Polymorphism of Interleukin 28 and HCV: Implementation of a method

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Polimorfismo di Interleuchina 28 e HCV: Messa a punto di un metodo

SUMMARY
HCV infection represents a global health problem. Only 20-30% of patients affected by HCV infection recovers spontaneously, while the remaining patients develop a chronic infection with risk of evolution to cirrhosis and hepatocarcinoma.

Nowadays, approximately 50% of individuals with hepatitis C infection is not responding to therapy with pegylated alpha interferon and ribavirin.

Polymorphisms (SNPs) of the gene coding for the interleukin 28 (IL28) have recently been described and they are in strong relationship with the outcome of HCV infected patients. In particular, the polymorphism rs12979860 (C/T), located 3 kb upstream of the gene, is associated to a rapid and early response to therapy (genotype C/C).

Consequently we have developed a method in high resolution melting (HRM), which allows a simple and rapid screening of polymorphism rs12979860.

The validation of the method was carried out by analyzing the IL28 genotype of 50 patients already determined by sequencing.

Sensitivity and specificity of the method were found to be equal to 100%. Accuracy, precision within and between series were equal to 100%. Compared to other methods described for the analysis of polymorphisms the HRM has the advantage of being faster and safe, relatively cheap and very simple in the optimization phase, therefore, applicable to a large throughput.

Up till now we have analyzed 329 patients of which 46 co-infected HCV-HIV.

The distribution of the polymorphism obtained is the following: 37% C/C, 51% C/T, 12% T/T. The distribution in the co-infected patients does not differ from that of the total HCV positive patients. We suggest a careful follow up of the therapeutic response of these patients to confirm the clinical usefulness of the test and to determine its true predictive value.

INTRODUZIONE
L’infezione da virus dell’epatite C (HCV) colpisce 170 milioni di persone in tutto il mondo (14). Di queste solo il 20-30% guarisce spontaneamente; il rimanente sviluppa un’infezione cronica con rischio di evoluzione a cirrosi ed epatocarcinoma (7, 9). Il 50% dei pazienti affetti da epatite C cronica risulta non rispondere alla terapia con pegylated alpha interferone e ribavirina.

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Green dye.
Per la PCR sono stati utilizzati 4 ng di DNA genomico in un volume finale di 10 µl. Per l’HRM sono stati aggiunti 5 µl di Master Mix HRM 1X e i primers 5’-CTGCACAAGTCTGGGATTCCT-3’ e 5’AGGCTCAGGGGTCAATCACAG-3’ (Invitrogen) (concentrazione finale 0.7 µM).
Il profilo utilizzato per la PCR prevede uno step di denaturazione iniziale a 95°C per 5 secondi, seguito da 40 cicli così costituiti: denaturazione a 95°C per 10 secondi, annealing a 55°C per 30 secondi e allungamento a 72°C per 10 secondi, con l’acquisizione in fluorescenza a singolo canale.
Alla PCR fa seguito un’analisi di melting da 85°C a 95°C (Figura I).
La validazione del metodo è stata effettuata confrontando il genotipo di IL-28 di 50 pazienti precedentemente determinato tramite sequenziamento (ABI Prism 310 – Applied Biosystems).

**RISULTATI**
Sensibilità e specificità del metodo sono risultate pari al 100% dal momento che non abbiamo riscontrato discordanze tra HRM e sequenziamento. La ripetibilità intra e interserie ottenuta è stata del 100%.
Nei 329 pazienti analizzati la distribuzione del polimorfismo ottenuta è stata la seguente: 37% C/C, 51% C/T, 12% T/T.
La distribuzione dei coinfetti non si discosta da quella della popolazione HCV positiva totale.

**DISCUSSIONE**
L’infezione da HCV rappresenta un problema sanitario globale.
Ci proponiamo un attento follow up della risposta terapeutica di questi pazienti per confermare l’effettiva utilità clinica del test e determinare il suo reale valore predittivo.
Rispetto ad altri metodi descritti per l’analisi di polimorfismi l’HRM ha il vantaggio di essere più rapido e sicuro, relativamente poco costoso e molto semplice nella fase di ottimizzazione, quindi applicabile ad un ampio throughput.

![Figura I. HRM for IL28B polymorphisms normalized temperature-shifted melting curves.](image-url)
BIBLIOGRAFÍA


