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New findings in HCV genotype distribution in selected West European, Russian and Israeli regions

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ABSTRACT

Background: HCV affects 185 million people worldwide and leads to death and morbidities. HCV has a high genetic diversity and is classified into seven genotypes and 67 subtypes. Novel anti-HCV drugs (Direct-Acting-Antivirals) eligibility, resistance and cure rates depend on HCV geno/subtype (GT).
Objectives: Analysis of epidemiological information and viral GT from patients undergoing viral genotyping in 2011–2015.
Study design: Anonymized information from 52 centers was analyzed retrospectively.
Results: 37,839 samples were included in the study. We show that the GT distribution is similar throughout Western European countries, with some local differences. Here GTs 1 and 2 prevalences are lower and of GT4 higher than in all previous reports. Israel has a unique GT pattern and in South Russia the GT proportions are more similar to Asia. GTs 5 and 6 were detected in very low proportions. Three cases of the recombinant genotype P were reported in Munich (Germany).

In addition, we observed that GT proportion was dependant on patientsí gender, age and transmission route: GTs 1b and 2 were significantly more common in female, older, nosocomially-infected patients, while GTs 1a, 3 and 4 were more frequent in male, younger patients infected by tattooing, drug consume, and/or sexual practices. In infections acquired by drug consume, GTs 1a (35.0%) and 3 (28.1%) prevailed. In infections related to sexual practices lower proportion of GT3 (14.0%) and higher of GT4 (20.2%) were detected. GT4 was mostly abundant in MSM (29.6%). HIV coinfection was significantly associated with higher proportions GTs 1a and 4 (42.5% and 19.3%, respectively).
Conclusion: Genotype prevalence evolves and correlates to epidemiological factors. Continuous surveillance is necessary to better assess hepatitis C infection in Europe and to take appropriate actions.

1. Background

Hepatitis C virus (HCV) infects >185 million infections worldwide, though occult infection may increase this number up to 20–30% [1]. Persistent HCV infection is associated with the development of liver cirrhosis, liver failure or hepatocellular carcinoma, and is the most common indication for liver transplantation.

HCV is a single stranded RNA virus belonging to the family Flaviviridae. HCV displays a high genetic diversity due to the high mutation rate of the viral polymerase and the high turnover of the virus. Viral variants are classified into seven genotypes (named 1–7) and then further into at least 67 subtypes, (labelled as a, b, c, etc.) [2].

HCV genotyping is performed by in-house sequencing followed by phylogenetic analysis or use of internet-based genotyping tools such as geno2pheno$_{[HCV]}$ (http://hcv.geno2pheno.org/index.php),

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or with commercial tests [3–7]. In spite of small differences in the geno/subtype (GT) results among methods, the use of all these methods for clinical purposes has been extensively validated.

The licensing of the first Direct-Acting-Antivirals (DAAs) targeting HCV proteins has significantly increased the cure rates [8]. However HCV eradication by therapy implementation is hampered not only by high DAA-therapy costs but also by its genotype-dependent implementation and success rates.

In spite of this critical role in HCV therapy, data on global HCV GT-distribution are limited. Most epidemiological studies are restricted to regional level. Recent supranational works are meta-analyzes based in overlapping datasets and cover no or very few epidemiological factors [9–13].

2. Objectives

In this work we aimed to gain large-scale knowledge on GT distribution based on current data from centers routinely diagnosing and treating HCV-infected patients. Additionally, we linked the HCV genotype distribution to epidemiological parameters.

3. Study design

3.1. Study design

A list of the clinical sites providing samples and their genotyping methods are provided in Supplementary Table 1. Sample inclusion criteria were: successful genotyping performed in the years 2011–2015 and report of genotyping method. Only one sample per patient was included in the analysis. If available, additional information was collected: patientsí year of birth or age at testing, HCV transmission route/risk group, and Human Immunodeficiency Virus (HIV)- or Hepatitis B virus (HBV)-coinfections. Markers for present or past HBV infection were HBs-antigen and/or anti-HBc. Data were anonymized at the origin and then sent to the University of Cologne for analysis and storage in the Arevir database.

3.2. Statistical analysis

We used a log-linear model to analyze multivariate dependencies between variables. In a first analysis the GT was used as the dependent variable and country, gender, date of birth, HIV-coinfection, HBV-coinfection, and transmission route/risk group as independent variables. Performance of the log-linear model was evaluated by *p*-values computed from the Chi-squared distribution, using the residual degrees of freedom and deviance of the model. Model variates were selected based on the changes in degree of freedom and deviance resulting from adding multivariate terms to the model. Only terms with a *p*-value below 0.05 were retained. We used Bonferroni correction to adjust the z-critical values for significance according to the size of the confusion table. We considered three different *p*-values reflecting significant ($p \leq 0.05$), very significant ($p \leq 0.01$), and highly significant ($p \leq 0.001$) residuals.

For the multivariate dependency analysis, all models included the genotype as a variable, since our main objective was the study of genotype-dependent associations. Associations between year of birth and country were not considered due to the intensity of the associated computations.

4. Results

We collected 37,839 genotypes from 52 centers from Austria, Belgium, Germany, Israel, Italy, Luxembourg, Portugal, Russia, Spain, and the UK, comprising. Raw and statistical data are summarized in Supplementary Tables 1–3. Baseline characteristics are shown in Table 1.

Table 1
Data overview.

	Number and percentage of samples
Year of testing	
2011	8301 (21.9%)
2012	7939 (20.9%)
2013	8410 (22.2%)
2014	8761 (23.1%)
2015	4533 (12.0%)
Genotype	
GT1*	3860 (10.2%)
GT1a	9910 (26.2%)
GT1b	10832 (28.6%)
GT2	1952 (5.2%)
GT3	7754 (20.5%)
GT4	3415 (9.0%)
GT5	70 (0.2%)
GT6	36 (0.1%)
non-1, 2 or 3**	10 (0.0%)
Gender	
M	20282 (53.6%)
F	9279 (24.5%)
unknown	8278 (21.9%)
transmission route/risk group	
parenteral	2282 (9.9%)
vertical	13 (0%)
nosocomial	442 (2.0%)
tattoo/piercing	93 (0.5%)
IVDA***	1231 (5.8%)
sexual	257 (0.9%)
unknown	34685 (91.7%)
age at testing	
≥65	4496 (11.9%)
64–55	6663 (17.6%)
54–45	10423 (27.5%)
44–35	6034 (15.9%)
34–25	2209 (5.8%)
24–15	307 (0.8%)
≤14	78 (0.2%)
unknown	7629 (20.2%)
coinfections	
HIV+	2095 (5.5%)
HIV−	1311 (3.5%)
HIV unknown	34433 (91.0%)
HBV+	1314 (3.5%)
HBV−	9325 (24.6%)
HBV unknown	27200 (71.9%)

* GT1 comprises genotype 1 samples not classified as 1a or 1b.
** Three samples of GT-P were detected in Munich and seven samples of GT non-1, -2 or -3 in Rostov on the Don.
*** Intravenous drug abusers.

4.1. Geographic distribution of geno/subtypes

The overall proportion of GTs is shown in Fig. 1. The most prevalent was GT1, followed by GT3 and GT4. Three samples classified as the recombinant genotype-P were identified in Munich. Some geographic variations in GT proportions were observed (Table 2). Geographic differences in the proportion of genotypes 1, 1a, and 1b might be the result of differences in the specificity of genotype testing rather than actual differences in the proportions. For example, nearly 30% (51/174) of GT1 measurements from Luxembourg did not differentiate between genotype 1a and 1b, while this was only the case for less than 4% (60/1773) measurements from Portugal.

4.2. Distribution of geno/subtypes related to date of testing and gender

The number of samples and GT proportion per year of testing was homogeneous (Table 3). For 29,561 (78.1%) of the samples gen-

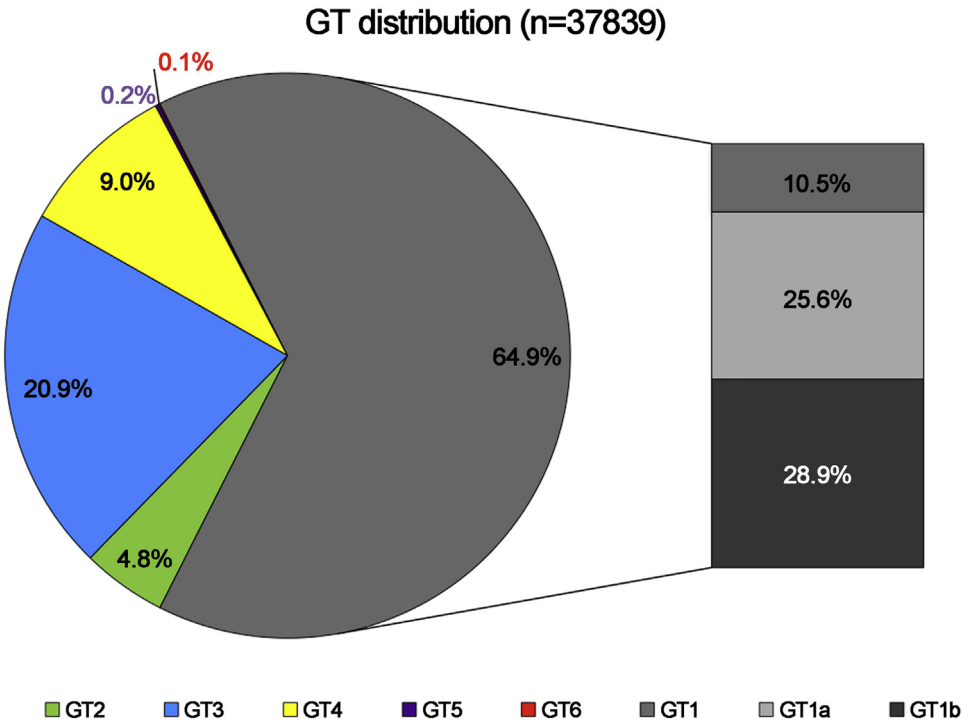

Fig. 1. geno/subtype distribution in the dataset.
GT1 in the pie graph corresponds to all samples classified as GT1.
(*)GT1 in the bar corresponds to all samples not classified as 1a or 1b.

der was reported, whereof 20,282 (68.6%) corresponded to male patients and 9279 (31.4%) to females. GT distribution depended on gender: GTs 1b and 2 were more prevalent in females, while GTs 1a, 3, and 4 were more frequent in males.

4.3. Distribution of geno/subtypes related to patientsí age

The year of birth/age at testing was known for 31,150 (82.3%) patients. GT distribution was highly dependent on patientsí age or date of birth (Table 4), and the pattern was similar in all centers. GT1b associated with births not later than 1958 and GT2 with patients born before 1954. We observed a highly significant association of GT1a in patients born between 1958–1974, and a less significant association in individuals born between 1974 and 1980. The proportion of GT3 infections increased significantly in patients with birth up to 1967. GT4 associated with patients born between 1962 and 1974.

4.4. Distribution of geno/subtypes related to coinfections with HBV/HIV

HBV-status was reported for 10,705 (28.3%) of the patients, whereof 1320 were positive for HBV markers of infection (Table 4). HCV GT distribution was independent of HBV coinfection.

HIV-status was known for 13,622 patients, with 2412 (17.7%) HIV-positive and 11,210 (82.3%) not-infected patients. In HIV-infected patients, the proportion of GTs 1a and 4 infections was significantly higher compared to HIV-negative patients (42.5% vs. 20.6% and 19.3% vs. 9.6%, respectively). This effect was observed in all countries.

The total prevalence of GT3-infection was similar in both HIV-infected and HIV-negative groups.

4.5. Distribution of geno/subtypes related to route of transmission

Data for HCV transmission route/risk group was available in 19 centers, and for 2547 patients, whereof 1525 patients reported more than one possible transmission/risk group (Table 4). For 606 patients transmission was labelled as “unknown” and for 34,686 individuals no transmission/risk group was available.

Parenteral transmission of HCV represented the main known mechanism of viral spread (2283 cases) and revealed a GT distribution very similar to the general pattern.

442 patients infected nosocomially and exhibited a different genotype distribution, with a significant majority of GT1b infections and fewer GTs 1a, 3, and 4.

56 infections occurred through tattoo/piercing, 1194 through drug consume (IVDA) and for 37 cases both routes were reported. For the tattoo/piercing risk group, the only association found was with GT1b. Drug use significantly correlated with a higher abundance of GTs 1a, 3, and 4, while GTs 1b and 2 were more uncommon.

Transmission in the context of sexual practices occurred in 257 patients, whereof 135 were specifically MSM. We observed no association between specific GT and transmissions in the context of sexual practices. However, for MSM with sexual-associated transmission, GT1a (57.0%) and GT4 (29.6%) were present in significantly higher proportions, while GTs 1b and 3 were rarely detected.

4.6. Multivariate dependency analysis

We created eight models to analyze the interdependence of the variables (Supplementary Table 3). These models confirmed that the main transmission route was age-dependent: nosocomial infections were significantly more common in females, older patients (born 1900–1954); drug use and MSM were significantly more common in middle-age male patients (born 1964–1974 and

Table 2
Geno/subtype distribution by country.

Country	N	GT1*	GT1a	GT1b	GT2	GT3	GT4	GT5	GT6	non−1, 2, or 3**
Austria	447	23 (5.1%) [+++]	122 (27.3%)	176 (39.4%)	17 (3.8%) [−]	87 (19.5%)	21 (4.7%) [−]	0 (0%)	1 (0.2%)	0 (0%)
Belgium	781	0 (0%)	153 (19.6%) [−−−]	292 (37.4%) [+++]	58 (7.4%) [+++]	178 (22.8%)	77 (9.9%)	23 (2.9%) [+++]	0 (0%)	0 (0%)
Germany	8332	165 (1.9%) [−−−]	2989 (35.9%) [+++]	2550 (30.6%) [+]	336 (4.0%)	1710 (20.6%) [++]	538 (6.5%) [−−−]	18 (0.2%)	23 (0.3%) [+++]	3 (0%)
Israel	84	0 (0%)	17 (20.2%)	66 (78.6%) [+++]	0 (0%)	0 (0%)	1 (1.2%)	0 (0%)	0 (0%)	0 (0%)
Italy	2592	73 (2.8%) [−−−]	567 (21.8%) [−−−]	970 (37.4%) [+++]	396 (15.4%) [+++]	386 (14.9%) [−−−]	198 (7.6%) [−−]	2 (0.1%)	0 (0%)	0 (0%)
Luxembourg	293	51 (17.4%) [+++]	78 (26.6%)	45 (15.4%) [−−−]	9 (3.1%)	93 (31.7%) [+++]	16 (5.5%)	0 (0%)	1 (0.3%)	0 (0%)
Portugal	2892	60 (2.1%) [−−−]	1267 (43.8%) [+++]	446 (15.4%) [−−−]	44 (1.5%) [−−−]	743 (25.7%) [+++]	326 (11.3%)	5 (0.2%)	1 (0%)	0 (0%)
Russia	4085	982 (24.0%)	289 (7.1%)	849 (20.8%) [−]	576 (14.1%) [+++]	1382 (33.8%)	0 (0%)	0 (0%)	0 (0%)	7 (0.2%) [+++]
Spain	17175	2467 (14.4%) [+++]	3973 (23.1%) [−−−]	5254 (30.6%)	453 (2.6%) [−−−]	2885 (16.8%) [−−−]	2119 (12.4%) [+++]	20 (0.1%)	4 (0%) [−−−]	0 (0%)
UK	1158	39 (3.4%) [+++]	455 (39.3%) [−−−]	184 (15.9%)	63 (5.4%) [+++]	290 (25.0%)	119 (10.3%)	2 (0.2%) [++]	6 (0.5%)	0 (0%)

The total number, percentage (in brackets), and statistical significance (in square brackets) are shown. We considered three levels of significance: significant ($p \leq 0.05$, + or −), very significant ($p \leq 0.01$, ++ or −−), and highly significant ($p \leq 0.001$, +++ or −−−), where "+" signs are used for prevalence above the expected value and "−" for underrepresentation.

* GT1 comprises genotype 1 samples not classified as 1a or 1b.

** Three samples of GT-P were detected in Munich and seven samples of GT non-1, -2 or -3 in Rostov on the Don.

1974–1980, respectively); and infection through tattooing/piercing was significantly increased in male younger patients born after 1980.

HIV-coinfection was more prevalent in Spanish and British patients, but infrequent for Germans, Italians, and Portuguese. It was also overrepresented in MSM.

Country-specific differences in GT distribution were found for HIV-positive patients in Germany: GT4; Italy: GT1a; Spain: GT1a and GT4.

5. Discussion

HCV coinfection is frequent in HIV-infected patients. HIV/HCV coinfection was diagnosed in 18% of our cases where a HIV test had been performed, though HIV/HCV coinfection rates ranging from 15% to 53% have been estimated by others [14,15]. HIV/HCV coinfection is associated with worse disease progression [16,17], and also challenges both HCV and antiretroviral therapy due to strong drug-drug interactions [18].

With the licensing of potent DAAs, the global public health community embraced the possibility of eradicating a virus without a vaccine. However, we now know that some roadblocks stand in the way, and one major obstruction is the genetic variability of the virus. HCV is classified into seven genotypes and 87 subtypes. Currently, GT is the only factor accounting for DAA eligibility. In addition, need for the adjuvants ribavirin or interferon, duration of treatment, DAA-resistance (prevalence of baseline mutations, development under treatment and persistence after treatment), as well as cure rates still remain highly dependent on HCV genotype and subtype [19–24]. In spite of its relevance, the contemporary global distribution of genotypes is not fully characterized. A number of previous literature revisions have reported global prevalence of HCV GTs [9–13]. They may have a biased GTs inclusion, such as higher proportion of GT1, because of its higher susceptibility to DAAs, or GTs more prevalent in the past (1b or 2a) since those patients are in higher need of treatment, while GTs becoming more prevalent in the very last years (GT4) may be underrepresented.

GT1 has been reported to account for the vast majority of HCV infections in Western Europe, with prevalences ranging 75–90% [9,12,25], and indeed only DAAs eligible for treatment of GT1 viruses have reached the market. In our study, GT1 accounted only for 66% of the infections in Western Europe and Israel. We could confirm that the subtypes 1a/1b ratio was dependent on patient age and transmission route [11,26–29].

GT3 is the second most prevalent GT in Western Europe in our and other studies [9–13], representing 20% to 28% of the infections. It is also one of the most challenging GT for therapy, as only sofosbuvir and daclatasvir are licensed for its treatment, and this GT associated with faster rates of fibrosis progression and higher prevalence of severe steatosis and hepatocellular carcinoma. GT3 has been diagnosed more frequently in drug consumers in certain areas [26,27]. Our Study has shown that this association occurs in most West European countries.

GT2 has been considered the third most frequent genotype with prevalences ranging from 8% to 11% [9,11–13]. However, GT2 was more infrequent in our dataset: 4.5% in total or 4.1% when excluding Russia. It significantly associated to females, nosocomial infection and was mostly detected in older patients. Higher proportions of GT2 were only found in Russia, more in accordance to the Asian GT distribution, and in Italy [30,31]. GT2c was probably introduced in

Table 3
xxx.

	N	GT1*	GT1a	GT1b	GT2	GT3	GT4	GT5	GT6	Non−1, −2, or −3**
Year										
2011	7971	1015 (12.7%)	1853 (23.2%)	2230 (28.0%)	383 (4.8%)	1713 (21.5%)	750 (9.4%)	18 (0.2%)	8 (0.1%)	0 (0%)
2012	7834	833 (10.6%)	1946 (24.8%)	2401 (30.6%)	339 (4.3%)	1550 (19.8%)	746 (9.5%)	12 (0.2%)	4 (0.1%)	3 (0%)
2013	8410	1027 (12.2%)	2112 (25.1%)	2254 (26.8%)	431 (5.1%)	1853 (22.0%)	710 (8.4%)	13 (0.2%)	9 (0.1%)	0 (0%)
2014	8665	861 (9.9%)	2399 (27.7%)	2366 (27.3%)	394 (4.5%)	1814 (20.9%)	794 (9.2%)	20 (0.2%)	13 (0.2%)	0 (0%)
2015	4533	223 (4.9%)	1359 (30%)	1464 (32.3%)	239 (5.3%)	824 (18.2%)	414 (9.1%)	7 (0.2%)	2 (0%)	0 (0%)
2010–2015***	426	0 (0%)	5 (1.2%)	209 (49.1%)	46 (10.8%)	166 (39%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
gender										
male	20282	1753 (8.7%) [−−−]	6749 (33.3%) [+++]	4489 (22.1%) [−−−]	673 (3.3%) [−−−]	4259 (21.0%) [+++]	2298 (11.3%) [+++]	36 (0.2%)	22 (0.1%)	3 (0.0%)
female	9279	1064 (11.5%) [+++]	1839 (19.8%) [−−−]	3694 (39.8%) [+++]	584 (6.3%) [+++]	1384 (14.9%) [−−−]	673 (7.3%) [−−−]	27 (0.3%)	14 (0.2%)	0 (0%)
unknown	8278	1142 (13.8%)	1086 (13.1%)	2741 (33.1%)	575 (6.9%)	2277 (27.5%)	443 (5.4%)	7 (0.1%)	0 (0%)	7 (0.1%)

The total number, percentage (in brackets), and statistical significance (in square brackets) are shown. We considered three levels of significance: significant ($p \leq 0.05$, + or −), very significant ($p \leq 0.01$, ++ or −−), and highly significant ($p \leq 0.001$, +++ or −−−), where "+" signs are used for prevalence above the expected value and "−" for underrepresentation.

* GT1 comprises genotype 1 samples not classified as 1a or 1b.

** Three samples of GT-P were detected in Munich and seven samples of GT non-1, -2 or -3 in Rostov on the Don.

*** 426 samples from Rostov-on-the-Don were analysed between 2010 and 2015 but no specific year was reported.

Italy as a result of population movements during Italian colonialism at the end of the 19th century, and it did not spread there through intravenous drug use [32].

GT4 has been traditionally associated to Central Africa and the Middle East, with a West European proportion of around 5% [9,10,12], with the exception of Spain with a prevalence of 8–11% [12,13,25]. Our data indicate that GT4 proportion in Belgium, Portugal, and UK is similar to Spain, and in Italy and Germany is about 7%. Overall, the West Europe prevalence was calculated as 9%. We observed that GT4 transmission is related to sexual practices, especially in MSM, and in HIV-coinfected patients [13,28]. A detailed study by de Bruijne and colleagues has shown that GT4 infections may be the consequence of three concomitant processes: increase in immigration from Northern and Central Africa, the use of drugs, and the introduction of GT4d viruses into European networks of MSM and injection drug users. This work identified three GT4 clusters in the current Dutch epidemiology: (i) GT4a-infected Egyptian immigrants; (ii) GT4d-infected Dutch IVDA patients; and (iii) HIV-positive MSM with GT4d [33]. Further studies at molecular level are required to clarify the origin of the increased proportion of GT4 in other European countries.

GTs 5 and 6 were detected in extremely low frequencies and no association with independent epidemiological parameters was found. However, new recombinant forms such as GT P, first isolated in St. Petersburg (Russia) [34], are being detected in Germany. In this GT the 5′ region up to the first part of NS2 corresponds to the subtype 2k, while the rest of the genome corresponds to 1b. Presently, HCV genotyping does not take into consideration recombination at all as most methods are based on amplification of single subgenomic fragments [3–7]. Our genotype P samples were identified by genotyping based on regions located in on the 5′ genomic regions followed by resistance testing of the DAA-target proteins. Our results suggest that recombinant genotypes we are unaware of may be spreading. Their role in the HCV epidemiology and their response to treatments is to date fully unexplored. Future studies are required to scrutiny the existence or spreading of recombinant GTs. Should the existence of recombinants be demonstrated, the HCV genotyping commercial kits and in-house protocols will have to be improved.

Our study has detected significant associations of GTs and epidemiological parameters using more than clinical 37,000 samples. However, it also shows that epidemiological data collection outside clinical studies is poor. Transmission route/risk group was documented only for 6.5% of the patients. Clear subtype classification was not available for 40% of the samples impeding a reliable subtype distribution analysis in GTs 2–6, and "unresolved" or "mixed" infections (signals corresponding to more than one GT in commercial kits) were not always clearly separated from double infections (unmistakable co-existence of two or more HCV strains). Therefore, additional studies are required to analyze whether our observations concur to other centers in West Europe. Epidemiological studies are important to identify the extent of current difficult-to-treat collectives such as GT3-infected or HIV co-infected patients in order to optimize vaccine and drug design as well as therapy policies.

In summary, the current HCV genotype distribution is a dynamic process influenced by traditional genotype prevalence and evolving transmission trends. The early-nineties epidemics of GTs 1b and 2a spread by nosocomial transmission have been replaced by a scenario of GTs 1a, 3a and 4 where IVDA and high-risk sexual practices are the main risk factor for HCV (and HIV) transmission [29,35–37]. However, other issues may also shape epidemiology within the next years. The role of past and current immigration, increase in sex/drug consume-tourism, infections in homeless people and prisoners, HCV re-infections rates in IVDA, generation of new (recombinant) GTs, as well as selection of certain GTs by the current DAAs should be clearly elucidated [25,33,34,38–41]. More regional but very detailed studies have shown the importance of accurate risk group assessment and viral subtype determination, but furthermore the utility of sequencing/phylogenetic analysis for deeper insights in the spread of HCV [22,29,32,33].

Table 4
Geno/subtype distribution by age at testing, viral coinfection and transmission route/risk group.

	N	GT1*	GT1a	GT1b	GT2	GT3	GT4	GT5	GT6	Non−1, −2, or −3**
Age at testing										
≥65	4910	584 (11.9%)	476 (9.7%)	2954 (60.2%)	463 (9.4%)	222 (4.5%)	165 (3.4%)	41 (0.8%)	5 (0.1%)	0 (0%)
64–55	6473	531 (8.2%)	1823 (28.2%)	2228 (34.4%)	311 (4.8%)	1065 (16.5%)	493 (7.6%)	11 (0.2%)	10 (0.2%)	1 (0%)
54–45	10922	852 (7.8%)	3805 (34.8%)	2170 (19.9%)	295 (2.7%)	2362 (21.6%)	1422 (13.0%)	8 (0.1%)	8 (0.1%)	0 (0%)
44–35	6165	584 (9.5%)	1994 (32.3%)	1145 (18.6%)	181 (2.9%)	1501 (24.3%)	747 (12.1%)	4 (0.1%)	7 (0.1%)	2 (0%)
34–25	2287	243 (10.6%)	701 (30.7%)	463 (20.2%)	61 (2.7%)	609 (26.6%)	203 (8.9%)	1 (0%)	6 (0.3%)	0 (0%)
24–15	316	48 (15.2%)	85 (26.9%)	68 (21.5%)	9 (2.8%)	77 (24.4%)	27 (8.5%)	2 (0.6%)	0 (0%)	0 (0%)
≤14	80	12.0 (15%)	21 (26.3%)	17 (21.3%)	3 (3.8%)	15 (18.8%)	11 (13.8%)	1 (1.3%)	0 (0%)	0 (0%)
unknown	6686	1105 (16.5%)	769 (11.5%)	1879 (28.1%)	509 (7.6%)	2069 (30.9%)	346 (5.2%)	2 (0%)	0 (0%)	7 (0.1%)
Viral coinfec-tions										
HIV positive	2412	118 (4.9%) [−−−]	1026 (42.5%) [+++]	346 (14.3%) [−−−]	41 (1.7%) [−−−]	414 (17.2%)	465 (19.3%) [+++]	0 (0%)	2 (0.1%)	0 (0%)
HIV negative	11210	2219 (19.8%) [+++]	2308 (20.6%) [−−−]	3103 (27.7%) [+++]	421 (3.8%) [+++]	2058 (18.4%)	1080 (9.6%) [−−−]	15 (0.1%)	6 (0%)	1 (0%)
HIV unknown	24217	1622 (6.7%)	6340 (26.2%)	7475 (30.9%)	1370 (5.7%)	5448 (22.5%)	1869 (7.7%)	55 (0.2%)	28 (0.1%)	0 (0%)
HBV positive	1320	83 (6.3%) [−−−]	416 (31.5%)	360 (27.3%)	44 (3.3%)	259 (19.6%)	152 (11.5%)	2 (0.2%)	4 (0.3%)	2 (0%)
HBV negative	9385	2000 (21.3%) [+++]	2191 (23.3%)	2121 (22.6%)	360 (3.8%)	1688 (18.0%)	1003 (10.7%)	15 (0.2%)	7 (0.1%)	0 (0%)
HBV unknown	27134	1876 (6.9%)	7067 (26.0%)	8443 (31.1%)	1428 (5.3%)	5973 (22%)	2259 (8.3%)	53 (0.2%)	25 (0.1%)	10 (0.1%)
Transmission route/risk group										
Parenteral	2283	215 (9.4%)	691 (30.3%)	513 (22.5%) [−−−]	66 (2.9%) [−]	526 (23.0%) [++]	270 (11.8%)	2 (0.1%)	0 (0%)	0 (0%)
vertical	13	0 (0%)	3 (23.1%)	7 (53.8%)	0 (0%)	1 (7.7%)	2 (15.4%)	0 (0%)	0 (0%)	0 (0%)
nosocomial	442	62 (14.0%) [+]	54 (12.2%) [−−−]	239 (54.1%) [+++]	24 (5.4%)	41 (9.3%) [−−−]	21 (4.8%) [−−]	1 (0.2%)	0 (0%)	0 (0%)
tattoo/piercing	93	15 (16.1%)	28 (30.1%)	12 (12.9%)	4 (4.3%) [−]	28 (30.1%)	6 (6.5%)	0 (0%)	0 (0%)	0 (0%)
IVDA	1231	132 (10.7%)	431 (35.0%) [+++]	130 (10.6%) [−−−]	27 (2.2%) [−−]	346 (28.1%) [+++]	164 (13.3%) [+++]	1 (0.1%)	0 (0%)	0 (0%)
sexual	257	15 (5.9%)	111 (43.2%)	36 (14.0%)	6 (2.3%)	36 (14.0%)	52 (20.2%)	0 (0%)	1 (0.4%)	0 (0%)
unknown	34685	3728 (10.7%) [−]	8729 (25.2%) [+++]	10154 (29.3%) [−]	1680 (4.8%)	7238 (20.9%) [−]	3043 (8.8%) [+++]	68 (0.2%)	35 (0.1%)	0 (0%)

The total number, percentage (in brackets), and statistical significance (in square brackets) are shown. We considered three levels of significance: significant ($p \leq 0.05$, +or −), very significant ($p \leq 0.01$, ++ or −−), and highly significant ($p \leq 0.001$, +++ or −−−), where "+" signs are used for prevalence above the expected value and "−" for underrepresentation.

* GT1 comprises genotype 1 samples not classified as 1a or 1b.

** Three samples of GT-P were detected in Munich and seven samples of GT non-1, -2 or -3 in Rostov on the Don.

This work is the first attempt for the establishment of a laboratory network for real-time collection of European HCV data to provide reliable information about the current GT prevalence situation, and it is also a call to join efforts and encourage further observational studies of HCV GT prevalence at supra-national level to gain reliable knowledge on HCV epidemiology.

Conflicts of interest

None declared.

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Ethical approval

Not required, as this is an anonymised, retrospective and non-interventional study.

Contributors

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HCV EuResist Study group

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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.jcv.2016.05.010.

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