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BRIEF REPORT

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A particular hepatitis C virus protease NS3 gene pattern in a patient not responding to interferon-ribavirin therapy

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Hepatitis C virus (HCV) is characterized by a high degree of genetic heterogeneity. This is a consequence of its rapid turnover in vivo and of the low fidelity of its RNA-dependent RNA polymerase, which lacks proofreading activity. Its high propensity to mutate is also reflected by the existence of closely related genomes, known as quasispecies [1]. NS3 protease is essential for HCV replication. It acts via a catalytic triad consisting of a histidine, an aspartate and a serine. It is also one of the most promising targets for specific anti-HCV therapy [2]. Although the NS3 protease is considered to be one of the least variable genes in the HCV genome, we previously found an appreciable polymorphism in this genomic region in 294 HCV genotype 1 clones isolated from the serum of 17 infected patients [3]. Reported here is a particular pattern, including three deletions and one insertion, found in four clones among 19 NS3 protease variants of a quasispecies detected in one of these patients.

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The 63-year-old male patient was hospitalized in January 2000 for chronic hepatitis C. His only risk factors for hepatitis C were acupuncture in 1981 and a stay in North Africa between 1957 and 1959, during which he had received intramuscular injections. A liver biopsy showed features of moderately active chronic hepatitis and extensive fibrosis (A2F3 according to the METAVIR scale). The infection was caused by HCV subtype 1b and the viral load was 3.98×10^6 IU HCV RNA/ml, as measured by quantitative PCR (Amplicor HCV Monitor test, version 2.0; Roche Diagnostics, Meylan, France). Treatment was initiated in March 2000 and consisted of 3 mega units of alpha-2b interferon 3 times a week plus ribavirin (1,200 mg per day). At the end of month 3 of treatment, antiviral therapy was stopped due to the development of severe asthenia, absence of biochemical response and only partial virological response in the patient. His HCV viral load was 3.16×10^5 IU HCV RNA/ml at this time.

The three observed deletions (sequences 1, 2, and 3, Fig. 1a) were all single-nucleotide deletions that resulted in a frameshift event and the appearance of a premature stop codon several residues downstream (Fig. 1b). This was confirmed by sequencing both DNA strands. In general, shifts in the translational reading frame, which almost invariably result in premature termination at some distance downstream of the mutation, are expected to inactivate a gene [4]. The histidine, aspartate and serine residues from the catalytic triad were preserved in, respectively, three, two and none of these variant proteases (Fig. 1b). Therefore, these three clones carrying single-nucleotide deletions may carry no functional NS3 protease. The single-nucleotide insertion detected in another clone also led to a frameshift event, but did not result in a premature stop codon (sequence 4, Fig. 1b). Translation of the nucleotide sequence generated a mutant NS3 protease, which may not be functional for proteolytic processing, notably because of the substitution of the serine from the catalytic triad.

No other insertions and only one deletion were found in the remaining 290 previously analyzed NS3 protease clones. The sequenced fragment of the NS3 region corresponds to only 181 of the 3,011 HCV residues and thus

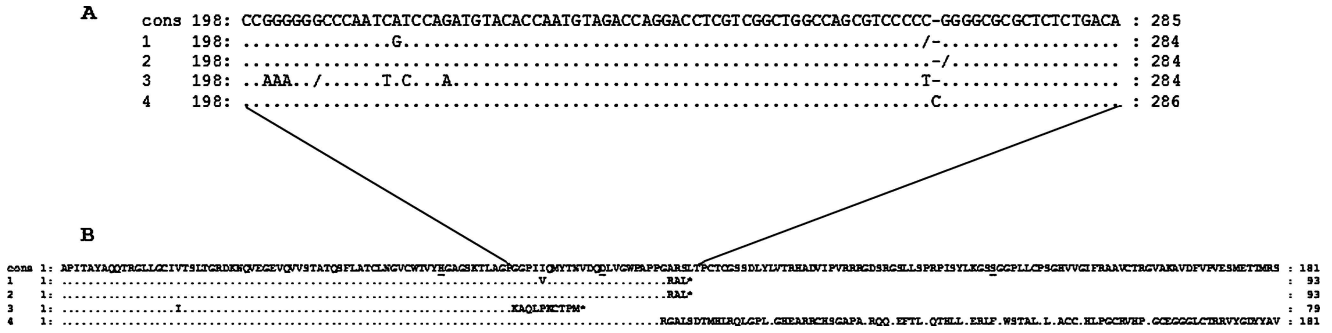

Fig. 1 Alignments of representative nucleotide (**a**) and deduced amino acid (**b**) sequences of the HCV NS3 protease gene of four variants detected in the quasispecies of the studied patient. Nucleotide and amino acid consensus sequences of the 15 remaining clones are referred as "cons". The positions within the HCV NS3 protease gene and protein are shown on both sides of the sequences. (·) indicates sequence identity. **a** (/) indicates nucleotide deletion at that position. **B** NS3 catalytic triad residues are underlined. (*) indicates stop codon

represents 6% of the coding capacity of the HCV genome. Although variation in the NS3 protease gene may not be representative of the entire HCV genome, the finding that 1.4% (4/294) of these sequences include stop codons or frameshifts suggests that most circulating viral populations contain defective genomes. Thus, defective HCV genomes have already been detected during quasispecies studies targeting various regions of the genome, i.e., in circulating blood [1, 5] as well as in confined compartments like hepatocellular carcinoma tissue [6] and ascitic fluid [7].

The NS3 protease has many structural and functional constraints. Consequently, substitutions that occur at essential residues, like those belonging to catalytic sites or substrate binding sites, are not fixed in quasispecies populations. Hence, the occurrence of these deletions and this insertion in our patient's pre-treatment sample may have particular significance. It is possible that defective clones, such as those we detected, influence persistent HCV infection and play a role in the absence of viral response to interferon. These defective viruses may be complemented by standard viruses providing elements essential for replication in trans, enabling production of the defective viruses. In either case, the lack of an efficient replication system for HCV in cultured cells prevents straightforward functional analysis of natural NS3 protease variants, and it is still not known whether the HCV genetic sequences found in patients belong to the infectious viruses.

Even if our patient has had hepatitis C for over 20 years, his circulating quasispecies is quite homogeneous, except for the presence of these four mutants. The mutants may have been selected a long time ago and persisted, which would suggest they are not deleterious for the viral population. Such deleted or inserted clones may be resistant to future HCV NS3 protease inhibitors as the resulting protease is truncated or chimeric. It is important to remember that defective clones belonging to the HCV quasispecies are circulating or confined in infected humans and that these clones probably play a role in viral persistence.

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