Detection of the NS3 Q80K polymorphism by Sanger and deep sequencing in hepatitis C virus (HCV) subtype 1a strains in the United Kingdom

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HCV heterogeneity

- HCV is genetically heterogeneous and classified into 7 genotypes and several subtypes (1a-m, 2a-r, 3a-l, 4a-t, 5a, 6a-u, 7a)

- HCV strains belonging to different genotypes differ at 30-35% of nucleotide sites, whereas strains that belong to the same subtype differ at <15% of nucleotide sites

- Different HCV genotypes and subtypes show distinct geographical distribution and association with risk categories in the population

Global geographic distribution of HCV genotypes

The NS3 Q80K polymorphism

- HCV NS3 protease enzyme exhibits a high degree of genetic variability
- Only 47% of its amino acids are conserved among circulating HCV
- Natural resistance to NS3 inhibitors is rare (<8%) among DAA naive patients infected with HCV-1
- Q to K substitution at codon 80 (Q80K) occurs as a polymorphism in the NS3 gene of HCV-1a strains
- The Q80K polymorphism reduces susceptibility to simeprevir plus pegylated interferon alpha and ribavirin (P/R)
- Screening for Q80K is therefore recommended before starting simeprevir

Aims

① Determine the prevalence of Q80K in NS3 sequences obtained from HCV-1a infected patients attending for care in two regions of the United Kingdom (UK)

② Investigate the occurrence of Q80K as a low frequency variant by next generation sequencing

③ Investigate the phylogenies of NS3 sequences from the UK in relation to publically available sequences from the rest of Europe and North America
Methods

**Study population**

238 adults (median age 44 years) HCV-1a infected

70/238 (29.4%) in the North-West (NW)

168/238 (70.6%) in the South-East (SE)

HCV RNA load median 6.3 log10 IU/ml (IQR 5.8-6.8)

All naïve to any anti-HCV therapy

All coinfected from SE
Methods

Deep sequencing
- aa180 of NS2 to aa204 of NS3
- Nextera XT
- Illumina MiSeq using v2 reagents
- Cutadapt v1.2.1
- Sickle v1.2
- BAMStats
- VirVarSeq pipeline v17
- 727bp HCV-1a molecular clone
- Error rate at the codon level mean 0.6\% (SD 0.2\%)

Population sequencing
- aa180 of NS2 to aa204 of NS3
- BigDye Terminator CS Kit
- ABI Prism 3730 Genetic Analyser
- SeqScape (v2.7)
- Geno2pheno
The Q80K prevalence

Overall 80K prevalence 44/238, 18.49% (CI 13.49%-23.43%)

- 28/178 (16.2%) of the samples tested by both population and NGS (≥1%)
- 16/60 (26.7%) tested only by population sequencing

- 28 samples identified 80K using both had by NGS mutant frequencies >40%

- 2 samples showing mixed 80Q/K by Sanger: one had a Q:K ratio of 54:46, and the other showed Q,K and L at a ratio of 57:41:2 by NGS

- Q80K increased 2% (1 more sample) for 80K with an interpretative cut-off between ≥0.5% and <1%, and 10% (3 more samples) with ≥0.2% and <0.5%

- BUT... estimated error rate (0.6%, SD 0.2%)
Q80K prevalence in European countries

Q80K prevalence (%)
- 0
- <5.0
- 5.1–10.0
- 10.1–15.0
- 15.1–20.0
- <20 HCV genotype 1a and <40 HCV genotype 1 patients with data
- No data

Leggewie M et al, AIDS 2013; Sarrazin et al, Antiviral Research 2014
The 80K prevalence by region / risk group

**% detection rates**

**North-West region (Liverpool)**
- 19/70
- 27.14%

**South-East region (London)**
- 25/168
- 14.88%

**HIV/HCV co infected**
- 18/107
- 16.82%

**HCV mono infected**
- 7/61
- 11.48%

*p=0.04 *

*p=0.38*
Other Q80 codon substitutions

- One sample showing 80L by Sanger had codon frequencies 88.2% L/11.5% Q
- Three more samples showing 80L by NGS with interpretative cut-off >1% (mutant freq 1.38%-5.59%)
- Three samples showing 80R by NGS (mutant freq 1.06% - 1.73%)

- Clinical significance ???
Other NS3 mutations

![Graph showing the distribution of NS3 mutations detected by Sanger sequencing and NGS with different coverage thresholds.](image-url)
Phylogenetic analysis

Methods

- HCV-1a NS3 sequences from North America and Europe
- 882 sequences from the Los Alamos HCV sequence database
- Maximum-likelihood (ML) phylogenies (with and without the Q80K codon)
- FastTree v.2.1.7 - local branch support by the Shimodaira–Hasegawa-like (SH-like) test
- Clusters (≥3 seqs) were identified by a bootstrap support >75%
Sampling NS3 sequences
UK sampling NS3 sequences
HCV-1a NS3 global phylogeny

Clade II / 80Q

Clade I / 80K
HCV-1a NS3 global phylogeny

Pickett et al, JVH 2011; McCloskey et al, JID 2014 (advance access view)
HCV-1a NS3 clades dating

Clade I
- 1966
  - (1952–1972)

- 1964
  - (1941–1976)

Clade II
- 1975
  - (1961–1989)

DeLuka et al, OFID 2015 (advance access view)
HCV-1a NS3 sequences 80K <1%

Clade II / Q80

Clade I / 80K
UK NS3-1a transmission clusters

Clusters (≥3 seqs) were identified by a bootstrap support >75%

- 4 NW, 8 SE, and 11 inter-regional clusters
- 3-20 sequences
- 148/238 (62.2%) of UK sequences (49/70, 70.0% in the NW and 99/168, 58.9% in the SE)
Conclusions

① Q80K at high prevalence among treatment-naïve HCV-1a carriers attending for care in UK

② The Q80K prevalence varied by geographical region, being higher in Northwest region in UK

③ Regional transmission networks may lead to these differences

④ No samples showing Q80K at a frequency below the detection threshold of Sanger sequencing (≥20%) and above the typical ≥1% interpretative cut-off for NGS
Acknowledgments

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• Simon King
• Kate Childs
• Athanassios Papadimitropoulos
• Mark Hopkins
• Mark Atkins
• Kosh Agarwal
• Mark Nelson
• Prof. Anna Maria Geretti

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• Institute of Infection and Global Health (IGH), University of Liverpool
• Centre for Genomic Research (CGR), University of Liverpool
• Liverpool Specialist Virology Centre, Royal Liverpool and Broadgreen University Hospitals NHS Trust
• Institute of Liver Studies, Department of HIV Medicine and Sexual Health, King's College Hospital
• Department of Clinical Virology, Frimley Park Hospital NHS Foundation Trust
• Institute of Liver Studies, Department of Liver Diseases, King's College Hospital
• Department of HIV medicine and Sexual Health, Chelsea and Westminster NHS Foundation Trust