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Potential conflict of interest: Nothing to report.

Reply:

We would like to thank Cusinato et al. for their thoughtful comments.

The relative prevalence of hepatitis C virus (HCV) genotypes was 87% (genotype 1), 7% (genotype 2), 5% (genotype 3), and 1% (genotype 4).

Clearly, HCV genotype is strongly associated with sustained virological response (SVR) rates and with the likelihood of receiving antiviral treatment. HCV genotype was also associated with development of cirrhosis or hepatocellular carcinoma (HCC) in our data set, but the results were not statistically significant. Specifically, for cirrhosis, the adjusted odds ratio (OR) was 0.74 (95% confidence interval [CI]: 0.52-1.06) for genotype 2 patients and 1.06 (95% CI: 0.71-1.56) for genotype 3 patients, relative to genotype 1 patients as the reference group. For HCC, the adjusted OR was 0.53 (95% CI: 0.16-1.73) for genotype 2 patients and 0.82 (95% CI: 0.25-2.7) for genotype 3 patients, relative to genotype 1 patients.

We did not find the proportion of human immunodeficiency virus (HIV)/HCV coinfected patients who ever received antiviral treatment (18%) surprisingly low. Only 22% (36,898 of 170,119) of all HCV mono-infected patients who were in Veterans Affairs (VA) care in 2011 ever received antiviral treatment. Lack of antiviral treatment may be partly explained by the presence of absolute and relative contraindications to antiviral treatment, which may be more common in VA than non-VA patients, by the relatively low success rate of antiviral treatment and its significant side effects. It is beyond the scope of this work to examine the patient, provider, and facility predictors of receipt of antiviral treatment. However, it is crucial to point out that these represent treatment rates in an unselected population of HIV/HCV coinfected patients (i.e., all patients who were seen at least once in any VA facility throughout the country in year 2009) and cannot be compared with treatment rates from tertiary referral centers or prospectively followed cohorts of HIV-infected patients. A more-appropriate comparison would be the proportion of all patients with HIV/HCV coinfection in the entire United States who ever received antiviral treatment.

Shire et al. reported that SVR rates to HCV antiviral treatment with pegylated interferon (Peg-IFN) and ribavirin (RBV) in HIV/HCV coinfected patients in five randomized controlled trials and two prospective cohort studies ranged from 27% to 44%. We reported that among all patients in VA care in 2009 who ever received antiviral treatment with either Peg-IFN or regular IFN with or without RBV (defined as at least two prescriptions of IFN), 17% achieved SVR. The lower SVR rate that we reported is the result of differences in treatment regimens and the fact that we did not report on results of clinical trials; rather, we reported SVR rates in unselected patients in a real-world setting. There are multiple viral, patient, provider, and facility factors that might account for these differences in SVR rates observed in clinical trials versus SVR rates observed in real clinical practice. The fact that we defined antiviral treatment as receipt of two or more prescriptions of IFN ("intention to treat") also accounts for lower SVR rates, as Cusinato et al. rightly point out. An analysis of predictors of SVR is fairly complicated and not directly relevant to the presented research. We merely wanted to determine whether achieving SVR is associated with clinical outcomes (cirrhosis and HCC).

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Circulating MicroRNA-122 Signature in Nonalcoholic Fatty Liver Disease and Cardiovascular Disease: A New Endocrine System in Metabolic Syndrome

To the Editor:

We read with great interest the article by Bala et al. about the role of circulating microRNAs (miRNAs) as markers of alcohol- and acetaminophen-induced liver damage in a mouse model; the investigators concluded that circulating miR-122 and miR-155 may serve as biomarkers of liver injury and inflammation, respectively.[1]

The concept that miRNAs in serum and plasma are powerful potential biomarkers for liver diseases has expanded very quickly in recent years, and the role of circulating miR-122 in predicting liver damage has been replicated in liver diseases of different etiologies, including human nonalcoholic fatty liver disease (NAFLD).[2] In fact, we evaluated the circulating expression of a panel of 84 miRNAs in serum of patients with NAFLD proven through biopsy in a case-

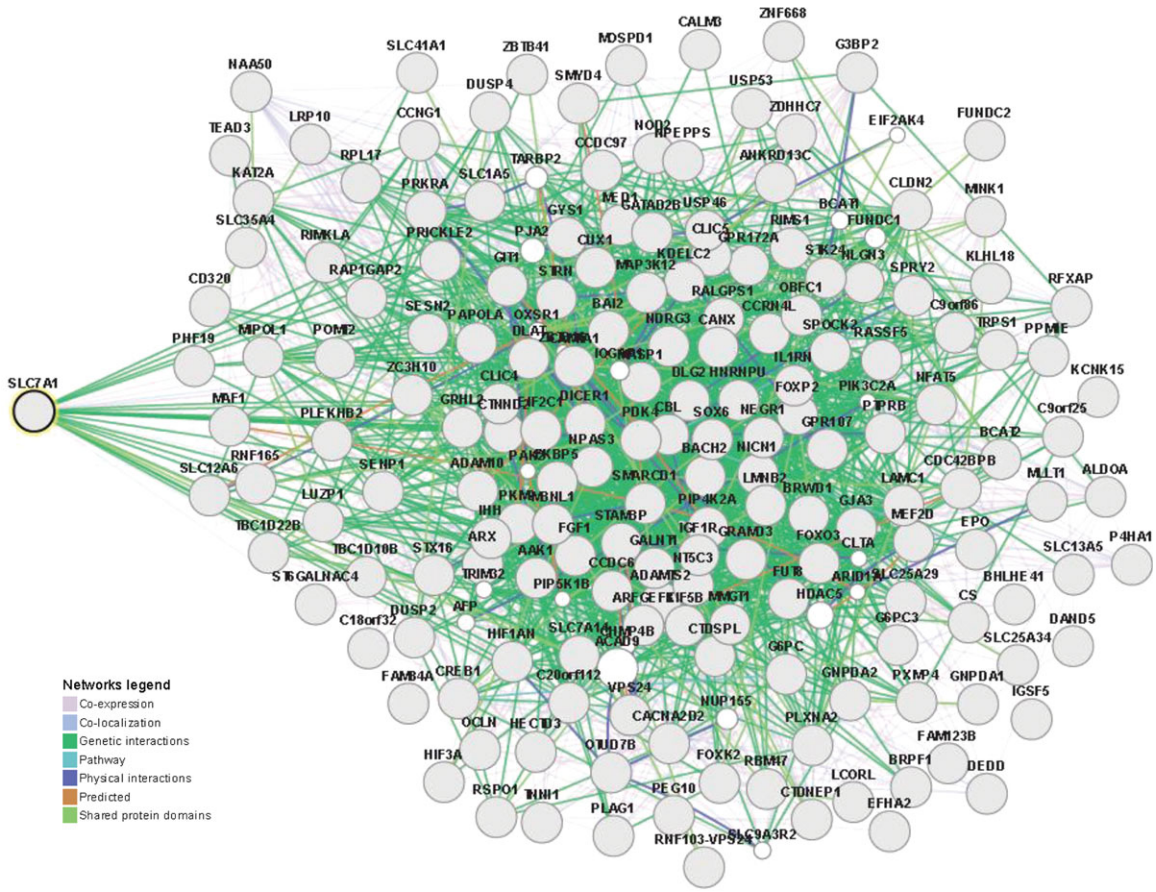

Fig. 1. Functional association analysis of genetic interactions among the 172 conserved gene targets of human miR-122. Prediction of biological targets of miR-122 was performed by the bioinformatics resource, TargetScan (http://www.targetscan.org), by searching for the presence of conserved 8- and 7-mer sites that match its seed region; the gene-interaction network was analyzed by the bioinformatics resource, GenMANIA (http://www.genemania.org).

control design, and we observed that miR-122 was significantly up-regulated in NAFLD patients, compared to control subjects, and the fold increase was strongly related to the disease severity (NASH versus simple steatosis 3.14 and versus control subjects 7.2, fold change). Thus, we agree that circulating miR-122 is a robust biomarker for predicting NAFLD progression and perhaps is able to solve the dilemma of how useful are aminotransferases to decide patients' monitoring and liver biopsy indication because its performance seems to be much better.[1,2]

Certainly, the role of circulating miRNAs in clinical scenarios is not restricted to disease monitoring. miRNAs circulate in the bloodstream and are taken up by distant cells; therefore, they have the enormous potential of regulating gene expression simultaneously in different tissues and cells like a truly new endocrine system, and, for example, miR-122 may regulate the expression of more than 170 highly interacting genes (Fig. 1).

In this scenario, we provide some preliminary evidence that circulating miR-122 could be also regarded as a powerful biomarker for cardiovascular disease (CVD) in patients with NAFLD. For instance, we explored, in a case-control study of 300 individuals with NAFLD, a gene variant (rs41318021) in the 3'-UTR (untranslated region) of human L-arginine transporter *SLC7A1*, which was associated with genetic predisposition to essential hypertension. The 3'-UTR of human *SLC7A1* contains a predicted miR-122-binding site that may play a role in controlling gene expression.[3] Interestingly, we found that, in patients with NAFLD, rs41318021 was significantly associated with arterial systolic and diastolic hypertension (odds ratio [OR], 2.057; 95% confidence interval [CI]: 1.279-3.294; $P < 0.000001$) or isolated diastolic hypertension (OR, 2.147; 95% CI: 1.245-3.702; $P < 0.00075$), even after adjusting for age and body mass index.

In conclusion, we suggest that circulating miR-122 is not only useful in predicting liver damage, but might integrate metabolic signaling in a putative "endocrine" fashion to mediate CVD. Hence, in patients with NAFLD, circulating miRNAs can be explored for improving diagnosis of liver injury and population screening of CVD.

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Reply:

We appreciate the feedback from the Sookoian group. Indeed, previous studies have demonstrated that plasma levels of miRNA-122 were increased in patients with nonalcoholic fatty liver disease (NAFLD).[1] It was proposed by Pirola et al.[2] that circulating miRNA-122, a marker of liver disease/damage, could also be used to predict the risk for cardiovascular disease in patients with NAFLD. A gene variant in the 3′ untranslated region of human L-arginine transporter SLC7A1, which is associated with hypertension, also contained an miRNA-122 binding site, and therefore the authors suggest that circulating miRNA-122 might be a bridge between metabolic signaling and cardiovascular disease.

It is well known that miRNA-122 regulates a broad array of genes involved in fatty acid and cholesterol metabolism (e.g, FASN, ACLY, ACC, DGATS, AGPATS, HMGCR). Recent studies suggest that a deficiency of miRNA-122 in mice (germ line and hepatocyte-specific) results in hepatosteatosis, hepatitis, and hepatocellular carcinoma.[3] Furthermore, miRNA-122 also regulates mitochondrial metabolism and tumor suppressor genes, and loss of miRNA-122 leads to hepatocellular carcinoma.[3,4] Given the multifactorial role of miRNA-122 in liver homeostasis, it might not be surprising that miRNA-122 could be a potential link between metabolic disorders and cardiovascular disease. Nevertheless, the notion that circulating miRNAs can modulate genes in a distant organ and/or mediate the disease has to be thoroughly investigated. To date, studies are very limited on the functional relevance of circulating miRNAs *in vivo*. Several principal questions still remain unanswered, such as the origin of circulating miRNAs (i.e., cells versus organs), their specificity, their *in vivo* biodistribution, and their turnover.

Remarkably, a recent study demonstrated the induction of plasma miRNA-122 in an ischemic porcine cardiogenic shock model.[5] It is most likely that liver ischemia triggered by cardiogenic shock resulted in the release of miRNA-122 to the circulation. However, this possibility was not explored. Further studies are warranted to examine the circulating miRNA in scenarios where multiple organs are affected.

In conclusion, we cautiously support the hypothesis that circulating miRNAs may have paracrine effects extending to organs other than the organ of origin and thus the ability to modulate genes at distant organs. However, due to the lack of sufficient data on the functional relevance of circulating miRNAs, this remains a speculation at this time.

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Overestimation of Hematopoietic Stem Cell Frequencies in Human Liver Grafts

To The Editor:

We read with great interest the article by Wang et al.[1] demonstrating evidence of blood chimerism of donor origin after liver transplantation potentially as a result of donor liver-derived hematopoietic stem cells (HSCs). The adult liver harbors progenitor cells that enable hematopoiesis.[2,3] Our group identified liver-derived CD34+ cells with hematopoietic potential, in agreement with earlier published work,[2] which we presented at the American Association for the Study of Liver Diseases (AASLD) annual meeting in 2008.

The field of human HSC biology has progressed considerably since the discovery that the majority of HSCs are found within the CD34+ compartment.[4] To date, cells with the marker profile $Lin^{-}CD34^{+}CD38^{-}CD90^{+}CD45RA^{-}$ satisfy the most stringent criteria for HSC appellation.[2] Wang et al.[1] report surprisingly high levels of HSCs within donor livers based on the antigenic profile $Lin^{-}CD34^{+}CD38^{-}CD90^{+}$. However, besides omitting CD45RA, the authors fail to