ABSTRACT

Introduction

Hepatitis C virus infection affects 150 million persons worldwide and represents a major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Genetic factors play an important role in the disease progression and response to therapy in patients with chronic hepatitis C (CHC). In this work, I studied genetic and molecular markers associated with the main aspects of clinical evolution in CHC: progression of liver injury (especially fibrosis) and response to antiviral treatment. Marker selection was performed according to their functional association with the pathological processes of chronic HCV infection, and their previously validated association with disease progression in CHC patients. The genetic markers included in this study were single nucleotide polymorphisms (SNPs) present in the *IL28B* region, and in the genes of adiponutrin (*PNPLA3*), bile salt export pump (*ABCB11*) and RING finger protein 7 (*RNF7*). miR-122 was chosen as an emerging molecular marker in CHC.

The aim of this work was to develop and validate assessment methods for these markers, in order to facilitate their inclusion in the clinical practice. I also evaluated the potential clinical utility of the genetic risk information obtained with these assays.

Methods

I developed a series of genotyping assays based on multiplex real-time PCR and high resolution melting analysis for the selected SNPs. First, I validated a duplex genotyping protocol for rs12979860 (*IL28B*) and rs738409 (*PNPLA3*), the most studied polymorphisms in relationship with CHC. Subsequently, I extended the assay to include a third SNP, rs8099917 (*IL28B*), and also validated a second triplex assay for the simultaneous genotyping of rs12979860 (*IL28B*), rs2287622 (*ABCB11*), and rs16851720 (*RNF7*). The analytical validation of the proposed genotyping assays included detailed reproducibility studies and was performed against two different reference methods: allele-specific PCR and the „gold standard” Sanger sequencing. Overall, 132 samples from CHC patients were genotyped. To illustrate the clinical utility of the genotyping assays, I developed predictive models for the presence of advanced fibrosis and the success of the antiviral treatment (sustained virological response, SVR), based on routine laboratory data and the selected genetic risk factors. For miR-122 quantification in biopsy, plasma and serum samples, I designed a hemi-nested qPCR method with SYBRGreen.

Results

The results of the newly developed genotyping assays were 100% concordant with the reference methods. Sensitivity and specificity were both 100%. The assays had excellent reproducibility. Addition of genotyping results to classical predictors brought significant improvement to the ability to predict the main clinical aspects in CHC: fibrosis development and response to treatment. Triplex genotyping of rs12979860 (*IL28B*) + rs2287622 (*ABCB11)* + rs16851720 (*RNF7*), combined with routine laboratory data, enabled accurate detection of advanced

fibrosis and SVR prediction, with an AUROC of 0.869 (CI95: 0.807 – 0.931), and 0.930 (CI95: 0.883 – 0.976), respectively. The second triplex assay, rs12979860 (*IL28B*) + rs8099917 (*IL28B*) + rs738409 (*PNPLA3*), together with basic laboratory results, enabled SVR prediction with similar accuracy (AUROC of 0.928, CI95: 0.882 – 0.974), but better stability. Using the proposed predictive SVR models to identify patients with high probability (>70%) of response to PEG-IFN/RBV may lead to an improvement of treatment allocation by at least 12%, compared with classical predictors, including the *IL28B* rs12979860 genotype.

The importance of the described predictive markers is that they enable the use of the classic therapy for the treatment of patients with high chances to clear the infection, whereas newer, much more expensive medications can be reserved to those with low chances of response to interferon and those with advanced liver disease. Moreover, risk assessment for rapid disease progression in untreated patients would enable their appropriate medical monitoring for early diagnosis and treatment of complications.

Conclusions

The assays presented in this work have several advantages over alternative options, with no decrease in analytical performance: they are more rapid and easier to perform, limit the risk of cross-contamination, and are less expensive.

This is the first report on the study of genetic markers in the combinations described herein, in the same group patients. The predictive algorithms described in this work are also novel in the field of CHC. They enable a better assessment of liver injury and of chances of response to antiviral therapy based on PEG-IFN and ribavirin. The proposed models could contribute to the improvement of clinical management and resource allocation in CHC.

Keywords: chronic hepatitis C, genetic marker, risk factor, diagnostic assay, predictive model, high resolution melting, single nucleotide polymorphism