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Interferon λ 3 and 4 Genotyping Using High-Resolution Melt Curve Analysis Suitable for Multiple Clinical Sample Types

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Many people living with hepatitis C virus (HCV) infection will continue to rely on interferon-based regimens until effective strategies to minimize the cost of directly acting antivirals (DAAs) and to improve treatment access are implemented. Host single-nucleotide polymorphisms related to *IFNL3* and *IFNL4* are associated with spontaneous clearance of HCV, and pegylated interferon— and DAA-based treatment outcomes. We describe a simple and rapid genotyping method for *IFNL* rs12979860, rs8099917, and rs368234815 using high-resolution melting analysis for DNA extracted from whole blood, buffy coat, plasma, serum, and dried blood spots. This assay successfully detected all three polymorphisms on DNA extracted by the automated platform easyMAG from all samples when compared to sequenced amplicons. Analysis of 126 participants with recent HCV infection from the Australian Trial in Acute Hepatitis C study demonstrated the prevalence of favorable single-nucleotide polymorphisms were 62%, 51%, and 45% for rs8099917 TT, rs12979860 CC, and rs368234815 TT/TT, respectively. The genotyping assay described here provides a rapid and affordable *IFNL3* and *IFNL4* genotyping method for a range of clinical sample types. Until global access to DAAs is achieved, *IFNL3* and *IFNL4* genotyping could identify those likely to clear naturally and in whom treatment could be delayed, or help prioritize DAA treatment to those less likely to respond to interferon-containing regimens. *(J Mol Diagn 2015, 17: 583—589; http://dx.doi.org/10.1016/j.jmoldx.2015.05.003)*

Directly acting antivirals (DAAs) have revolutionized hepatitis C virus (HCV) treatment. However, high associated costs and restricted access mean that many of those living with HCV may not receive the benefit of these new therapies. As such, affordable diagnostic tools are required to prioritize and select cost-effective HCV treatment. Diagnostic tools, such as *IFNL3* and *IFNL4* genotyping, can predict those most likely to spontaneously clear HCV, for whom treatment could be delayed. These tools may also stratify individuals and prioritize DAA treatment to those less likely to respond to interferon-containing regimens, or identify those likely to respond well to therapy with shortened treatment. A combination of strategies that improve harm reduction and prevention programs, increase HCV screening rates, and optimize treatment regimens are required to reduce the transmission and burden of disease in populations most affected by HCV infection.

Spontaneous HCV clearance occurs in 25% of individuals.[1,2] Polymorphisms in *IFNL3* [single-nucleotide polymorphism (SNP) rs8099917, located near *IFNL3* gene and SNP rs12979860] or called IL-28B, are the strongest host factors

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Table 1 Primer Sequence, Amplicon Size, and Melting Temperature for IFN-λ Amplicons Used for HRM

Primers	Amplicon size	Primer sequences	Melting range temperature
rs1297860 4F	184 bp	5′-ATTCCTGGACGTGGATGGGTACT-3′	90.5°C to 91.5°C
rs1297860 4R		5′-AGGCTCAGGGTCAATCACAGAA-3′	
rs8099917 1F	207 bp	5′-AGTAAGTCTTGTATTTCACCTCCTGG-3′	78.0°C to 79.0°C
rs8099917 5R		5′-GCTGGGCCCTAACTGATACGCTATAAT-3′	
rs368234815 F2	151 bp	5′-TTTGGCTTCCCTGACGTCTC-3′	92.5°C to 94.2°C
rs368234815 R1		5′-GCCTGCTGCAGAAGCAGAGAT-3′	

HRM, high-resolution melting.

predicting both spontaneous HCV clearance in acute infection[3] and response to interferon-based treatment in chronic infection.[2,4,5] Recently, Prokunina-Olsson et al[6] described a new dinucleotide variant rs368234815 (previously designated as ss469415590) that creates (ΔG) or disrupts (TT) an open reading frame of a gene encoding the interferon λ-4 (IFN-λ4) protein. The dinucleotide variant rs368234815 is in high linkage disequilibrium with rs12979860, but is more strongly associated with both HCV clearance, and pegylated interferon-α and ribavirin treatment response in individuals of African ancestry than rs12979860.[6] Recent findings showed that recombinant IFN-λ4 protein strongly stimulates Jak-STAT signaling and interferon-stimulated gene induction through binding to the IFN-λ receptor.[7] The active IFN-λ4 protein may therefore be the driver of high baseline hepatic interferon-stimulated gene expression at the time of infection and consequently poor HCV clearance.[8] Those with the polymorphism resulting in an impaired IFN-λ4 (*IFNL4*) variant respond better to treatment and have improved rates of spontaneous clearance compared with those who express the active IFN-λ4 variant.[8]

There is recent evidence to suggest that *IFNL* polymorphisms may retain clinical relevance for management of interferon-containing DAA therapy. In treatment-naive patients receiving interferon-containing DAA treatment, the *IFNL3* genotype continues to be significantly associated with sustained virological response and treatment duration required to achieve this response.[9] Recently, the *IFNL3* rs12979860 genotype has been shown to be a strong predictor of tissue inflammation and fibrosis during chronic HCV infection,[10] potentially broadening the clinical relevance of *IFNL* genotypes to disease progression. The authors conclude that the *IFNL3* genotype may be an important part of the development of an individualized patient management algorithm.

Our aim was to develop a rapid, reliable, and inexpensive method to genotype *IFNL3* and *IFLN4* SNPs using real-time high-resolution melting (HRM) analysis[11,12] on a range of clinical sample types. This assay was then used to estimate the prevalence of favorable *IFNL3* and *IFNL4* in recent HCV, using a well-characterized cohort of participants from the Australian Trial in Acute Hepatitis C (ATAHC) study.

Materials and Methods

Study Population and Study Design

Assay validation and comparison of sample types was completed on 10 healthy volunteers (St Vincent's Hospital, Sydney; ethics approval LNR/12/SVH/358). The prevalence of *IFNL3* and *IFLN4* genotypes in recent hepatitis C virus infection was assessed in the ATAHC study. The ATAHC study enrolled 163 participants with acute and early chronic HCV infection. The study design has been described previously in detail.[13] Acute and early chronic infection was defined as either anti-HCV antibody seroconversion in the past 2 years, or clinically documented acute HCV infection. Acute clinical HCV infection was defined as a positive HCV antibody and symptomatic seroconversion illness or alanine transaminase level >400 IU/L, with exclusion of other causes of acute hepatitis, at most 12 months before the initial positive anti-HCV antibody. All participants with available peripheral blood mononucleocytes (PBMCs) were identified for the extraction of genomic DNA for this study (N = 126).

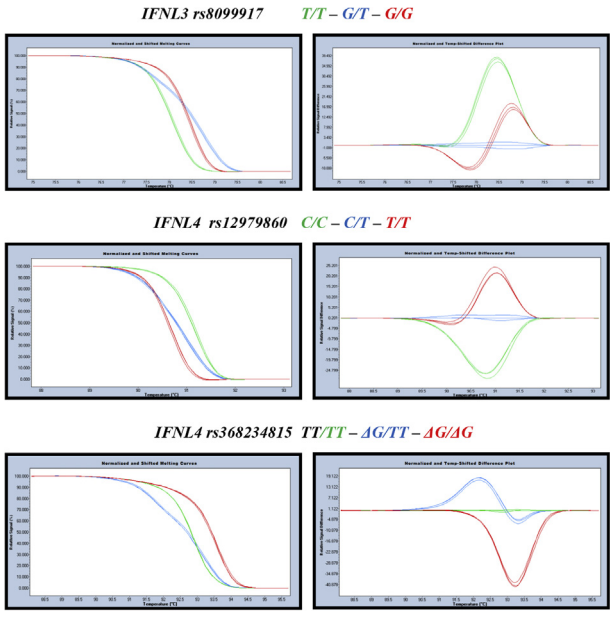

Figure 1 High-resolution melting (HRM) analysis curves. HRM analysis enables homogeneous genotyping without probes, even when the sequence change is only a single base. With saturation dyes, the PCR product is labelled along its entire length so that all melting domains are detected. Single-base genotyping is identified by the difference of melting curves as shown by the normalized melting curve (**left graphs**) and temperature shift (**right graphs**) for rs8099917, rs12979860, and rs368234815, with favorable homozygotes as green curves (TT, CC, and TT/TT, respectively), heterozygotes in blue curves (GT, CT, and ΔG/TT, respectively), and unfavorable genotype as red curves (GG, TT, and ΔG/ΔG, respectively). The assay was performed using the Roche LightCycler 480 System and the HRM analysis performed with the Roche Gene Scanning software.