Prevalence of the hepatitis C virus NS3 polymorphism Q80K in genotype 1 patients in the European region

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Abstract
Hepatitis C virus (HCV) NS3 polymorphism Q80K is mainly found in patients with HCV genotype (G) 1a, and has been associated with a reduced treatment response to simeprevir with pegylated interferon (P) and ribavirin (R). Prevalence of Q80K among G1 patients may vary geographically. Q80K prevalence in the North-American G1 population in a recent study was 34%.

We conducted a post hoc meta-analysis of Q80K polymorphism prevalence among HCV G1-infected patients enrolled in simeprevir and telaprevir Phase II/III studies. Baseline HCV NS3/4A protease sequences were analysed by population sequencing to determine Q80K prevalence.

Overall, of 3349 patients from 25 countries in the European region analysed, 35.8%, 63.8% and 0.3% of patients had G1a, G1b and other/unknown HCV G1 subtypes, respectively. Q80K was detected at baseline in 7.5% of HCV G1 patients overall. Examination by subtype showed that 19.8%, 0.5% and 18.2% of patients with G1a, G1b and other/unknown HCV G1 subtypes had the Q80K polymorphism, respectively. Among countries in the European region with sequencing data available for either P or 20 patients with G1a and/or P or 40 G1 patients overall, the Q80K prevalence in G1 ranged from 0% in Bulgaria to 18.2% in the UK. Q80K prevalence also varied within G1a across different countries.

HCV subtype 1a was correctly determined in 99% of patients by the LiPA v2 assay.

A low overall prevalence of Q80K was observed in HCV G1-infected patients in the European region, compared with North America. However, the prevalence varied by country, due to differing ratios of G1a/G1b and differing Q80K prevalence within the G1a populations.

1. Introduction

HCV NS3/4A protease inhibitors (PIs) are used as part of different regimens to treat patients with chronic hepatitis C virus (HCV) infection. Naturally occurring amino acid substitutions in the NS3 protease domain, also referred to as polymorphisms, have been reported (Bartels et al., 2013; Gaudieri et al., 2009; Kuntzen et al., 2008). These polymorphisms can reduce the antiviral activity of PIs (Jacobson et al., 2014; Manns et al., 2014; De Meyer et al., 2012; Barnard et al., 2013).

Simeprevir is an approved PI, indicated for use in combination with other medicinal products for the treatment of chronic hepatitis C genotype (G) 1 and 4 infection in adult patients (Simeprevir Summary of Product Characteristics). Phase III trials of simeprevir in combination with pegylated interferon and ribavirin (PR) in HCV G1- and G4-infected treatment-naïve and prior relapse patients showed sustained virologic response after 12 weeks (SVR12) rates of approximately 80% (Jacobson et al., 2014; Manns et al., 2014; Forns et al., 2014; Moreno et al., 2014).

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Q80K is an NS3 polymorphism, mainly present in HCV G1a, that confers low level resistance to in vitro simeprevir activity. G1a isolates with a baseline Q80K polymorphism resulted in a median fold change in simeprevir EC50 value of 11 in a transient replicon assay (Verbinnen et al., 2014). It is the most common polymorphism among the NS3 polymorphisms associated with reduced activity of NS3/4A PIs (Bae et al., 2010).

The presence of Q80K at baseline has only a minor effect on initial response to treatment with simeprevir in combination with PR but may facilitate the emergence of additional mutations and subsequent failure to simeprevir/PR therapy (Lenz et al., 2014). Among patients treated with simeprevir in combination with PR, SVR rates were reduced in HCV G1 patients with Q80K compared with G1a patients without this polymorphism. SVR12 was lower in patients with Q80K than those without Q80K (58% versus 84% in HCV G1a treatment-naïve patients and 47% versus 79% in G1a prior relapsers) (Forns et al., 2014; Jacobson et al., 2013). In the same studies G1b patients had SVR12 rates of 85% in HCV treatment-naïve and 86% in prior relapsers. Most other PIS do not appear to be affected by Q80K. For example, both telaprevir (Shafran, 2014) and faldaprevir (Berger et al., 2014) have similar SVR rates with and without Q80K polymorphism at baseline when combined with PR, while the virologic response to asunaprevir was shown to be less robust in patients with Q80K than patients without Q80K (McPhee et al., 2012). However no data to show the effect of Q80K on SVR in asunaprevir-containing regimens have been published. In paritaprevir, EC50 was increased 3-fold in K80 compared with Q80t, but no data is currently available for the effect of Q80K on SVR with regimens containing this PI (Pilot-Matias et al., 2014). Structural differences between PIs account for the different impacts of amino acid substitutions, such as Q80K, on drug activity.

Alternative therapy to simeprevir plus PR should be considered in HCV G1a patients who have detectable Q80K polymorphism at baseline according to both the European Association for the Study of the Liver (EASL recommendations) and the joint American Association for the Study of Liver Diseases (AASLD) and Infectious Diseases Society of America (IDSA) recommendations (AASLD/IDSA recommendations). However, recent results with simeprevir-containing, interferon-free therapy showed that the effect of Q80K on SVR12 rates seems to be substantially attenuated or possibly eliminated (Lawitz et al., 2014). The COSMOS study, which evaluated simeprevir combined with sofosbuvir with or without ribavirin for 12 or 24 weeks, among HCV G1 prior-null responders with METAVIR F0–4, and treatment-naïve patients with METAVIR F3–4, showed SVR rates of 88% and 94% in G1a patients with and without baseline Q80K, respectively, in the intent-to-treat (ITT) population (Lawitz et al., 2014). Of the six patients (4%) who experienced viral relapse in the COSMOS study, four had Q80K at baseline. Recent interim real-life data of simeprevir in combination with sofosbuvir with or without ribavirin in American patients has shown similar results to COSMOS with overall SVR+4 (SVR achieved four weeks or more after end of treatment) of 89% (269/303) in HCV-TARGET (Sułkowski et al., 2014a). This cohort notably has a high proportion of difficult-to-treat patients including 61% having HCV G1a and 57% being cirrhotic (Sułkowski et al., 2014a). Similarly in the US TRIO Health cohort, 82% (n = 276, intent to treat population), of HCV G1 patients achieved SVR12 with the same regimen (SVR12 was 90% in per protocol population, n = 252). Again, this cohort was made up of difficult-to-treat patients, and of those selected for this regimen, 63% were infected with HCV G1a and 45% had cirrhosis (Dieterich et al., 2014). Data relating to the effect of Q80K on efficacy are not available in either HCV-TARGET or TRIO cohorts as Q80K testing was rarely performed.

This study aimed to provide Q80K prevalence data across the European region. The presented results inform physicians on their local prevalence of HCV G1a versus 1b, together with the frequency of Q80K in the overall G1 population, HCV G1a and other rare HCV G1 subtype isolates. Additionally, the concordance of two commonly used HCV G1 subtyping assays (TRUGENE and Versant LiPA v2) with a NS5B or NS3/4A sequence-based HCV genotype subtyping assay was determined.

2. Materials and methods

Demographic and baseline data from patients in the European region were pooled from 14 telaprevir and simeprevir Phase Ib and III clinical studies. Studies of patients who were HCV treatment-naïve were QUEST-1 (Jacobson et al., 2014), QUEST-2 (Manns et al., 2014), PILLAR (Fried et al., 2013), C208 (Marcellin et al., 2011), OPTIMIZE (Buti et al., 2014), PROVE2 (Hézode et al., 2009), ADVANCE (Jacobson et al., 2011), ILLUMINATE (Sherman et al., 2011) and REPLACE (clinicaltrials.gov). Studies of patients who had received prior HCV interferon-based treatment were ASPIRE (Zeuzem et al., 2014), PROMISE (Forns et al., 2014), ATTAIN (Reddy et al., 2014), REALIZE (Zeuzem et al., 2011) and PROVE3 (McHutchison et al., 2010). For each of the studies the ITT population was analysed, defined as all patients who received at least one dose of study drug. Descriptive statistics on the prevalence of Q80K across different subgroups were tabulated by HCV genotype subtype. Population sequencing was performed as described previously (Koletzki et al., 2013), HCV genotype subtypes were determined at screening by TRUGENE or Versant LiPA v2 assay (both Siemens Healthcare Diagnostics, IL, USA), depending on the study. HCV genotype subtypes were also determined at baseline by sequencing a 329 base pair region within NS5B, or by sequencing the NS3/4A region, depending on the study, both followed by basic local alignment search tool (BLAST) analysis. For the prevalence analyses reported here, the HCV genotype subtypes determined by the NS3/4A or NS5B region were used, and if not available, the results from the LiPA v2 or TRUGENE assay were used instead.

The concordance of results of the TRUGENE and LiPA v2 HCV genotype subtyping assays (which are commercially available) with results from NS3/4A or NS5B sequence-based genotype subtyping assays was also analysed. Only samples that had results available with both the LiPA v2 and NS3/4A or NS5B sequence-based assays or both the TRUGENE and NS3/4A or NS5B sequence-based genotype subtyping assay were included in this analysis.

3. Results

The ITT population of these studies included 3462 patients from 25 countries in the European region: Austria, Belgium, Bulgaria, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Israel, Italy, the Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Turkey, Ukraine and the UK. Baseline NS3/4A population sequencing data were available for 3349 (96.7%) patients and HCV genotype subtype and Q80K prevalence data is reported for this subset. Country-specific data are only shown for countries that had sequencing data available for at least 20 G1a patients (Austria, Belgium, France, Germany, Italy, the Netherlands, Norway, Poland, Portugal, Spain, Sweden and the UK) or at least 40 G1 patients overall (Bulgaria, Romania and Russia).

3.1. HCV genotype subtype and Q80K prevalence

Overall, 35.8% (1200/3349) of patients had G1a, 63.8% (2138/3349) G1b and 0.3% (11/3349) had another or unknown G1 subtype. Of the 11 patients with another or unknown G1 subtype, two were G1c, two were G1d, three were G1e, one was G1i and one was G6e (this patient was determined to be G1b by TRUGENE
assay). The G1 subtype of the remaining two patients was unknown. For these two patients the NS5B sequence-based HCV genotype subtype assay failed and only results from the TRUGENE assay were available and are reported here.

The overall prevalence of Q80K was 7.5% (250/3349), broken down as 19.8% (237/1200) in G1a, 0.5% (11/2138) in G1b and 18.2% (2/11) in other or unknown G1 subtypes (two patients with HCV G1d; none of the other patients with other or unknown G1 subtype had Q80K). Overall prevalence of baseline Q80K among G1-infected patients in countries with sequencing data for at least 20 G1a patients and/or at least 40 overall G1 patients ranged from 0% in Bulgaria to 18.2% in the UK (Fig. 1 and Table 1). Prevalence of Q80K was higher when only considering the G1a population compared with the overall population. In countries with sequencing data for at least 20 G1a patients, Q80K prevalence in G1a patients ranged from 4.8% in Norway to 75.0% in Poland.

HCV G1b prevalence also varied throughout the European region, impacting on Q80K prevalence in the overall HCV G1 population. When considering countries with sequencing data for at least 40 G1 patients, Poland, Romania and Russia had high G1b prevalences of 95.5% (444/465), 100% (92/92) and 96.7% (177/183), respectively, amongst the overall G1 population. These resulted in low overall Q80K prevalences of 3.7% (17/465), 1.1% (1/92) and 2.2% (4/183), respectively. While other countries, such as Spain and Bulgaria, had a substantial proportion of HCV G1a of 31.2% (82/263) and 28.6% (14/49), respectively, due to low prevalence of Q80K among the G1a population. Q80K prevalence (0.0% [0/82] in Spain and 0% [0/49] in Bulgaria). In contrast, the UK, where overall Q80K prevalence in the HCV G1 population amounted to 18.2%, had a low G1b prevalence of 19.7% (39/198) and a Q80K prevalence within the G1a population of 22.6% (36/159).

No baseline demographics or disease characteristics analysed, other than country or G1 subtype, appeared to be linked to Q80K prevalence, (Supplementary Table). This included race, ethnicity, IL28B genotype, METAVIR score, whether the patient was PR-therapy experienced and, for patients who had prior PR therapy, the response to treatment. (Supplementary Table).

3.2. HCV genotype subtype assay concordance

A total of 3368 patients had results available with both the LiPA v2 and NS3/4A or NS5B sequence-based assays or both the TRUGENE and NS3/4A or NS5B sequence-based genotype subtyping assay, and were included in this analysis.

In total, 1909 samples had results available with both LiPA v2 and NS3/4A or NS5B sequence-based assays (Table 2). Overall, 98.8% of samples (1887/1909 samples; 657 G1a and 1230 G1b) had the same HCV genotype subtype with both the LiPA v2 and the NS3/4A or NS5B sequence-based assays. Eleven patients (0.6%) determined as HCV G1b by LiPA v2 were classified as G1a by NS5B or NS3/4A sequence-based assay. Nine of these eleven patients had Q80K data available. Of these nine patients 33.3% (3/9) had Q80K. An additional three samples determined as HCV G1b by LiPA v2 were classified as G1d, G1e and G1l with the NS3/4A or NS5B sequence-based assays. Three samples determined as G1a with LiPA v2 were classified as G1b with the NS3/4A or NS5B sequence-based assay. For four patients the HCV G1 subtype could not be determined via LiPA v2; three of these were G1a and one G1b based on NS3/4A or NS5B genotype subtyping assays. None of these patients had a Q80K polymorphism.

A total of 1461 samples had results available with both TRUGENE and NS3/4A or NS5B sequence-based assays (Table 3). Overall, 79.6% of samples (1163/1461 samples; 405 G1a, 756 G1b, one G1c and one G1g) had the same HCV genotype subtype with both assays. The TRUGENE assay identified 79 samples as HCV G1b that were classified as either HCV G1a (74/79); unknown HCV G1 subtype (2/79), G1d (2/79) or G6e (1/79) by the NS3/4A or NS5B sequence-based assay. Of the 74 patients determined as G1b with TRUGENE but shown to be G1a by NS3/4A or NS5B sequence-based
assay, 66 had Q80K data and 0% (0/66) of those had Q80K. Additionally, 43 samples determined as G1a with the TRUGENE assay were classified as G1b (37/43), other HCV genotype subtypes (5/43) or unknown HCV G1 subtype (1/43) when tested with the NS3/4A or NS5B sequence-based assay. In addition, of 176 samples in which G1 subtype was not determined with the TRUGENE assay, 77 were G1a, 98 were G1b and one was G1d with the NS3/4A or NS5B sequence-based assay.

4. Discussion

The prevalence analyses reported here, comprised a large dataset of 3349 patients from 25 countries in the European region and showed a low overall prevalence of Q80K (7.5%). This low Q80K prevalence is due, in part, to the relatively high (63.8%) occurrence of G1b and the lower Q80K prevalence in G1a patients in the European region. This is in contrast to previously reported data, which generally included a high proportion of sequences from North America, such as a reported Q80K prevalence of 47% (121/268) in G1a (Bae et al., 2010).

Overall, G1 Q80K prevalence varied by country from 0% to 18.2%. In countries with a low prevalence (Romania, Russia and Poland) this was driven by the very low percentage of G1a patients in these countries. These data are consistent with previous reports of high G1b prevalence in Romania of 93.5% (143/153) and Poland 97.1% (100/103) (Grigorescu, 2009; Chlabicz et al., 2008). Of note, Norway and Portugal had the lowest HCV G1b prevalences but also low Q80K prevalences due to a low Q80K prevalence within their respective HCV G1a populations.

A recent analysis of Q80K in HCV G1 patients showed that Q80K prevalence was higher in both the overall North American population (34.4%) and in the North American G1a population (48.1%) than in European patients overall (6.1%) and the European G1a population (19.4%) (European data were a subset of the study presented here (Lenz et al., 2014)).

The prevalence of Q80K within HCV G1a patients also varied, ranging from low prevalence in Norway, Spain and Portugal of 4.8%, 8.5% and 8.1%, respectively to higher prevalence of 75.0%, 29.0%, 22.6% in Poland, Germany and the UK, respectively. Despite the very high Q80K prevalence in G1a patients in Poland, its overall HCV G1 Q80K prevalence (3.7%) is comparable to Spain (2.7%) and Portugal (6.5%) due to very low G1a prevalence. Other data on Q80K prevalence by countries are limited and frequently based

### Table 1

<table>
<thead>
<tr>
<th>Country</th>
<th>All HCV G1 N (%)</th>
<th>HCV G1a N (%)</th>
<th>HCV G1b N (%)</th>
<th>Other/unknown N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All countries</td>
<td>3349 (7.5)</td>
<td>1200 (19.8)</td>
<td>2138 (19.8)</td>
<td>11 (0.5)</td>
</tr>
<tr>
<td>Q80K</td>
<td>290 (6.8)</td>
<td>237 (8.4)</td>
<td>53 (1.9)</td>
<td>0 (0.0)</td>
</tr>
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<td>Austria</td>
<td>204 (5.7)</td>
<td>190 (9.3)</td>
<td>14 (0.7)</td>
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<td>211 (5.7)</td>
<td>61 (28.9)</td>
<td>147 (3.3)</td>
<td>3 (0.1)</td>
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<td>14 (28.6)</td>
<td>35 (7.1)</td>
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<td>275 (5.4)</td>
<td>244 (4.7)</td>
<td>2 (0.4)</td>
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<td>241 (41.1)</td>
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<td>60 (28.6)</td>
<td>150 (7.1)</td>
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<td>26 (63.4)</td>
<td>14 (34.1)</td>
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<td>46 (78.0)</td>
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<td>36 (100)</td>
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CI: confidence interval.
Data are shown per country for countries with at least 20 genotype 1a patients and/or at least 40 genotype 1 patients overall.
HCV geno/subtype is based on the NS5B or NS3/4 assay, and if not available on LiPA v2 or TRUGENE results.

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<th>N (%)</th>
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### Table 2

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<th>HCV genotype subtype (LiPA v2 assay)</th>
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<th>lb</th>
<th>lc</th>
<th>lg</th>
<th>Overall</th>
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<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
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<tr>
<td>G1a</td>
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<td>11</td>
<td>1</td>
<td>1</td>
<td>672</td>
</tr>
<tr>
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<tr>
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<td>n</td>
<td>n</td>
<td>Overall</td>
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<th>Overall</th>
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<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
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<td>G1b</td>
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<td>556</td>
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<tr>
<td>Mixed genotype</td>
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<td>n</td>
<td>n</td>
<td>n</td>
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<td>Overall</td>
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<th>CI: confidence interval.</th>
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<td>Data are shown per country for</td>
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<td>countries with at least 20</td>
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<td>genotype 1a patients and/or at</td>
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<td>least 40 genotype 1 patients</td>
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<td>overall.</td>
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<td>NS5B or NS3/4 assay, and if not</td>
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<td>available on LiPA v2 or TRUGENE</td>
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<td>results.</td>
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Table 3

Cross-tabulation of HCV genotype subtype determined by NS3/4A or NS5B sequence-based assay versus HCV genotype subtype determined by LiPA v2 (ITT population).

- Includes one patient determined as genotype 1b and genotype 6e with TRUGENE and NS5B based assay, respectively.
on limited sample size. In general, the prevalences of Q80K in HCV genotype 1a in France (14%) Italy (20%), Sweden (15%) and UK (23%) in our study were similar with those previously reported in French (7%), Italian (14%), Swedish (6%) and Scottish (16%) patients (Berger et al., 2014) as well the more recent French cohort (11% prevalence) (Morel et al., 2014). These data show that the ratio of G1a to G1b is not the only factor that determines Q80K prevalence in HCV G1 overall, but also that the Q80K prevalence within the HCV G1a population varies by country.

Different prevalences of G1a clades and the Q80K prevalence in these clades may provide some explanation for Q80K being less frequent in G1a-infected patients from Europe and the variation of Q80K prevalence within the European region. Two distinct clades of G1a (clade 1 and clade 2) have been identified by phylogenetic analysis (Pickett et al., 2011). A different distribution of naturally occurring amino acid substitutions exist between clades. The Q80K polymorphism is only found in clade 1 and not clade 2 (48.9% versus 0%; n = 293 sequences) (De Luca et al., 2013). The Q80K polymorphism is believed to have originated in the US, with 96% of HCV infections in an international cohort of 677 patients with Q80K present analysed descending from a single lineage originating in the 1940s (McCloskey et al., 2014). A higher prevalence of HCV genotype 1a clade 1 (72.7%) was seen in the Los Alamos database (which includes sequences mainly from the USA) than in an equivalent Italian cohort (47.3%) (De Luca et al., 2013), and in a French cohort (43.2%) (Morel et al., 2014) suggesting a difference in HCV G1a sequences between North American and European populations. It is likely that clade 2 originated in Europe (De Luca et al., 2013).

Additional epidemiological studies are needed to further determine the prevalence of the two GT1a clades as well as of Q80K across Europe but also to assess potential regional differences which may be present within a specific country.

Baseline testing for Q80K is recommended in HCV G1a patients before initiating treatment with simeprevir plus PR (Simeprevir Summary of Product Characteristics). However, our data suggest that Q80K baseline testing may not be cost-effective in certain regions (e.g. Eastern Europe), although full cost-effectiveness studies would be needed to determine this. Another consideration when assessing the need for Q80K testing is the use of on-treatment stopping rules, which should be applied when initiating simeprevir plus PR (Simeprevir Summary of Product Characteristics). Patients with HCV RNA \( \geq 25 \) IU/mL at Week 4 have a low chance of achieving SVR; therefore, if a treatment response (i.e. HCV RNA <25 IU/mL) to simeprevir is not reported at Week 4, all treatment should be stopped and an alternative management plan agreed upon. Conversely, patients with an early treatment response have a high chance of achieving SVR, irrespective of baseline predictive factors (including Q80K) (Jacobson et al., 2013; Forns et al., 2014). Hence, determining early responses to simeprevir plus PR provides a useful tool for assessing the utility of this treatment in individual patients, especially in the setting where Q80K testing has not been performed. The effect of Q80K on SVR is substantially attenuated or possibly eliminated when simeprevir is taken with sofosbuvir with or without ribavirin (Lawitz et al., 2014).

Alternative interferon-free therapies currently approved in HCV G1 include sofosbuvir in combination with daclatasvir, and sofosbuvir in a fixed dose combination with ledipasvir. Both combinations have shown to be effective in patients with mutations associated with resistance to protease inhibitors (Sulkowski et al., 2014b; Afdhal et al., 2014). Additionally, the drug combination of paritaprevir (boosted with ritonavir), dasabuvir and ombitasvir is expected to be approved soon with other therapies in clinical development. It is unlikely that there will be one regimen that will be suitable for all patients due to the range of baseline characteristics, the genetic differences between genotypes and the naturally occurring polymorphisms that can infer resistance to various drugs.

Since population sequencing rarely fails to detect the Q80K polymorphism when it is present, this eliminates the need for more sensitive deep sequencing technologies. In a subset of patients \( n = 274 \) from pooled Phase II and III simeprevir studies, 70 patients (26%) had Q80K polymorphism detected by population sequencing, and only two additional patients (0.7%) having Q80K detected by deep sequencing (Fevry et al., 2014). Similarly in a telaprevir study, out of 185 samples that had no Q80K detected at baseline by population sequencing, only one had Q80K detected by deep sequencing (Dierynck et al., 2014). The clinical relevance of such minority species harbouring Q80K is unknown.

Given that Q80K testing is only recommended in HCV G1a patients when considering treatment with simeprevir and PR, this dataset was also used to determine the consistency of the commercially available LiPA v2 and TRUGENE assays compared with NS3/4A or NS5B sequence-based genotype subtyping assays, which are considered the most accurate methods for standard HCV genotype subtyping. The LiPA v2 assay had very high concordance (98.8%) with NS3/4A or NS5B assays. Concordance of the TRUGENE assay with NS3/4A or NS5B assays was lower than LiPA v2, but still remained high (79.6%). These findings are consistent with previous reports where the LiPA v2 assay was a more accurate method of determining HCV genotype subtype than the TRUGENE assay (Germers et al., 2003; Stetzer et al., 2007; Hedskog et al., 2014). Among our samples that could not be subtyped with TRUGENE, 43.8% were G1a and 55.8% were G1b; this ratio was similar to the overall results (35.9% and 63.5%) indicating no apparent error bias for a specific subtype. This study also showed that there is a very low risk of erroneously subtyping a HCV G1a with Q80K as G1b with either assay. Of HCV G1a (determined by NS3/4A or NS5B sequence based assay) patients with Q80K, 1.3% (3/237) and 0% (0/237) were erroneously subtyped as G1b by the LiPA v2 and TRUGENE assays, respectively.

5. Conclusions

Overall, there was a low prevalence of the Q80K polymorphism in HCV patients infected with HCV G1 living in the European region. However, Q80K prevalence varied between countries due to different Q80K prevalence within the HCV G1 populations and differing ratios with G1a compared with G1b.

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De Meyer, and Oliver Lenz are employees of Janssen Pharmaceuticals and may be Johnzon and Johnson stockholders.

Glossary

Q80K is a naturally occurring amino acid substitution occurring in the NS3 protease domain. This NS3 polymorphism is mainly present in HCV G1a, conferring low level resistance to in vitro simprevir activity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2015.01.003.

References


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References
resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naïve patients. Hepatology 48, 1769–1778.


