Misidentification of a Hepatitis C Virus (HCV) recombinant form leading to treatment failure with new direct acting antivirals

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INTRODUCTION

The use of new **Direct Acting Antivirals** (DAA) specific of **Hepatitis C Virus** (HCV), has greatly improved the virological suppression, and for the most recent ones, the biological and clinical tolerance of HCV treatment. Most of the **DAA do not haveyet a pangenotypic action**, which means that most of them have a predominant action on specific HCV genotype. This observation led to different recommendations in the treatment of HCV genotype 1, 2, 3, and 4 infections.

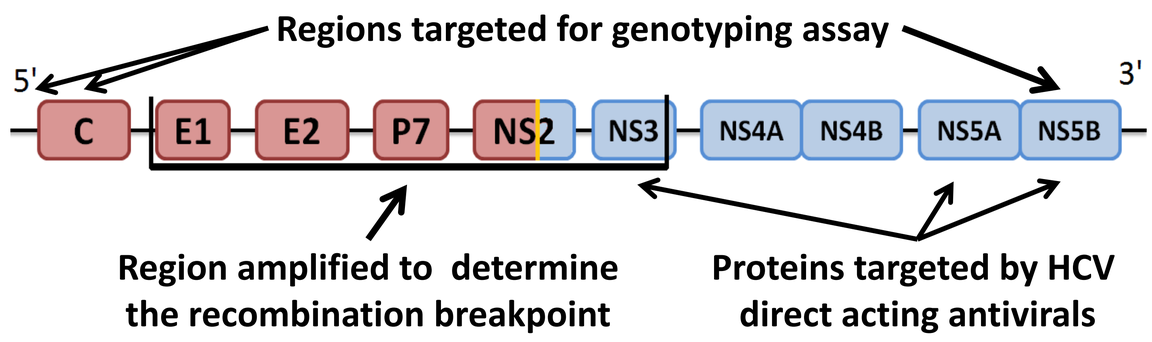
Genotyping methods may target different regions of the HCV genome, although only the **whole genome sequencing** could confirm the **correct genotype**. We report the case of a patient, infected with an unusual recombinant 2k/1b recombinant HCV form which was falsely diagnosed with an HCV genotype 2 infection, and treated with an unadapted treatment, leading to a treatment failure.

PATIENT

In 2005, a 42-year-old Ukrainian male, was diagnosed with an HCV infection, which was genotyped as a **genotype 2** using the *Genekit HCV 5'NC* (Visible). In May 2014, he started a **12 week treatment** with **sofosbuvir (400 mg daily)** and **ribavirin (600 mg twice daily)**, following our national guidelines for genotype 2 treatment. The pre-treatment HCV RNA plasma viral load (pVL) was 5.7 log UI/mL. Four weeks after, the pVL was <12 UI/mL and remained undetectable until the end of treatment in August 2014. Two weeks after, the pVL was detectable, and rose to 6.1 log UI/mL eight weeks after the end of the treatment. **After this virological failure**, a retreatment was decided in July 2015 with the same drug association but for 24 weeks. In the meantime, **we decided to check the genotype** using a previously described method targeting the NS5B region.

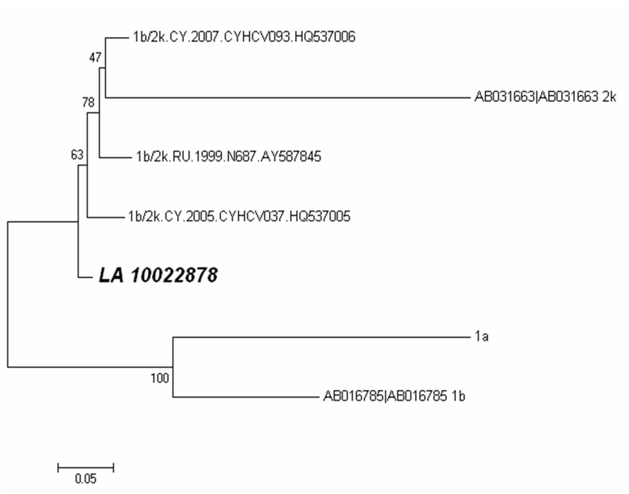
METHODS

- Both 5' **Core** and 3' **NS5B** region of the genome were sequenced to confirm the presence of a putative recombinant virus
- A cDNA covering the region from **E1** to **NS3** was synthesised, and sequenced by the Sanger method with a set of 15 primers adapted from previously published methods
- **Bioinformatic analysis** was performed by NCBI Genotyping, BLAST, Neighbour Joining phylogenetic analysis and SimPlot sequences

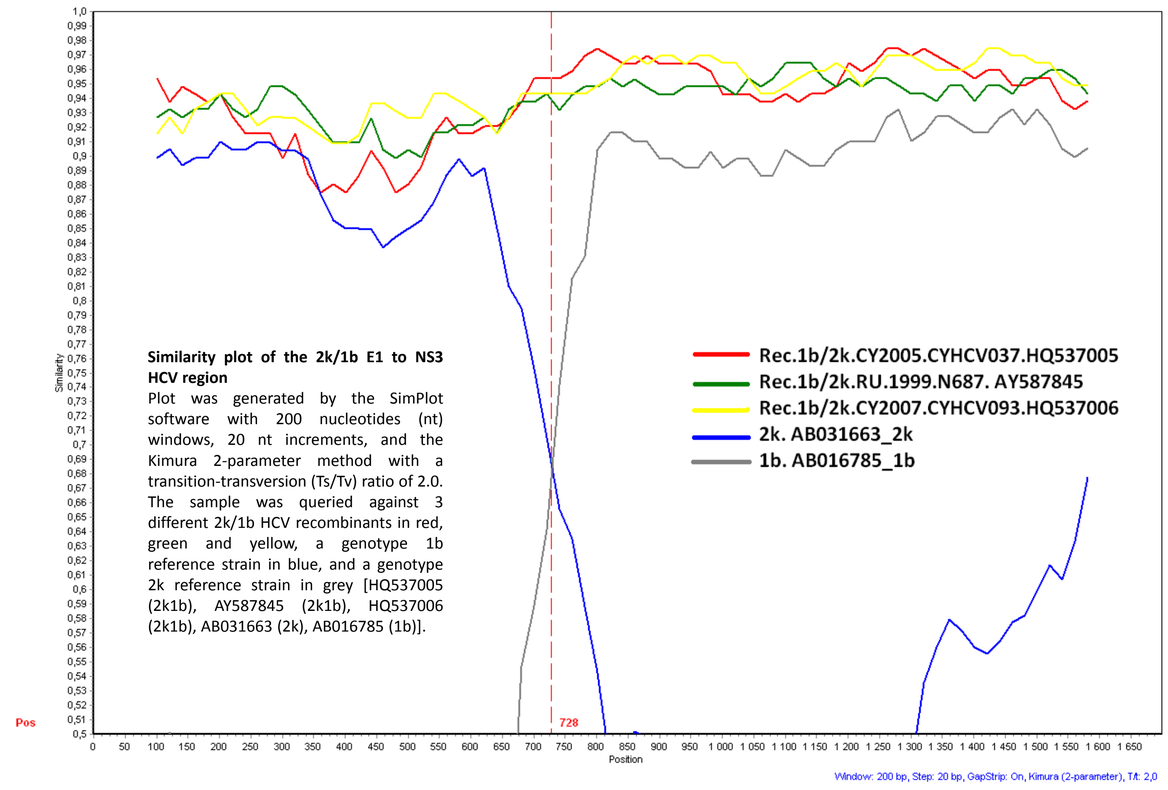


RESULTS

- Sequencing of the **Core region** at the **beginning of the genome** confirmed the phylogenetic **proximity to genotype 2k HCV**
- Sequencing of the **NS5B region** at the **end of the genome** confirmed the phylogenetic **proximity to genotype 1b HCV**
- Comparison of the NS5B gene to HCV 1a and 1b reference strains, and recombinant 2k/1b described in Russia and Cyprus made us focus the region from E1 to NS3 to search for the recombination breakpoint, as the breakpoint is usually situated in this part of the genome in these circulating strains



- Similarity of the complete sequence of 1699 nucleotides, from **E1 to NS3**, was compared to different genotype query sequences to identify the recombination breakpoint using SimPlot software
- SimPlot analysis showed a **recombination breakpoint** at the final part of the **NS2 gene** as described below



CONCLUSION

- **We confirm that analysing a single region of the genome to extrapolate the HCV genotype leads to misidentification of HCV recombinant forms**
- **The misidentification of these forms leads to treatment failure. In the case presented here the additional cost of the treatment, follow-up and laboratory analysis was estimated above 70 000 euros**
- **We urgently need rapid and easy methods for the whole genome sequencing of HCV to avoid such costly consequences**
- **All suspicions of recombinant strains can be investigated by the method described herein**