (results not shown). In the MAT1A knockouts, the steady-state mRNA level of MAT2A is induced by 3-fold ($P < 0.05$ vs. wild type). Because MAT2A expression is associated with rapid growth and dedifferentiation of the liver, we examined the expression of AFP. In the knockouts, AFP mRNA levels are also markedly increased (Fig. (Fig.2A).

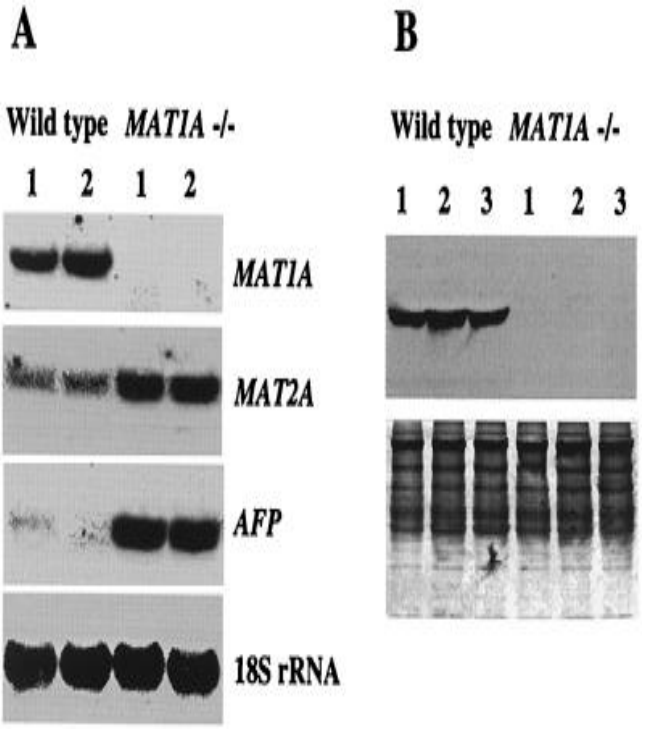

Figure 2: (A) Northern blot analysis of MAT1A, MAT2A, and AFP in MAT1A knockout and wild-type littermates. Liver RNA (30 μg each lane) samples obtained from two wild-type and two MAT1A knockout mice were analyzed by Northern blot hybridization with a 32P-labeled MAT1A cDNA probe, as described in Methods. A representative Northern blot is shown. (B) Hepatic steady-state liver-specific MAT protein level in MAT1A knockout and wild-type littermates. Liver cytosol (50 μg/lane) obtained from three wild-type and three MAT1A knockout mice was analyzed by Western blot analysis by using anti-rat liver-specific MAT antibodies, as described in Methods. Equivalent protein loading was assured by Coomassie-blue staining of gels after transblotting (Lower).

Cite this article: Detection by Suppression-Subtractive Hybridization: Genes in the Liver of Patients with Chronic Hepatitis C Virus Infection. Inte J Panc & Liv Dis. 2019; 1(1): 001-008.

REFERENCES

1. Early Alzheimer's Disease: Patterns of Functional MRI Activation-The Neural Substrates of Semantic Memory Deficits. Am J Bra Dis and Tum. 2018; 1(1): 001-010.
2. H Chahal, S W D Souza, A J Barson and P Slater. How to develop human brain using magnesium of N-methyl-D-aspartate receptors, Am J Bra Dis and Tum. 2018; 1(1): 001-005.
3. F S LaBella, et al.Concepts and correlations related to general anaesthesia and cytochrome P450 oxygenases. Am J Anest and Pai med. 2018; 1(1): 01-05.
4. Hazim J Safi, et al. The long term method with the elephant trunk for the repair of aortic aneurysms. Am J Anest and Pai med. 2018; 1(1): 001-008.
5. Yoshitaka Fujii, et al. Diaphragmatic Fatigue is treated with Inhaled Aminophylline Therapy in an Experimental Canine procedure. Am J Anest and Pai med. 2018; 1(1): 001-003.
6. O Demirkiran, et al. Complications in patients with Crush syndrome after the Marmara earthquake. Am J Anest and Pai med. 2018; 1(1): 001-005.
7. Qi Wei, et al. Laparoscopic choledochotomy after Biliary drainage: Study. Am J Anest and Pai med. 2018; 1(1): 001-007.
8. Mark Palazzo, et al. Unilateral Babinski/Plantar Reflex - Acute Inflammatory Demyelinating Polyneuropathy. Am J Anest and Pai med. 2018; 2(1): 01-02.
9. Hakan Alfredson, et al. Achilles and patellar tendon operations performed in local anestesia, Am J Anest and Pai med. 2018; 1(1): 001-002.
10. Naemeh Nikvarz, et al. Evaluation The Analgesic Effect of Duloxtine Drug in Burn Pationts. Am J Anest and Pai med. 2019; 2(1): 01-07.
11. Chuandong Zheng, et al. Intravascular Plaque: Cause for Radial Arterial Catheterization Failure. Am J Anest and Pai med. 2019; 2(1): 01-05.
12. Laura Tyler Perryman, et al. Wireless Dorsal Root Ganglion Stimulation: An Introduction and Early Experience with the New Approach for Chronic Pain Management. Am J Anest and Pai med. 2019; 2(1): 01-04.
13. Lazraq Mohamed, et al. Pediatric Pre-Anesthesia Consultation: What are Parents Expectations?. Am J Anest and Pai med. 2019; 2(1): 01-02.
14. Alaa Ali M. Elzohry, et al. Safety and Efficacy of Intraperitoneal Irrigation of Levo-Bupivacaine plus Morphine in Patients Undergoing Major Abdominal Cancer Surgeries. Am J Anest and Pai med. 2019; 2(1): 01- 07.
15. Yıldız K, et al.Comparison between Anesthesia Methods In Orthopaedics Initiatives of Upper Extremity. Am J Anest and Pai med. 2019; 2(2): 01-03.
16. Jianming Liu, et al. The Analgesic Effects Nalbuphine Hydrochloride Combined With Sufentanil for Patients after Thoracoscopic Lobectomy. Am J Anest and Pai med. 2019; 2(2): 01-03.
17. Fudong Shi, et al. The Patient Controlled Intravenous Analgesia of Dezocine on the Elderly Patients After Orthopedic Surgery. Am J Anest and Pai med. 2019; 2(1): 01-04.
18. GE Meglia, et al. Investigation in blood Leukocytes and Neutrophils in Periparturient Dairy Cow. Sci J of Ani and Vet Sci. 2018; 1(1): 001-009.
19. G E Duhamel, et al.DNA Sequence Analysis of an Immunogenic Glucose-Galactose Mglb. Sci J of Ani and Vet Sci. 2018; 1(1): 001-009.
20. David G. White, et al. Chloramphenicol and Florfenicol Resistance in Escherichia Coli of Characterization . Sci J of Ani and Vet Sci. 2018; 1(1): 001-006.
21. N B Alhaji, et al. Anophthalmia and Choanal Atresia In Two Months Old Kid. Sci J of Ani and Vet Sci. 2018; 1(1): 001-004.
22. Christopher W Olsen, et al.Isolation and Characterization of H4N6 Avian and Influenza Viruses. Sci J of Ani and Vet Sci. 2018; 1(1): 001-0025.
23. Teresa Lopez-Arteaga, et al. Apathy as a Psychiatric Manifestation of Meningioma. Am J Bra Dis and Tum. 2018; 1(1): 001-004.
24. David R Murdoch, et al. The Use of Brain Natriuretic Peptide- Whole Blood can be Measured, Am J Bra Dis and Tum. 2018; 1(1): 001-003.
25. Stefan Brocke, et al. Antibodies to Integrin α4 and CD44, but not CD62L, Prevent CNS Inflammation and Experimental Encephalomyelitis by Blocking Secondary Leukocyte Recruitment. Am J Bra Dis and Tum. 2018; 1(1): 001-006.
26. Andrew J Saykin, et al. Early Alzheimer's Disease: Patterns of Functional MRI Activation-The Neural Substrates of Semantic Memory Deficits. Am J Bra Dis and Tum. 2018; 1(1): 001-010.
27. P Slater, et al.How to develop human brain using magnesium of N-methyl-D-aspartate receptors, Am J Bra Dis and Tum. 2018; 1(1): 001-005.
28. Clyde W Hodge, et al. The Paraventricular Nucleus Interactively Modulate Ethanol Consumption -Norepinephrine and Serotonin Receptors, Am J Bra Dis and Tum. 2018; 1(1): 001-005.
29. Paulo C Carvalho, et al. Bioinformatics grid application in simple - Squid. Sci J Biome and Biost. 2018; 1(1): 001-004.
30. Mahmoud A E Abdelrahman, et al. On The New Exact Solutions for the Nonlinear Models Arising In Plasma Physics. Sci J Biome and Biost. 2018; 1(1): 001-004.
31. Weicheng Shen, et al. Based on Personal Identification- Automated Biometrics. Sci J Biome and Biost. 2018; 1(1): 001-002.
32. V Prasathkumar, et al.Fingerprint Biometric System - Using of Personal Authentication. Sci J Biome and Biost. 2018; 1(1): 001-003.
33. SavitaChoudhary, et al. Software Development Environment : Design of Biometric Based Transaction System. Sci J Biome and Biost . 2018; 1(1): 001-003.
34. D J Lawrence, et al. Measuring the effectiveness in reliability and validity of a visual function outcomes instrument in cataract surgery. Sci J Biome and Biost. 2018; 1(1): 001-004.
35. Z Suvakovic, et al. Evaluation of early detection of gastric cancer requries more than gastroscopy. Anna of Can Ther and Phar. 2018; 1(1): 05.
36. Ho GY, et al. Informing and involving personalised computer based data for cancer patients. Anna of Can Ther and Phar. 2018; 1(1): 001-005.
37. Ray Jones, et al. Prostate Cancer Risk is associated with Polymorphism of Insulin gene. Anna of Can Ther and Phar. 2018; 1(1): 001-005.
38. : Jean-Pierre J. Issa, et al. Role of DNA Methylation in Tumor Suppressor Gene Silencing in Colorectal Cancer. Anna of Can Ther and Phar. 2018; 1(1): 001-008.
39. Jules J Berman, et al. Histological classification of tumour and molecular analysis meets Aristotle. Anna of Can Ther and Phar. 2018; 1(1): 001-005.
40. Kafil Akhtar, et al. Tuberculosis of the Tongue with Coexistent Squamous Cell Carcinoma: An Interesting Case Presentation, Anna of Can Ther and Phar. 2018; 1(1): 001-002.
41. Serafin Morales Murillo, et al. Vitamin D as A Prognostic Factor in Triple Negative Breast Cancer. Anna of Can Ther and Phar. 2019; 2(1): 01-08.
42. Ahmet Fuat, et al. A Qualitative Study of Accurate Diagnosis and Effective Management of Heart Failure in Primary Care. Am J of Card and Cardiovas Disc. 2018; 1(1): 01-05.
43. Jesús Millán Núñez-Cortés, et al. Prescription Habits for Statins in Patients with Impaired Glucose Metabolism. Results of a program with Focus Groups to Assess the Selection Criteria. Am J of Card and Cardiovas Disc. 2019; 1(1): 01-04.
44. G D Kolovou, et al. Evaluation of Postprandial hyper

Cite this article: Detection by Suppression-Subtractive Hybridization: Genes in the Liver of Patients with Chronic Hepatitis C Virus Infection. Inte J Panc & Liv Dis. 2019; 1(1): 001-008.

triglyceridaemia in patients withTangier disease. Am J of Card and Cardiovas Disc. 2018; 1(1): 01-04.
45. Brian O rourke, et al. Determination of The Mitochondrial Redox Waves and Subcellular Metabolic Transients in Heart Cells. Am J of Card and Cardiovas Disc. 2018; 1(1): 01-04.
46. Shuixiang Yang, et al. Radiofrequency Ablation Treating Atrial Fibrillation Can Reverse the Changes of Mirnas Regulating Ion Channel Proteins. Am J of Card and Cardiovas Disc. 2018; 1(1): 01-08.
47. Hadi abdulsalam Abo Aljadayel, et al. Penetrating War Cardiac and Great Vessels Injury, Surgical Outcome Analysis in 24 Patients. Am J of Card and Cardiovas Disc. 2018; 1(2): 01-05.
48. Hatice Yorulmaz, et al. Assessment of the Death Anxiety and Death Depression Levels of Cardiac Patients. Am J of Card and Cardiovas Disc. 2019; 2(1): 01-06.
49. Camara Abdoulaye, et al. Cardiomyopathie Du Peripartum Compliquee D'accident Vasculaire Cerebral Cas D'une Guinéenne De 19ans : Cas Clinique. Am J of Card and Cardiovas Disc. 2019; (1): 01-03.
50. Sergio F. Estrada-Orihuela, et al. Lasalocid, Interrupts and Reverses, Within One Minute, The Myocardial Damage Caused By Coronary Anoxia Reperfusion in Rat Heart. Am J of Card and Cardiovas Disc. 2019; (1): 01-05.
51. Jesus Millan Nunez-Cortes, et al. Prescription Habits for Statins in Patients with Impaired Glucose Metabolism. Results of a program with Focus Groups to Assess the Selection Criteria. Am J of Card and Cardiovas Disc. 2019; 1(1): 01-06.
52. Federico Cacciapuoti, et al. The Dilemma of Diastolic Heart Failure. Am J of Card and Cardiovas Disc. 2019; 1(1): 01-03.
53. Elad Boaz, Bowel Ischemia and Vascular Air-Fluid Levels. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-00.
54. Sinisa Franjic, et al. A Patient With A Maxillofacial Problem. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-004.
55. Davidson W, et al. Case Presentation: Hantavirus pulmonary syndrome [HPS]. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-005.
56. Farid ZM, et al. Uropathy Secondary Chronic obstructive to Ureter Inguinal Herniation. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-002.
57. De Letter DJ, et al. Cornual Molar Ectopic Pregnancy Diagnosis and Treatment. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-003.
58. Ameni Touati, et al. Silver Russell Syndrome: Case Reports from North Africa and Review on The Literature. Anna Cas Rep and Ima Surg. 2019; 1(1): 001- 004.
59. Kunst WM, et al. Case Reports and Review of Spontaneous Rupture of Hyperreactive Malarial Splenomegaly [HMS]. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-005.
60. F Hanefeld, et al. A Review of The Literature an Emerging Community Pathogen methicillin-Resistant Staphylococcus. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-0011.
61. Page W Caufield, et al. Evidence for a Discrete Window of Infectivity. Am J Den and Ora Car. 2018; 1(1): 001-006.
62. Robert T Dirksen, et al. Dihydropyridine Receptors and Ryanodine Receptors: Bi-Directional Coupling . Am J Den and Ora Car. 2018; 1(1): 001-009.
63. IJ Jacobs, et al. Cancer and Intraepithelial Neoplasia-Tissue-specific apoptotic effects of the p53 codon 72 polymorphism . Am J Den and Ora Car. 2018; 1(1): 001-003.
64. Iain L C Chapple, et al. Human Immunodeficiency Virus disease in oral health significances. Am J Den and Ora Car. 2018; 1(1): 001-007.
65. H Larjava, et al. Activity of αvβ6 Integrin in Oral Leukoplakia. Am J Den and Ora Car. 2018; 1(1): 001-005.
66. Siddharth Kothari, et al. Effectiveness of Counselling and Home Care Self-Management Strategies in Reducing Masticatory Muscle Pain: A Review. Am J Den and Ora Car. 2019; 2(1): 001-007.
67. Betania Maria Soares, et al. Use of Blue LED and Curcumin for Photosensitization of Candida Albicans. Am J Den and Ora Car. 2019; 2(1): 001-005.
68. Jing Guo, et al. Advances in Methods of Maxillary Transverse Expansion. Am J Den and Ora Car. 2019; 2(1): 01-05.
69. Dario C. Altieri, et al. Cell division by p34cdc2 phosphorylation of survivin- Regulation . Sci J of Der and Ven. 2018; 1(1): 001-005.
70. Axel Trautmann, et al. Eczematous dermatitis: T cell and keratinocyte apoptosis plays a key pathogenetic . Sci J of Der and Ven. 2018; 1(1): 001-007.
71. JD Fine, et al. Epidermolysis bullosa Cardiomyopathy in inherited . Sci J of Der and Ven. 2018; 1(1): 001-004.
72. NE Fusenig, et al. Human Skin Angiogenic Switch Occurs Squamous Cell Carcinomas . Sci J of Der and Ven. 2018; 1(1): 001-007.
73. Tapani Tuomi, et al. Water- Damaged Building and Mycotoxins in Crude Building Materials. Sci J of Der and Ven. 2018; 1(1): 001-005.
74. John S Davies, et al. The Use of Social Media among Doctors Under taking a Post-Graduate Endocrinology Diploma. Sci J Endo and Meta. 2018; 1(1): 001-004.
75. Juan J Gagliardino, et al. By Short-Term Dietary Manipulation: The Endocrine Pancreas Activity of Tyrosine Hydroxylase. Sci J Endo and Meta. 2018; 1(1): 001-005.
76. Colin A. Leech, et al. The Glucose Dependent in Pancreatic β-Cells : Voltage-Independent Calcium Channels Mediate Slow Oscillations of Cytosolic CalciumPancreatic β-Cells. Sci J Endo and Meta. 2018; 1(1): 001-009.
77. Colin A. Leech, et al. The Voltage-Independent Activation of Inward Membrane Currents and Elevation of Intracellular Calcium in HIT-T15 Insulinoma CellsPituitary Adenylate Cyclase-Activating Polypeptide Induces. Sci J Endo and Meta. 2018; 1(1): 001-008.
78. Suhail AR Doi, et al. Making Use Of Combined Criteria - Diagnostic Criteria For Diabetes. Sci J Endo and Meta. 2018; 1(1): 001-006.
79. Maria I Borelli, et al. Effect Of Endogenous Islet Catecholamines Possible Modulatory On Insulin Secretion. Sci J Endo and Meta. 2018; 1(1): 001-005.
80. Louis Irwin, et al. Effect of exercise in combination with dietary nopal and zucchini on chronic and acute glucohomeostasis in genetically obese mice. Inte J Expe Bio. 2018; 1(1): 001-005.
81. Vijaya Saradhi Settaluri, et al. Validation of Non Essential Amino Acids and Total Protein Content in Different Categories of Tea. Inte J Expe Bio. 2018; 1(1): 01-04.
82. Patrick D Craig, et al. T Antigen: Polyomavirus Middle of Natural Biology. Inte J Expe Bio. 2018; 1(1): 001-007.
83. Yoshinori Ohsumi, et al. The HIV Coreceptor CCR5 - Recycling and Endocytosis. Inte J Expe Bio. 2018; 1(1): 001-008.
84. Marino Zerial, et al. Elicitation of the Angiogenic Phenotype1: Transforming Myc Protein for In Vivo. Inte J Expe Bio. 2018; 1(1): 001-008.
85. Zhang Y, et al. Odorant Receptor In Mammali : The Caenorhabditis Elegans Seven-Transmembrane Protein ODR-10 Functions on Cells. Inte J Expe Bio. 2019; 1(1): 001-008.
86. Kazuo Maeda, et al. Improved Outcome with Novel Studies in Fetal Monitoring. Sci J of Gyne and Obste. 2019; 2(1): 001-004.
87. Sunil J. Wimalawansa, et al. Vitamin D Deficiency-Related Reproductive Consequences. Sci J of Gyne and Obste. 2019; 2(1): 001-006.
88. Munch A, et al.Investigation in blood Leukocytes and Neutrophils in Periparturient Dairy Cow. Sci J of Gas and Hepa. 2018; 1(1): 001-006.
89. Jie Song Hua, et al. Primary Helicobacter Pylori Resist

Cite this article: Detection by Suppression-Subtractive Hybridization: Genes in the Liver of Patients with Chronic Hepatitis C Virus Infection. Inte J Panc & Liv Dis. 2019; 1(1): 001-008.

ance to Clarithromycin and Metronidazole in Singapore. Sci J of Gas and Hepa. 2018; 1(1): 001-003.
90. Paul Moayyedi, et al. A Systematic Review and Economic Analysis: Proton Pump Inhibitors in Nonulcer Dyspepsia Efficacy. Sci J of Gas and Hepa. 2018; 1(1): 001-003.
91. Zhen-Ning Wang, et al.Gastric Cancer: Collagen IV Expression and Biological Behavior. Sci J of Gas and Hepa. 2018; 1(1): 001-002.
92. Zhen-Ning Wang, et al. A Possible Pathophysiologic Contribution to Necrotizing Enterocolitis: Human Intestine Inflammation. Sci J of Gas and Hepa. 2018; 1(1): 001-006.
93. Paul M Wassarman, et al. Egg Interaction during Mammalian Fertilization in the Molecular Basis of Sperm . Sci J of Gyne and Obste 2018; 1(1): 001-006.
94. Mary Lou Moore, et al. Breastfeeding Benefits Support -Research. Sci J of Gyne and Obste 2018; 1(1): 001-002.
95. Pepita Gimenez-Bonafe, et al. Preservation of Fertility in Patients with Cancer. Sci J of Gyne and Obste 2018; 1(2): 001-006.
96. Yueyang F Fei, et al. Non-Hemorrhagic Unilateral Adrenal Infarct In Pregnancy: A Case Report. Sci J of Gyne and Obste. 2019; 1(1): 001-002.
97. Karen Pierre, et al. Protein-Energy Adequacy of Dialysis Patients in Trinidad and Tobago. Am J of Nep and Ther. 2018; 1(1): 01-05.
98. Balakrishna N, Tenckhoff Catheter Surgical under Local Anesthesia. Am J of Nep and Ther. 2018; 1(1): 001-003.
99. J T Ohlsson, et al. Man in angiotensin and noradrenaline inhibits the Endothelin. Am J of Nep and Ther. 2018; 1(1): 001-005.
100. David J, et al. Apoptosis and Ischemic Renal Injury Reduce the Guanosine Supplementation. Am J of Nep and Ther. 2018; 1(1): 001-005.
101. R W Baldeweg, et al. Tumor-induced osteomalacia : Cloning and characterization of Fibroblast Growth Factor 23. Am J of Nep and Ther. 2018; 1(1): 001-006.
102. Amitabh Arya, et al. Post Pyeloplasty Follow Up In Children And Adolescents: Diuretic Renography Or Renal Ultrasonography? Am J of Nep and Ther. 2019; 2(1): 001-005.
103. Amitabh Arya, et al. Post Pyeloplasty Follow Up In Children And Adolescents: Diuretic Renography Or Renal Ultrasonography? Am J of Nep and Ther. 2019; 2(1): 001-005.
104. Richard Lechtenberg, et al. Tau Interferon in Multiple Sclerosis. Amer J Neur & Neurophysi. 2018; 1(1): 001-002.
105. Eva Guy Rodriguez, et al. Discussion of the differential diagnosis of bilateral thalamic lesions-Bilateral thalamic infarcts due to occlusion of the Artery of Percheron. Amer J Neur & Neurophysi. 2018; 1(1): 001-004.
106. Yhashi Chang, et al. IVIg for Miller Fisher syndrome: Cerebral infarction. Amer J Neur & Neurophysi. 2018; 1(1): 001-002.
107. Fredrick J. Seil, et al. T cell responses to Myelin Antigens and Antimyelin Antibodies. Amer J Neur & Neurophysi. 2018; 1(1): 001-005.
108. Y Niimi, et al. Embolization of Spinal Cord AVMs: Neurophysiologic Provocative Testing. Amer J Neur & Neurophysi. 2018; 1(1): 001-002.
109. Ameni Touati, et al. Some Reducibility Results for Differentiable Sets. Amer J Neur & Neurophysi. 2019; 1(1): 001-005.
110. Chrisostomos Sofoudis, et al. Sofoudis C. Septic Abortion Accompanied with Dessiminated Intravascular Coagulation and Acute Cardiomyopathy Presentation of a Rare Case and Mini Review. Am J Nur & Pract. 2018;1(1): 001-00.
111. Nick Jones, et al. Nurse Practitioners and Family Physicians Ethics Health Care Services. Am J Nur & Pract. 2018; 1(1): 001-005.
112. Thomas R A, et al. Human Infants Learning by Prenatal and Postnatal Flavor . Am J Nur & Pract. 2018; 1(1): 001- 006.
113. D. J Wise, et al. A Randomized, Double-Blind, Placebo-Controlled - Milk Production in Mothers of PremaTure Newborns Domperidone Drug effect. Am J Nur & Pract. 2018; 1(1): 001-005.
114. Bronagh Bufton, et al. Effects of Nursing Homes Ownership Compromise the Quality of Care. Am J Nur & Pract. 2018; 1(1): 001-005.
115. Kerstin Ekberg, et al. How Physicians Deal With the Task of Sickness Certification in Cause-Based and Comprehensive Disability Systems – A Scoping Review. Am J Nur & Pract. 2019; 2(1): 01-10.
116. Michael J. Vives, et al. Factors in Choosing the Surgical Approach: Cervical Spondylotic Myelopathy. Am J Orth and Rhe. 2018; 1(1): 001-004.
117. M. Runge, et al. Geriatric Patients in Balance Training and Exercise. Am J Orth and Rhe. 2018; 1(1): 001-003.
118. Ukoha Ukoha Ukoha, et al. Nutrient Foramina in Long Bones : Study. Am J Orth and Rhe. 2018; 1(1): 001-003.
119. Zhiquan An, et al. Human Humeral Diaphysis of the Nutrient Foramina : Anatomical Study : Study. Am J Orth and Rhe. 2018; 1(1): 001-007.
120. K-P Günther, et al. Hip Replacement in Rates: International Variation: Study. Am J Orth and Rhe. 2018; 1(1): 001-005.
121. Saeed Taj din, et al. Level of Physical Activity among Diabetic Patients of Rural and Urban Areas. Am J Orth and Rhe. 2019; 2(1): 001-004.
122. Carolina Caleza Jiménez, et al. Breastfeeding, Bed-Sharing and Early Childhood Caries. Is There An Association? A Review of the Literature. Am J Pedi and Heal care. 2018; 1(1): 001-004.
123. Katarzyna Niewiadomska-Jarosik, et al. Lipid Profile in Children Born As Small for Gestational Age . Am J Pedi and Heal care 2018; 2(1): 01-03.
124. Mustafa Aydin, et al. Antibiotic Susceptibility Pattern and Clinical Features of Klebsiella Sepsis in Newborn Infants. Am J Pedi and Heal care 2019; 1(1): 01-04.
125. H Dele Davies, et al. Necrotizing Fasciitis- Flesh-Eating Bacteria Disease. Am J Pedi and Heal care 2019; 1(1): 01-06.
126. Marie Westwood, et al. The diagnosis of urinary tract infection (UTI) in children under five years: Rapid tests and urine sampling techniques. Am J Pedi and Heal care 2019; 1(1): 01-09.
127. Folkert Fehr, et al. What Entrustable Professional Activities Add To a Primary Care Residency Curriculum. Am J Pedi and Heal care 2019; 2(1): 01-06.
128. Sonya Martin, et al. Spatially Modulated Illumination Microscopy□D measures the size of Biological Nanostructures . Ann of Phar Nano Tech and Nanomedi. 2018; 1(1): 01-05.
129. Sonya Martin, et al. Genetic analysis of Fis interactions with their binding sites. Ann of Phar Nano Tech and Nanomedi. 2018; 1(1): 01-07.
130. John H Reif, et al. Nucleation assembly of DNA tile complexes is directed by barcode-patterned lattices. Ann of Phar Nano Tech and Nanomedi. 2018; 1(1): 01-07.
131. Thomas H LaBean, et al. Self assembly of DNA nanotubes from triple-crossover tiles as templates for conductive nanowires. Ann of Phar Nano Tech and Nanomedi. 2018; 1(1): 01-05.
132. Ulrich Kettling, et al. Dual-Photon Fluorescence Coincidence Analysis: Rapid quantification of Enzyme activity. Ann of Phar Nano Tech and Nanomedi. 2018; 1(1): 01-05.
133. Ahmed R. Gardouh, et al. Design, Optimization and In-Vitro Evaluation of Antifungal Activity of Nanostructured Lipid Carriers of Tolnaftate Ann of Phar Nano Tech and Nanomedi. 2019; 2(1): 01-05.
134. Mohammed Khalid, et al. Khalid M. Predictors of Prognosis in Pulmonary Hypertension. Anna Pul and Crit Car Med.

Cite this article: Detection by Suppression-Subtractive Hybridization: Genes in the Liver of Patients with Chronic Hepatitis C Virus Infection. Inte J Panc & Liv Dis. 2019; 1(1): 001-008.

2018; 1(1): 001-004.
135. Abdullah Alsaeedi, et al. The Prevalence of Smoking among sample of Kuwait Asthmatics and its impact on the response of the treatment, Anna Pul and Crit Car Med. 2018; 1(2): 001-002.
136. Nicolau Beckmann, et al. Resolving the Oedematous Signals Induced by OVA Challenge in the Lungs of Actively Sensitised Rats. Anna Pul and Crit Car Med.. 2018; 1(1): 01-06.
137. Thomas J walsh, et al. Investigate the performance of non-invasive diagnostic tests such as galactomannan enzyme immunoassay and quantitative Caspofungin in the early diagnosis of invasive aspergillosis (IA). Anna Pul and Crit Car Med.. 2018; 1(1): 01-06.
138. Charles B. Huddleston, et al. Lung Transplantation in pediatrics. Anna Pul and Crit Car Med.. 2018; 1(1): 01-05.
139. Jeffrey P. Lamont, et al. Comparision of valved vs non-valved implantable ports for vascular access:A randomized trial. Anna Pul and Crit Car Med.. 2018; 1(1): 01-03.
140. D Inwald, et al. Risk and relevance of open lung biopsy in Nonneonatal extracorporeal membrane oxygenation (ECMO) patients. Anna Pul and Crit Car Med.. 2018; 1(1): 01-04.
141. Guillaume Mortamet, et al. Does Esophageal Pressure Monitoring Reliably Permit To Estimate Trans pulmonary Pressure In Children?. Anna Pul and Crit Car Med.. 2018; 2(2): 01-05.
142. Yang Jin, et al. Extracellular Vesicle-Shuttling MicroRNAs Regulate the Development of Inflammatory Lung Responses. Anna Pul and Crit Car Med.. 2018; 1(2): 01-04.
143. Nicola Clemente, et al. Pneumonectomy As A Salvage Therapy: A Rare Indication For A Gastric Malt Lymphoma Disseminated To The Lung. Anna Pul and Crit Car Med.. 2018; 1(2): 01-04.
144. Nicola Clemente, et al. Pneumonectomy As A Salvage Therapy: A Rare Indication For A Gastric Malt Lymphoma Disseminated To The Lung. Anna Pul and Crit Car Med.. 2018; 1(2): 01-04.
145. Victor Chew, et al. Pulmonary Cement Embolism. Anna Pul and Crit Car Med. 2019; 2(1): 01-02.
146. Victor Chew, et al. An Unusual Cause of a Tension Pneumothorax. Anna Pul and Crit Car Med. 2019; 2(1): 01-03.
147. Mark C. Lavigne, et al. A Performance Summary of Agents Used in Oral Care for Non-Ventilated and Mechanically-Ventilated Patients. Anna Pul and Crit Car Med. 2019; 2(2): 01-34.
148. Elisangela Hermes, et al. Psychomotricity in Vestibular Dysfunction Therapy (VDT): A Collective Health Question. Am J Rhin and Otolo. 2018; 1(1): 001- 005.
149. Ramtej J Verma, et al. Diethanolamine-Induced Hepatic Injury and Its Amelioration by Curcumin. Am J Toxi and Res. 2018; 1(1): 001-004.
150. Chee Kong Yap, et al. A Preliminary Screening of Cd and Pb Concentrations in the Some Traditional Chinese Herbal Medicines Bought From Selected Shops in Peninsular Malaysia. Am J Toxi & Res. 2018; 1(1): 001-004.
151. Geza Bozoky, et al. Acute Silent Non-Massive (submassive) Pulmonary Embolism. Am J Ang and Surg . 2018; 1(1): 001-003.
152. Muhammad Imran Qadir, et al. Is Hunting Lovering Associates with Pulse Rate. Am J of Viro and Dis. 2019; 1(1): 01.
153. Mujahid Rasheed, et al. Relation of Blood Group with Motion Sickness. Am J of Viro and Dis. 2019; 1(1): 02.
154. Mujahid Rasheed, et al. Views of University Paramedical Students about Causes of Pharyngitis, Its Transmission and Medicinal Control. Am J of Viro and Dis. 2019; 1(1): 02.
155. Kainat Rafaqat, et al. Views of University Paramedical Students about Causes of Pharyngitis, Its Transmission and Medicinal Control. Am J of Viro and Dis. 2019; 1(1): 02.
156. Sajid Ullah, et al. HCV Prevalence in the Volunteer Blood Donors in District Bajaur Khyber Pakhtunkhwa Pakistan. Am J of Viro and Dis. 2019; 1(1): 02.
157. Rabbia Aslam, Analogue of Breathing With Lizard Fright Am J of Viro and Dis. 2019; 1(1): 01.
158. Hurain Shaukat, et al. Linkage of Body Temperature with Exercise Am J of Viro and Dis. 2019; 1(1): 01.
159. Mariyam Javed, et al. How Breathe Rate Relates With Cricket Likeness? Am J of Viro and Dis. 2019; 1(1): 02.
160. Hakan Alfredson, et al. Achilles and patellar tendon operations performed in local anestesia, Am J Anest and Pai med. 2018; 1(1): 001-002.
161. Richard Lechtenberg, et al. Tau Interferon in Multiple Sclerosis. Amer J Neur & Neurophysi. 2018; 1(1): 001-002.

Cite this article: Detection by Suppression-Subtractive Hybridization: Genes in the Liver of Patients with Chronic Hepatitis C Virus Infection. Inte J Panc & Liv Dis. 2019; 1(1): 001-008.