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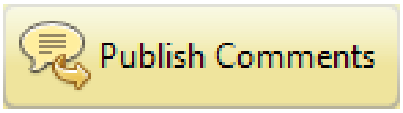

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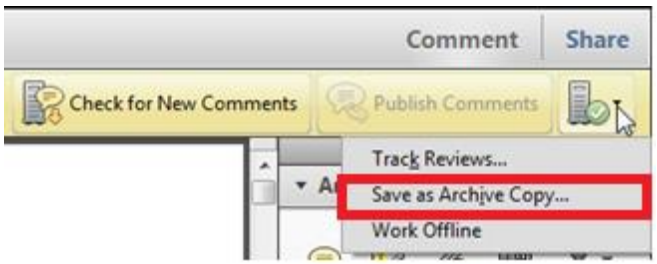

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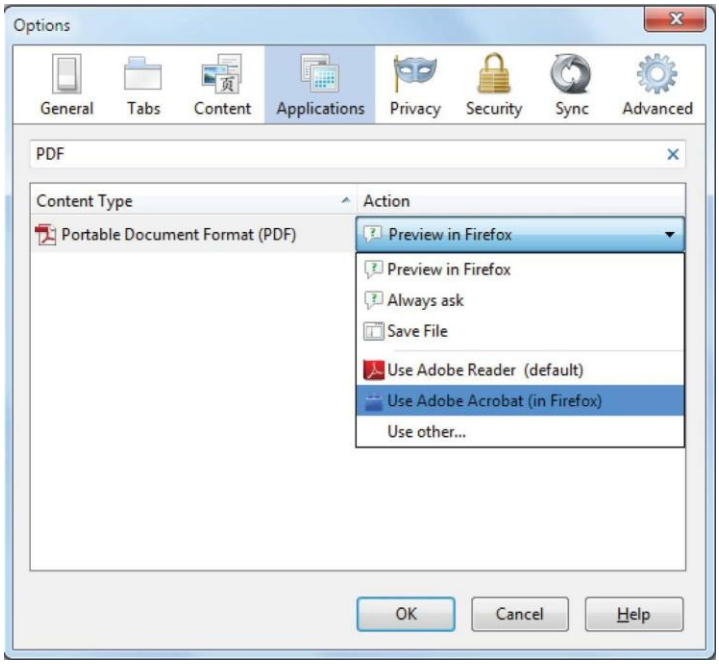

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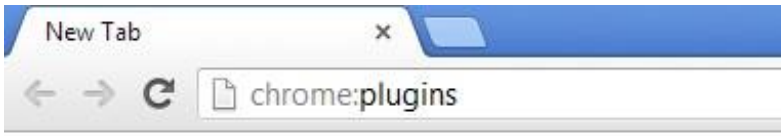

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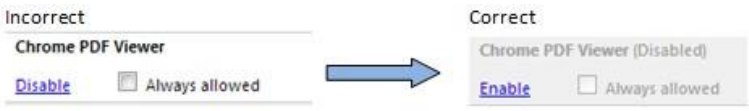

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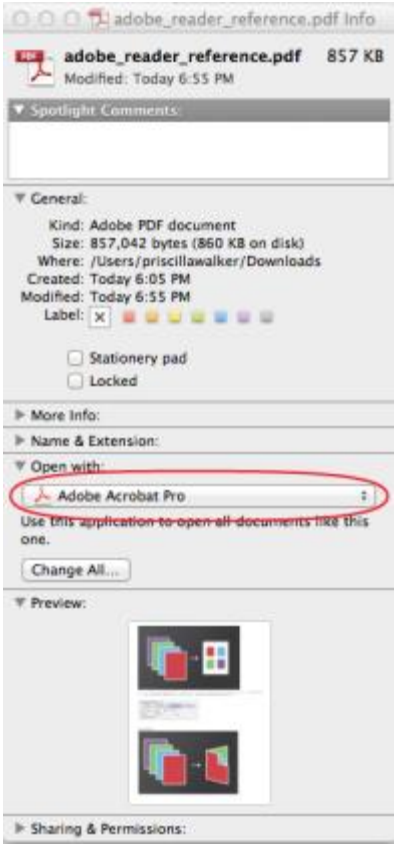

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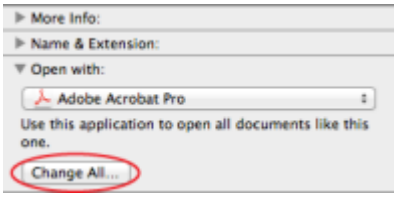

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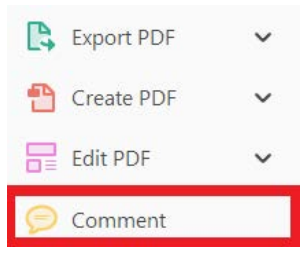

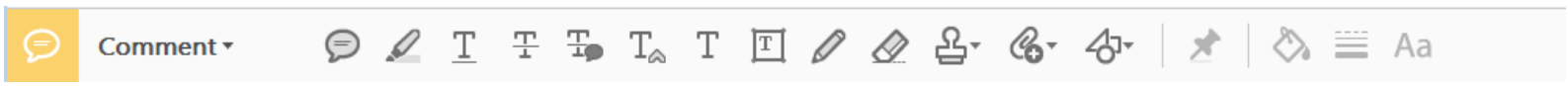

1. Replace (Ins) Tool – for replacing text.

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- Highlight a word or sentence.
- Click on .
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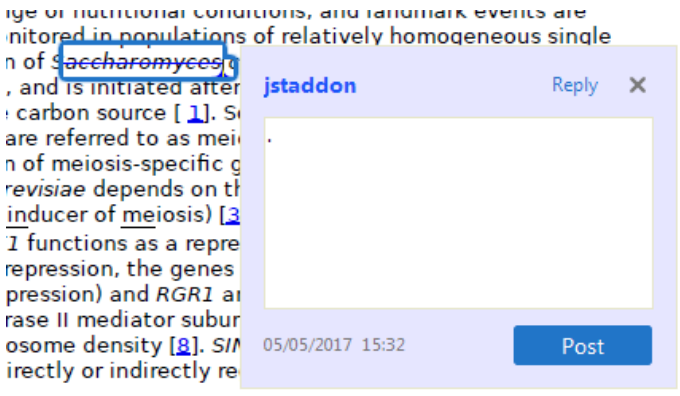

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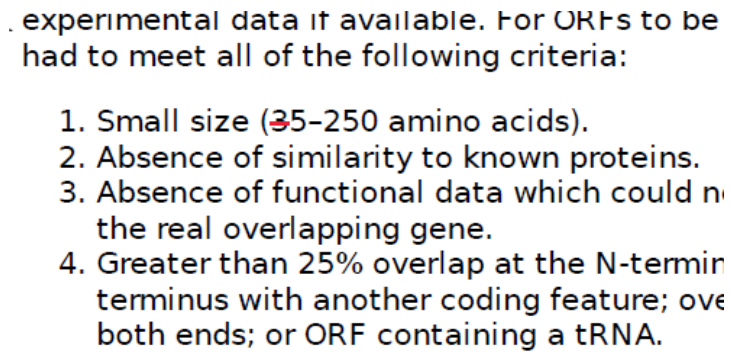
experimental data if available. For ORFs to be had to meet all of the following criteria:

1. Small size (~~3~~5–250 amino acids).
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3. Absence of functional data which could n the real overlapping gene.
4. Greater than 25% overlap at the N-termir terminus with another coding feature; ove both ends; or ORF containing a tRNA.

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Use these 2 tools to highlight the text where a comment is then made.

How to use it:

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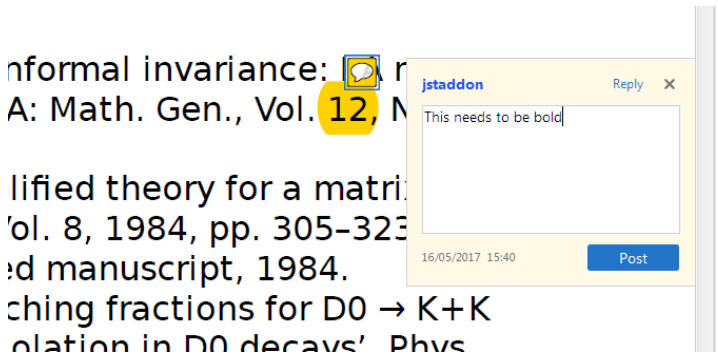
- Type any instructions regarding the text to be altered into the box that appears.



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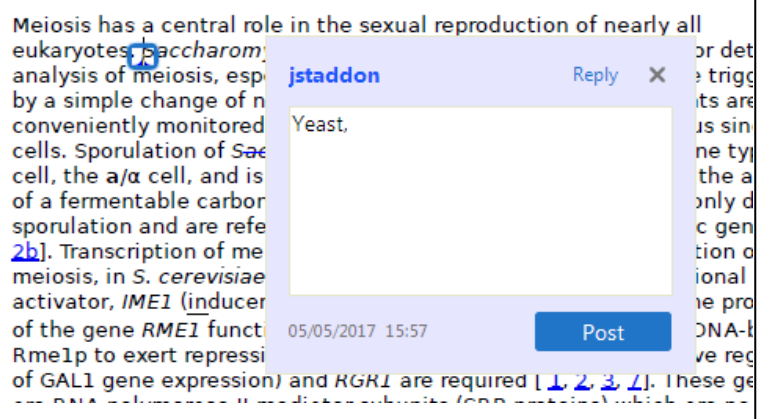
- Type the comment into the box that appears.



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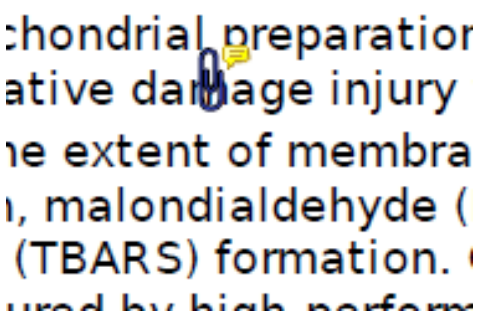

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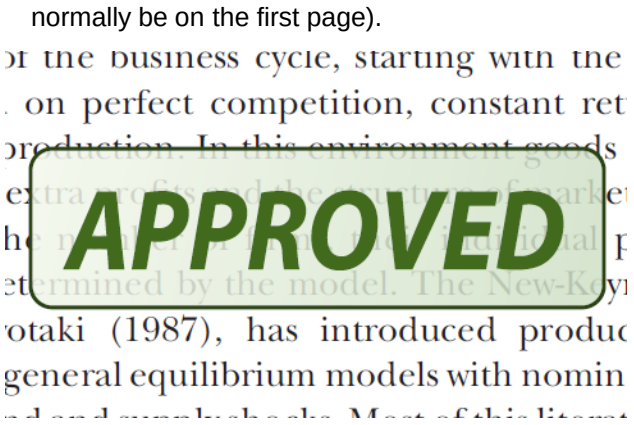

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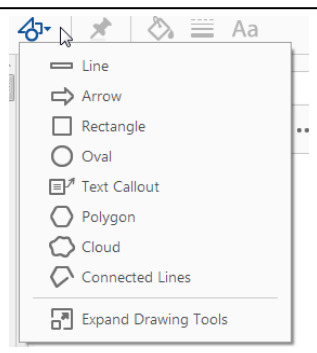

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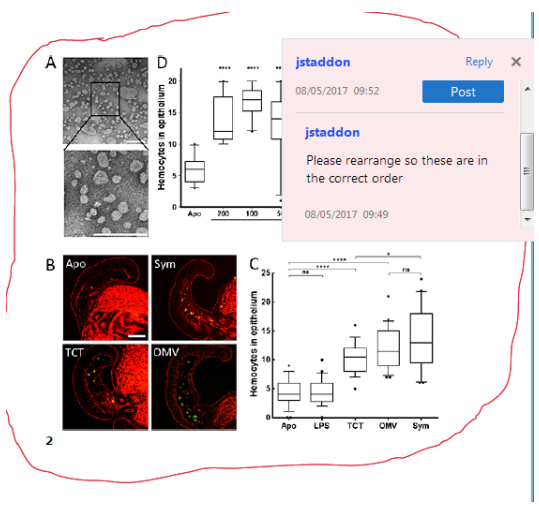

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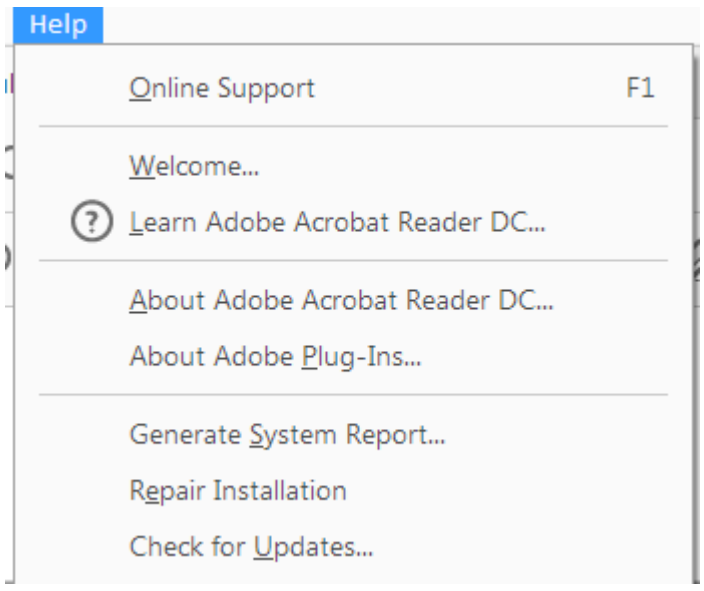

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REVIEW

WILEY JOURNAL OF MEDICAL VIROLOGY

What is changed in HBV molecular epidemiology in Italy?

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Hepatitis B virus (HBV) infection represents the most common cause of chronic liver diseases worldwide. Consequently, to the introduction of the universal HBV vaccination program, the prevalence of hepatitis B surface antigen was markedly reduced and less than 1% of the population of Western Europe and North America is chronically infected. To date, despite great advances in therapeutics, HBV chronic infection is considered an incurable disease. Ten hepatitis B virus genotypes (A-J) and several subgenotypes have been identified so far, based on intergroup divergences of 8% and 4%, respectively, in the complete viral genome. HBV-D genotype has been found throughout the world, with highest prevalence in the Mediterranean area. In the present review, several articles concerning HBV epidemiology, and phylogeny in Italy have been analyzed, mainly focusing on the changes occurred in the last decade.

KEYWORDS
epidemiology, evolution, hepatitis B virus

1 | INTRODUCTION

Hepatitis B virus (HBV) infection is a global public health problem causing chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC).[1–3]

To date, 10 HBV genotypes have been identified, from A to J, based on a genetic diversity of at least 8% in the viral genome.[4,5] These genotypes have a distinct geographic distribution worldwide (http://www.who.int/csr/disease/hepatitis/HepatitisB_whocdscsrlyo2002_2.pdf). HBV-genotype A predominates in Northern Europe and North America, genotypes B and C in central Asia, genotype D in Mediterranean countries, genotype E in sub-Saharan Africa and in Madagascar, genotype F in South and Central America, genotypes G and H in Mexico and some other countries in Central America[6,7] and genotypes I and J in Eastern Asia.[8] In particular, genotypes B and C prevails in highly endemic countries where perinatal HBV transmission plays an important role, whereas genotype A is frequent in areas with primarily horizontal transmission (ie, sexual contact).[4,9] All genotypes are present in countries of immigration with frequencies depending on the ethnicity.[10,11]

1.1 | brief summary on the usefulness of serum makers during HBV infection

Presence of hepatitis B surface antigen (HBsAg) in serum indicates an ongoing HBV infection, acute, or chronic. The serum titre of anti-HBc IgM (antibody to Hepatitis B core antigen-HBcAg) may help to diagnose between acute and chronic HBV replication, high titres indicating a recent active viral replication, and low titres a chronic lower HBV production.

The titre of serum HBV DNA measures the entity of viral replication (HBV serum load) which is currently considered useful for

Alessia Lai and Caterina Sagnelli contributed equally to the study.

therapeutic choices. The presence of hepatitis B e antigen (HBeAg) in serum and the detection of HBsAg serum titre have been used as surrogate markers of ongoing HBV replication in several studies, since the HBeAg is detectable only in patients with an active HBV replication and the HBsAg titre correlate with the HBV load. In patients with HBV acute hepatitis, the persistence of HBeAg in serum for more than 10 weeks is associated with a high likelihood of transition to chronicity, as does the persistence of HBV DNA and HBsAg for more than 6 months. Antibodies to HBeAg (anti-HBe) or IgG to HBcAg (anti-HBc IgG) are present in subjects with present or past HBV infection, whereas antibody to HBsAg (anti-HBs) indicates past HBV infection and protection to HBV reinfection. Anti-HBs and anti-HBc are frequently used in epidemiological surveys.

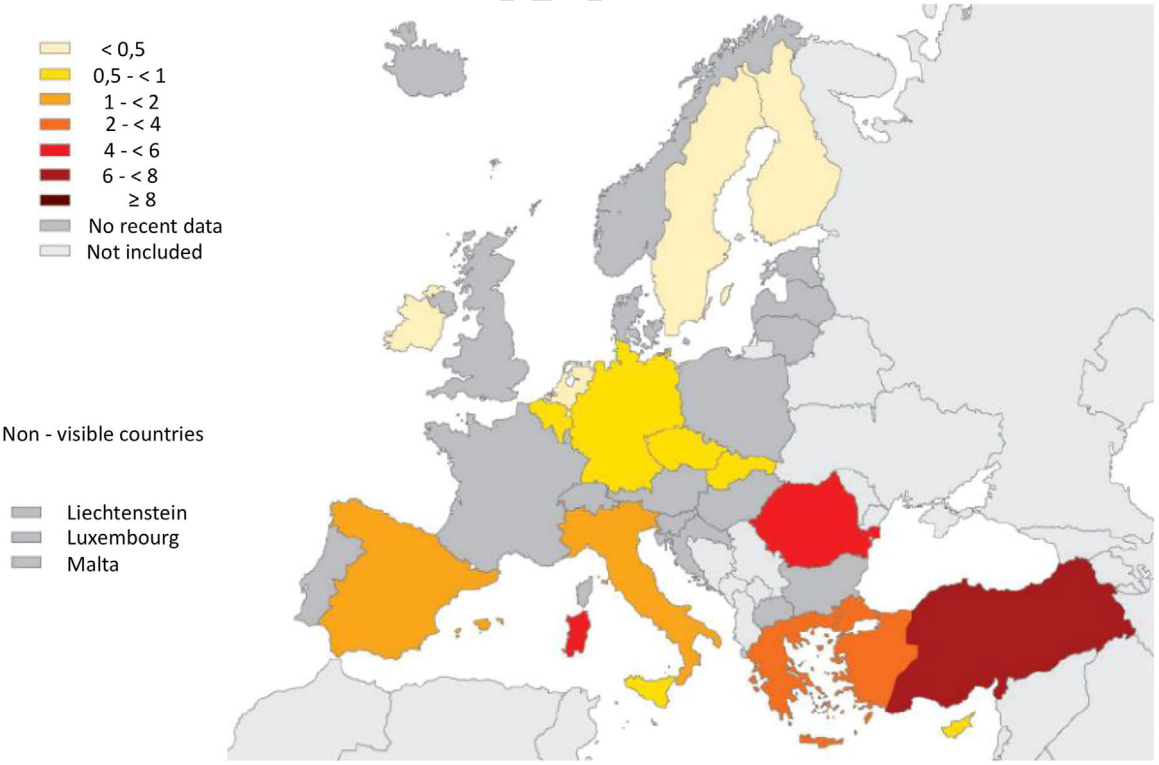
1.2 | HBV transmission and worldwide epidemiology

In 2015, the World Health Organization (WHO) estimated that about 257 million people were living with chronic HBV infection and about 887 000 died of associated liver diseases (http://apps.who.int/iris/bitstream/10665/255016/1/9789241565455-eng.pdf?ua=1). HBV spread affects mostly African and Western Pacific Regions whit a high endemicity level. Worthy of mention, nearly 7.4% of the 36.7 million people living with HIV are also coinfected with HBV (http://apps.who.int/iris/bitstream/10665/255016/1/9789241565455-eng.pdf?ua=1).

HBV circulates in peripheral blood of infected subjects and any parenteral or mucosal exposure to potentially infected blood or blood contaminated material should be considered a risk factor for HBV transmission to non-immune/non-infected subjects.[12,13] The virus is present at infectious concentrations in semen and cervical secretions, and is frequently transmitted by sexual and vertical routes.

The impact of the routes of transmission varies significantly from one country to another.[14,15] In countries with a high endemicity level, HBV infection is prevalently acquired at birth from a HBeAg-positive mother, becoming chronic in around 90% of cases, or by horizontal transmission in early childhood through household contact (most frequently between siblings), with a progression to chronicity from 10% to 40% of cases. Promiscuous unprotected sexual activity and intravenous drug use (IVDU) are the major risk factors for acquiring HBV infection in the adulthood in areas with a low endemicity, with a progression to chronicity from 2% to 5%. Worthy of note, the screening of blood donors for markers of HBV infection has dramatically reduced the risk of HBV transmission through the transfusions of blood and blood products. This risk is currently estimated as 1-4 cases per million blood components transfused in low-endemicity areas[16] and around 1 case per 20 000 blood transfusions in high-endemicity regions.[17]

The prevalence of HBsAg chronic carriers in the general population ranges from 0.1% to 7% in European countries), prevalence estimates that could be considered representative for the entire country for Belgium, Czech Republic, Denmark, Finland, Germany, Greece, Ireland, Italy, Netherlands, Romania, Slovakia, and Turkey (Figure 1 and Table 1). The HBsAg prevalence is lower than 1% in France, Hungary, Italy, the



COLOR

FIGURE 1 HBV prevalence in the general population according to WHO estimates

TABLE 1 Number of estimates for the prevalence of hepatitis B (HBsAg)

Country[a]	Number
Belgium	2
Bulgaria	1
Cyprus	2
Czech Republic	1
Denmark	4
Finland	1
France	1
Germany	6
Greece	9
Ireland	2
Italy	20
Lithuania	1
Netherlands	4
Poland	1
Romania	1
Slovakia	2
Spain	3
Sweden	1
Switzerland	1
Turkey	10
United Kingdom	4

[a]HBV: No recent data for Austria, Croatia, Estonia, the former Yugoslav Republic of Macedonia, Hungary, Iceland, Latvia, Liechtenstein, Luxembourg, Malta, Norway, Portugal, Slovenia.

Netherlands, Portugal, Spain, and the UK, between 1% and 5% in Turkey, Romania, and Serbia, and over 5% in Albania and Iran. Policies addressed for HBV diagnosis and treatment vary significantly across European countries. Ozaras et al[18] analyzed the differences existing among different medical facilities across Europe. Most of high-income and middle-income countries have no restrictions regarding HBV diagnostics, prophylaxis, drug availability and reimbursement, whereas Albania, Iran and Serbia have some restrictions.

Vaccination is the most effective measure to reduce the global incidence of hepatitis B. In 1991, the WHO recommended the introduction of the policy of universal hepatitis B vaccination to prevent and control HBV infection in all countries. Before universal vaccination, most of HBV chronic infection were acquired within the 5th year of age (Beasley Rp Fau—Trepo, #2) and the worldwide prevalence of HBsAg chronic carriers in this age group was estimated around 4.7%. Consequently, in several countries from the 1980s to the early 2000s, the universal HBV vaccination for all newborn babies was introduced and the prevalence of HBV infection in this age group was estimated around 1.3% in 2015, when the global coverage of HBV vaccination reached the 84% with some regional differences. In the African, Eastern Mediterranean and Eastern European regions, the coverage persists below the global rate.

The epidemiology of HBV infection is changing worldwide, due to the impact of universal infant vaccination programs[19,20] and to the migratory flows from countries with intermediate or high endemicity to those with a low endemicity.[21,22]

2 | HBV EPIDEMIOLOGY IN ITALY

2.1 | Studies on acute HBV infection

After a substantial improvement in HBV endemicity over the last few decades, Italy is currently a country at a low endemicity. According to the Acute Hepatitis National Surveillance System (SEIEVA), the incidence of acute hepatitis B (AHB) decreased from 12 per 100 000 in 1985 to 1.0 per 100 000 in 2011, and to 0.6 per 100 000 in 2016 as a consequence of the universal HBV vaccination introduced in 1991 for all newborns and for the12-year-old adolescents since 2004.

2.2 | Studies on chronic HBV infection

At the end of the 70s', the prevalence of HBsAg chronic carriers in the general population was estimated over 3%[23,24] with an increasing gradient from northern to southern Italy where this rate was close to 5%,[25,26] and the proportion of HBeAg-positive cases among HBsAg chronic carriers reached 40%.[12] HBV genotype D was responsible for nearly 90% of cases with chronic HBV infection, independently from the disease progression[27]; however, only few studies investigated on the viral strains circulating in recent years.[28] An impressive reduction in the rate of HBsAg-positive children and teenagers was documented at the end of the 80', due to an improvement in socio-economic conditions, a decrease in the family size, and the abolition of re-usable glass syringes. To date, the rate of HBsAg positive subjects in the open population is estimated between 0.8% and 1%. Studies on the percentage of HBsAg-positive cases among patients with chronic liver disease of different etiologies confirmed this progressive decrease: from the 61% in 1975[17] to 44% in 1980, 34% in 1989,[29] and 12.2% in 2001.[30–32] The clinical impact of universal HBV vaccination on this setting of patients has been recently assessed[33]: the rate of HBsAg inactive carriers decreased from 20.0% in 2001 to 3.3% in 2014, while that of cirrhotic patients increased from 22.6% to 33%.

Romanò et al[34] evaluated the sero-prevalence of markers of HBV infection in of 31 190 blood donors recruited in Italy between April 2004-March 2005 from 21 blood banks selected to achieve a representative distribution of the 300 000 national blood donors population. Authors found 102 (0.33%) subjects positive for HBsAg and 2.593 (8.3%) for anti-HBc-positive.

In a multicenter study[35] on inmates of several Italian prisons published in 2013 the participation to the screening reached the 65.3% and 4.4% of the 2265 subjects tested were HBsAg-positive.[35]

2.3 | Studies on HBV vaccination

Recent studies focused on the impact of universal hepatitis B vaccination on HBV epidemiology. Campagna et al[36] demonstrated

the high efficacy of the universal vaccination in Italy, as almost all the 806 individuals investigated showed anti-HBs protective titers over 10 years after HBV vaccination.

The comparison of two epidemiological survey performed in a town of southern Italy in 1996 and 2012 showed an overtime reduction of HBsAg rate from 0.8% to 0.6%[37] and of anti-HBc from 21.5% to 15.2%; no subject aged less than 30 years showed serum markers of HBV infection.

Of 1704 students attending the school of medicine of the Second University of Naples from September 2012 to December 2013, 588 had been vaccinated in infancy and 1116 when 12 years old. All vaccinated subjects were HBsAg/anti-HBc negative, 15.8% with an anti-HBs titer between 1 and 9 IU/L, 57.9% between 10 and 400 IU/L and 26.3% over 400 IU/L. A multivariate logistic regression identified the age at vaccination as the only factor independently associated with an anti-HBs titer <10 IU/L.[38]

Less effective were the HBV vaccination campaigns for categories of workers at risk of acquiring HBV infection. Worthy of note is a study on healthcare workers (HCWs) where only 34.8% of HCWs completed the vaccination schedule with a 64.7% protective anti-HBs response three months after vaccination.[39] In this study, an inadequate vaccination schedule resulted the strongest predictor of antibody loss during a long-term follow up.

2.4 | Studies on HBV infection in migrant population

El Hamad et al[40] performed a longitudinal prospective study on prevalence and risk factors for HBV infection in 3.728 mainly undocumented migrants from Non-European countries observed in Northern Italy from January 2006 to April 2010: 47% were male, the mean age was 31.3 years, 12.4% were from North Africa, 44% from Eastern Europe, 21.4% from Sub-Saharan Africa, 16.8% from Asia (of whom 37% were from China), and 5.4% from Central or South America. The rate of HBsAg positivity was 6%, independently associated with the prevalence of HBsAg carriers in the geographical area of origin. The distribution of HBV genotypes reflected that of the geographic area of provenance.

Coppola et al[3] performed a longitudinal prospective study on undocumented migrants and refugees living in southern Italy from January 2012 to June 2013. Of the 882 individuals enrolled, 9% were HBsAg positive and 47% of the HBsAg negative patients were anti-HBc positive. The rate of HBsAg-positivity was high (14%) in the 444 individuals from sub-Saharan Africa and intermediate in those from Eastern Europe (6% of 198), northern Africa (2% of 80) and Indo-Pakistan subcontinent (3% of 126).

Zermiani et al[41] found a prevalence of 3.5% for HBsAg and 40.6% for anti-HBc in migrant female sex workers in Verona, Northern Italy during the 1999-2007 period, without significant difference between women from Africa and those from Eastern Europe.

An observational study performed by Laganà et al[42] between January 2003 and December 2013 detected HBV infection in 9 (2.8%) of 320 migrant women attending as outpatients at a clinical center in Messina (Sicily), of whom only 10 (3.1%) had been immunized against HBV.

A cross-sectional study[43] performed from February to July 2008, investigated 3.760 HBV chronic carriers, of whom 932 migrants born outside Italy. Areas of origin were Far East (37.1%), Eastern Europe (35.4%), Sub-Saharan Africa (17.5%), North Africa (5.5%), and other sites in 4.5 %. Genotype D, was detected in 87 % of subjects born in Italy and in 40 % of migrants. The latter compared with the Italian subjects more frequently were inactive HBV carriers and less frequently had chronic hepatitis, cirrhosis and HCC.

2.5 | Studies on HBV molecular epidemiology

Because of the uninterrupted flux of migrants to Italy over the last two decades, about 10% of the resident population are immigrants predominantly from sub-Saharan Africa and EasternEurope. This has influenced the molecular epidemiology of acute hepatitis B in Italy, where HBV genotype D had been responsible of 95% of cases of AHB for decades.

In 2012, Ferraro et al[44] reported a high rate of HBV genotype non-D isolated from new cases of AHB in Sicilian patients infected mainly through unsafe sex, 44% with HBV-genotype A and 3% with genotype E (3%).

In 2015, Zuccaro et al[45] reported that half of 103 consecutive patients with AHB showed HBV genotype non-D, mainly A and F, reflecting the introduction of "new" genotypes from other countries. The unsafe sexual exposure was the most frequently risk factor, significantly associated with non-D genotypes.

Sagnelli et al[46] investigated for genetic diversity 53 HBsAg chronic carriers with HBV-genotype E living in Southern Italy from a median period of 33 months, 47 (88.7%) from different countries in sub-Saharan Africa, four (7.5%) from Eastern Europe (Albania, Romania, Russia, Ukraine), one (1.9%) from Central America (Dominican Republic), and one (1.9%) from Sri Lanka, indicating that HBV-genotype E has been spread from central/western sub Saharan Africa to several other countries.

Sticchi et al[47] evaluated the prevalence of HBV-S-gene escape mutants in Italy. Of 256 consecutive HBsAg positive serum samples collected from 2007 to 2011 in northern Italy, eight harbored HBsAg mutations, with a prevalence of 3.1%. The mutation G145R was found alone in five (62.5%) and accompanied by other mutations in the remaining three (37.5%).

2.6 | HBV infection in onco-hematologic diseases

Some Italian papers evaluated the clinical relevance of HBV infection in patients with onco-hematologic diseases.

Taborelli et al[24] described the potential association between chronic HBV infection and non-Hodgkin lymphoma in a case-control study performed in Italy from 1999 to 2014; 571 incident cases of histological confirmed NHLs and 1004 of cancer-free matched controls had been enrolled. The prevalence of HBsAg seropositivity was 3.7% in NHL cases and 1.7 % in controls, leading to an OR of 1.95 (95%CI:1.00-3.81).

Castelli et al[26] evaluated 85 newly diagnosed non-Hodgkin lymphomas (NHL) patients with resolved HBV infection receiving

Rituximab-containing chemotherapies, all under lamivudine prophylaxis. The determination of HBV DNA viral load at baseline and then every 4 weeks for 1 year after completion of the Rituximab containing regimens detected HBV reactivation in nine patients (10.5%).

2.7 | HBV structure, mutations, and pathogenetic mechanisms

The virus consists of a nucleocapsid carrying HBcAg, a DNA polymerase/reverse-transcriptase (Pol/ RT), the viral genome and some cellular proteins[32] and an outer envelope showing HBsAg and the pre-S1 and pre-S2 antigens, which play a prominent diagnostic role. The genome consists of 3200 base pairs in a partially double-stranded circular DNA containing four overlapping open reading frames encoding surface proteins, the core protein, the polymerase and a multifunctional non-structural protein called X. The pre-S region of the S gene consists of the pre-S1 and pre-S2 domains and partially overlaps the gene region encoding the polymerase. The enhancer and basic core promoter regions overlap with the X gene. The pre-S1, pre-S2, and S genes code for three antigens, pre-S1, pre-S, and HBsAg. The protein encoded by the pre-core/core gene yields the HBeAg through a post-translational modification. The core gene codes for HBcAg, the major structural protein of the nucleocapsid, and the X gene for a potent trans-activator of viral and cellular genes (HBxAg).[48,49]

The polymerase gene encodes for the viral reverse transcriptase DNA polymerase, viral replication proceeding through a reverse transcription of an intermediate pre-genomic RNA and the subsequent synthesis of viral DNA.[50,51] The transcriptional template of HBV is the cccDNA, which resides inside the hepatocyte nucleus as a mini-chromosome.[52] The maintenance of cccDNA is essential for the persistence of the virus. HBV replication implicates reverse transcription of the pre-genomic RNA intermediate into HBV DNA. Reverse transcriptase is error prone and the mutation rate is high (appendix). The receptor for HBV entry into hepatocytes is sodium taurocholate polypeptide.[53] The most common HBV mutations include a single nucleotide change at position 1896 in the pre-core region, which creates a stop codon, and a double nucleotide change at positions 1762 and 1764 in the basal core promoter region, which decreases the transcription of the pre-core mRNA.[54] These mutations abolish or downregulate the production of HBeAg without affecting the replication capacity of the virus and cause HBeAg negative chronic HBV infection. The pre-core and basal core promoter mutations can occur alone or together.

Humans are known to be the only natural host of HBV, which reaches the liver through the systemic circulation and replicate only in hepatocytes.[55] HBV is not cytopathic and both liver damage and viral control are immune-mediated. The clinical outcome of infection is dependent on the complex interplay between HBV replication and host immune response. HBV is a weak inducer of the innate immune response.[56] Resolution of acute infection is mainly mediated through the adaptive immune response. People with serological recovery from acute HBV infection have strong T-cell responses to several epitopes in different regions of the HBV genome. By contrast, patients chronically infected with HBV have weak T-cell responses to a few epitopes.[57,58] Recovery of immune function has been reported in patients who underwent spontaneous HBeAg seroconversion and in those with virologic responses to antiviral therapy.[59,60] However, the immune responses to HBV may also have negative effects. Overly aggressive immune response is considered the cause of fulminant HBV hepatitis and of the exacerbations of chronic disease. Although some flares are accompanied by falls in HBV DNA concentrations and by a successful subsequent HBeAg to anti-HBe seroconversion,[61] other flares represent ineffective attempts of immune clearance and are accompanied by transient falls in HBV DNA concentrations.[62] Recurrent flares increase the risk of cirrhosis and hepatocellular carcinoma. Extrahepatic manifestations, mainly glomerulonephritis, and vasculitis including polyarteritis nodose, can develop because of an imbalance of humoral responses generating circulating immune complexes.

2.8 | HBV variants in acute hepatitis B (AHB)

AHB clinical presentation varies according to the grade of reactivity of cell-mediated immunity, low or absent in new-born babies and infants, moderate in children aged under 12 years, normal or high in children aged over 12 years and in adults.[12] After an incubation of 45-180 days and some constitutional symptoms, jaundice and dark urine are signals of the symptomatic phase usually lasting a couple of weeks or longer in adults. Jaundice develops in less than 10% of children under 5 years and in more than 50% of older children and adults. The universal HBV vaccination currently covers the Italian subject under 35 and, occurring only in subjects over 35, AHB is frequently severe and seldom show a fulminant course.[63–67] Occasionally, extrahepatic features may complicate the clinical presentation, reflecting immune complex-related damage such as acute necrotizing vasculitis, membranous glomerulonephritis and papular acrodermatitis in children.[68,69] An over-reaction of the immune system may lead to a fulminant form nearly 1% of the cases,[70,71] with a high fatality rate, requiring liver transplantation in most cases. Female sex and being a young adult are considered predictors of more severe clinical presentation, like same lifestyles (alcohol intake, drug abuse) and concomitant viral infections (HDV, HCV, or HIV).[66,72,73] The frequent occurrence of severe AHB in HBsAg negative patients with HCV chronic infection strongly suggest HBV vaccination in this subset of patients.

Virologic factors have also been associated with a peculiar clinical presentation and disease outcome.[38,74–76] Genotype HBV-Ae seems more frequent in the normal form[38] and the Bj sub-genotype and genotype B in patients with fulminant hepatitis.[77,78] HBV-sub-genotype C2, basal core promoter and pre-core variants have been found associated with the progression to fulminant hepatitis.[79] In the USA, HBV-genotype D has been found twice as frequent in patient with a fulminant form than in those with a mild course.[79] The pre-core stop-codon mutation (G1896A) and core-promoter double mutation (A1762T/G1764A) were found to be significantly more frequent in patients with a fulminant than in those with a mild clinical course,[38] in full accordance with previous investigations showing fulminant HBV infection associated with a

G1896A pre-core stop codon mutation[80,81] or with two mutations in the core promoter region, A1762 T, and G1764 A.[82,83] In a case-control study on 50 patients with fulminant and 50 with a self-limiting AHB a multivariate analysis showed the G1896A mutation, serum HBV DNA level higher than 5.23 log copies/mL and total bilirubin serum value higher than 10.35 mg/mL were all independently associated with a fulminant outcome.[84] The M204V/I variant was also associated with the severe forms of AHB in an Italian study.[85]

Coppola et al[86] explored changes in molecular epidemiology of acute viral hepatitis B by sequencing 123 consecutive patients with acute hepatitis Naples and found an overtime increase of the prevalence of HBV non-D genotypes from 11.1% in 1999-2003 to 41.1% in 2004-2008 ($P < 0.005$). Unsafe sexual intercourse resulted the prevalent risk factor in these patients (72.3%), whereas the prevalence of patients with HBV-D genotype reporting intravenous drug use significantly decreased from 1999 to 2003 (88.3%) to 2004-2008 (11.7%).

Coppola et al[87] investigated 138 patients enrolled from 1999 to 2012. The multivariate logistic regression analysis identified that a pre-existing HCV chronic infection was the only factor independently associated with a severe or fulminant clinical course of AHB (OR 4.89, 95%CI 1.5-15.94, $P = 0.01$), most probably due to the combination of the liver lesions caused by both HBV and HCV.

Concluding on this point, the study of the impact of HBV genetic variants on the clinical presentation and outcome of AHB is at its beginning, but the published data, although preliminary and fragmentary, strongly suggest that that this topic deserves further investigation.

2.9 | Viral and host factors in chronic hepatitis B (CHB)

CHB presents a variable clinical presentation, from a healthy HBV carriage to the more severe forms of the disease and a variable clinical course ranging from a benign, indolent course over decades to a rapid evolution to cirrhosis and HCC.[9,88,89] Several factors are linked to the severity of CHB: viral factors (HBV genotype, viral strain, viral load, and length of infection), host factors (age at the time of infection, degree of immune response), co-morbidities (diabetes, immunosuppression), coinfections with other viruses (hepatitis delta virus, human immunodeficiency virus, hepatitis C virus), or environmental factors (IVDU, alcohol abuse).[90,91]

The clinical course of CHB may be divided into five phases, not sequential:

(a) The "immune tolerant" phase is characterized by HBeAg positivity, high levels of HBV replication, mild or no liver necroinflammation with normal or low aminotransferase levels, no or slow progression of liver fibrosis. This phase, more frequent and prolonged in subjects infected perinatally, is associated with preserved HBV specific T cell function at least until the young adulthood.[92] The rate of spontaneous HBeAg loss is very low in this phase and patients are highly contagious.

(b) The "immune reactive phase" characterized by HBeAg positivity, but with a lower level of HBV replication, moderate or severe liver necroinflammation and increased or fluctuating aminotransferase levels and by a more rapid progression of liver fibrosis. In this phase several patients can achieve the control of HBV replication and enter in a HBeAg-negative phase, while those who fail to control HBV replication progresses to the a HBeAg-negative CHB.

(c) The phase "HBeAg-negative CHB" characterized by fluctuating HBV DNA and aminotransferase levels and detectable anti-HBe. This phase is linked to an e-minus HBV variant with nucleotide substitutions in the pre-core and/or the basal core promoter regions that become unable to express HBeAg or expresses it at low levels.[93] The liver histology shows histological features of chronic active hepatitis.

(d) The "inactive HBV carrier state" characterized by an undetectable or low (<2000 IU/mL) serum HBV DNA levels with low or normal serum aminotransferases and in most cases detectable seroconversion to anti-HBe. A few cases may have HBV DNA levels >2000 IU/mL, but usually <20 000 IU/mL, with minimal hepatic necro-inflammation, persistently normal ALT, low fibrosis and low tendency to progress to cirrhosis or HCC, which however might have developed in a previous phase. HBsAg loss and/or seroconversion to anti-HBs may occur spontaneously in 1-2% of cases per year in patients who usually show low HBsAg serum levels (<1000 IU/mL).[94]

(e) Occult HBV infection (OBI) may be considered the fifth phase of CHB, characterized by a low HBV replication (low HBV DNA level in the liver and in some cases in the blood) and usually by normal serum aminotransferases[74,95,96] in HBsAg-negative/anti-HBc-positive subjects with or without detectable anti-HBs. The gold standard to diagnose OBI is the detection of HBV DNA in the hepatocytes by highly sensitive and specific techniques like real-time polymerase chain reaction (PCR) or nested PCR with the use of oligonucleotide primers specific for different HBV genomic regions.

In the everyday clinical practice, the detection of anti-HBc in serum of HBsAg-negative subjects, is considered a sign of previous acute hepatitis B and used as a surrogate serum marker of OBI.[97,98]

2.10 | HBV phylogeny in Italy

Pollicita et al[99] described a local epidemiological cluster, characterized by neighbour-joining and maximum-likelihood methods, of AHB, sexually transmitted in a short time period (November 2011-May 2014), involving eight individuals infected with subgenotype F1b in central Italy. This subtype, not commonly found in western countries, was carried by 6/8 Italian subjects.

Ferraro et al[44] examined 34 patients with AHB using a Neighbor-joining approach, found that 44% were genotype A, 53% genotype D, and 3% genotype E. This molecular analysis of isolates from AHB patients from South Italy showed a change in the local epidemiology of this infection, with an increase in HBV-genotype A infections and a

clustering effect for HBV-D2, possibly correlated to immigration. The introduction of new genotypes, could have an effect on HBV-correlated diseases due to the different association between genotype, liver disease and response to antiviral therapy. The main risk factor of these isolates was unsafe sex and these belong to a social-demographic setting (commercial sex workers) which is not reached by screening for HBV. Due to the ubiquitous presence of HBV-genotype A, Zehender et al[100] analyzed the origin and path of dispersion of HBV-A sub-genotypes on a reliable calendar timescale using a calibration based approach. They studied 111 Italian patients, eighteen were A1, one was A3, and 90 were A2, including 35 (28.9%) collected from Italian patients co-infected with HIV-1. Most Italian patients carrying sub-genotype A1 formed a highly significant clade that, according to the authors, suggests a founder effect characterized by a single introduction followed by autochthonous circulation. This could be explained by the fact that Italy was not involved in historical migrations from Africa, differently from other countries like Belgium, France, or Great Britain. Regarding the sub-genotype A2, the authors identified a series of small subclades, most of which with tMRCAs dating back the 1970s and the 1990s, confirmed by the demographic analysis obtained with the Bayesian Skyline Plot (BSP) that showed an exponential growth in the effective number of infections during the same years. In line with previous data of this group,[101] these data suggest the recent spread of HBV-A2 among subject sat high risk of infection, such as MSM. Moreover, the high frequency of HIV-positive patients in the Italian clades associated with young Romanian women without known risk, also suggests the potential role of sexual transmission in Italy and Romania.

Villano et al[102] performed a study on 43 CHB patients with the purpose to describe the molecular epidemiology of HBV in a chronically infected population of migrants living in Italy, using phylogenetic analysis. Sequences were classified as 25 (58.1%) of genotype D, 7 (16.3%) of genotype A, 4 (9.3%) of genotype B, 3 (7%) of genotype C, 1 (2.3%) of genotype E. Three (7%) sequences resulted unclassified. Among genotype A isolates, 4 (57.1%) were classified as sub-genotype A2 and 3 (42.9%) as sub-genotype A3. Among the genotype D sequences, 18 (72%) as sub-genotype D1, 6 (24%) were classified as sub-genotype D2 and 1 isolate (4%) as sub-genotype D3. Maximum Likelihood analysis indicated that most of the Italian sequences clustered together in a main clade separately from all others. By comparing the phylogenetic results with epidemiological data, indicating the date of arrival in Italy of migrants, the authors demonstrated that infection of migrants occurred in their country of origin.

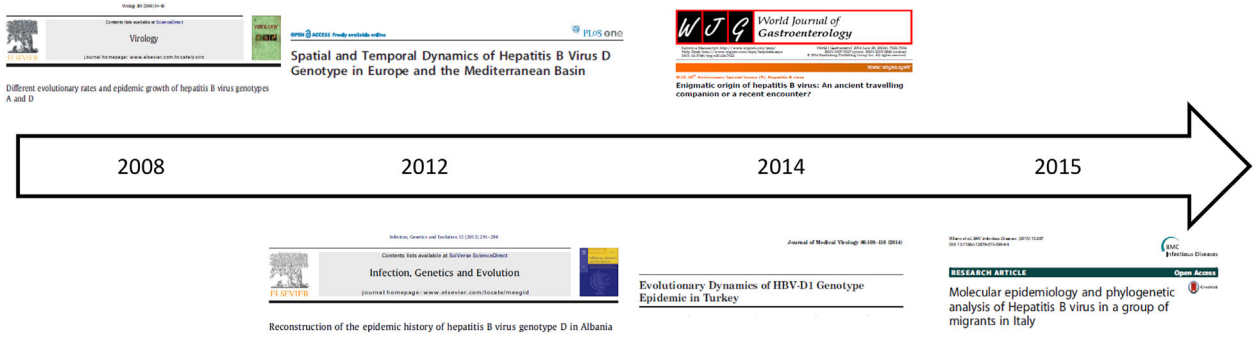
Ciccozzi et al[103] focusing on HBV-D1 in Turkey, identified a significant linkage between Italy and Turkey. Based on their phylogenetic results authors dated the spread of the HBV-D1 subgenotype around the early mid-1940s, corresponding to the European Second World War period. Thereafter, HBV-D1 infection spread on the entire Mediterranean area in the early 1970s. Probably most of infections were transmitted horizontally mainly using unsterilized needles and syringes, and house hold contacts as indicated for the countries where HBV-D is prevalent.

The genotype D supports 70-80% or more of HBV infection occurring in Italy.[104] By reconstructing the epidemic history of HBV-genotype D in Albania, Zehender et al[100] identified only one Italian clade with a tMRCA in 1980. They estimated a high value of basic reproductive number of infection (R_0) for the Italian HBV-D3 epidemic, probably consequently to the parenteral route of transmission in Italy.

In a study on 312 sequences of HBV-D isolated in various countries of the world and including 63 Italian isolates were analyzed. HBV-D1 strains grouped only in small clusters, each including no more than 5 isolates, with Turkey as most probable location. The phylodynamic analysis of HBV-D3 in Italy dated even before the epidemic of intravenous drug addiction in the early 1970s, raising questions about other possible routes of geographic dispersion of these strains that in this work resulted the earlier sub-genotype diverging in 1940s.[105]

The most recent investigation on HBV origin, evolution and phylodinamic have been represented as a timeline in Figure 2.

Phylogenetic and phylodinamic analysis suggested two distinct epidemic waves of HBV in Italy. The first dated at the time of World War II, when the HBV epidemic grew rapidly mainly due to sub-genotype D3; the second in the 1960s and 1970s, involving high risk subjects, characterized by the import of new viral strains as well as the spread of pre-existing variants.



COLOR

FIGURE 2 Timeline representation of the most recent investigation on HBV origin, evolution and phylodinamic

CONFLICTS OF INTEREST

All the authors declare that they have no conflicts of interest about this paper.

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