

REVIEW



The clinical implications of hepatitis B virus genotypes and HBeAg in pediatrics

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SUMMARY

Although a successful vaccine against HBV has been implemented in 184 countries, eradication of hepatitis B virus (HBV) is still not on the horizon. There are over 240 million chronic carriers of HBV globally. The risk of developing chronic hepatitis ranges from >90% in newborns of hepatitis Be antigen (HBeAg)-positive mothers, 25%–35% in children under 5 years of age and <5% in adults. HBeAg, a non-particulate viral protein, is a marker of HBV replication. This is the only HBV antigen to cross the placenta, leading to specific unresponsiveness of helper T cells to the capsid protein and HBeAg in newborns. HBeAg is tolerated *in utero* and acts as a tolerogen after birth. Perinatal transmission is frequent when mothers are HBeAg-positive, whereas it occurs less frequently when mothers are HBeAg-negative. Sequence heterogeneity is a feature of HBV. Based on an intergroup divergence >7.5% across the complete genome, HBV is classified phylogenetically into at least nine genotypes. With between ~4% and 8% intergroup nucleotide divergence, genotypes A–D, F, H and I are classified further into subgenotypes. HBV genotypes/subgenotypes may have distinct geographical distribution and can develop different mutations in the regions of the HBV genome that code for HBeAg. These differences can be related to the role of HBV genotypes to the natural history of infection and mode of transmission. Thus genotypes/subgenotypes of HBV can be responsible for the different natural history of infection and modes of transmission in children, found in various regions of the world, where different genotypes/subgenotypes prevail. © 2016 The Authors *Reviews in Medical Virology* Published by John Wiley & Sons, Ltd.

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GLOBAL EPIDEMIOLOGY AND PATHOGENESIS OF HEPATITIS B VIRUS (HBV) INFECTION

Two of the 7.3 billion of the world's population have been exposed the hepatitis B virus (HBV). It is estimated that 240 million individuals are currently chronically infected with this virus [1]. Twenty to thirty percent of the chronic carriers of HBV will develop complications including acute liver failure, hepatic decompensation, cirrhosis and hepatocellular carcinoma (HCC). Annually approximately 786 000 people die as a result of complications caused by chronic HBV infection. These deaths are ranked 15th in the Global Burden of Disease (GBD) 2010 [2] and account for 50% and 33% of deaths as a result of HCC and cirrhosis, respectively [2].

With the exception of Ecuador, Peru, Bolivia, and southern eastern Asia, Indonesia and East Timor, the prevalence of HBV infection in children (5 to 9 years) coincides with that in adults (19 to 49 years) (Figure 1) [3,4]. In these south American

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Abbreviations Used

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCP, basic core promoter; ELISA, enzyme linked immunosorbent assay; ϵ , encapsidation signal; GBD, global burden of disease; HBeAg, hepatitis B c antigen or core protein; HDV, hepatitis D virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B s antigen; HCC, hepatocellular carcinoma; LiPaTM, line probe assay; MTCT, mother-to-child transmission; P, polymerase; pgRNA, pregenomic ribonucleic acid; preC/C, precore/core; TLR-2, toll-like receptor 2; WHO, World Health Organization.

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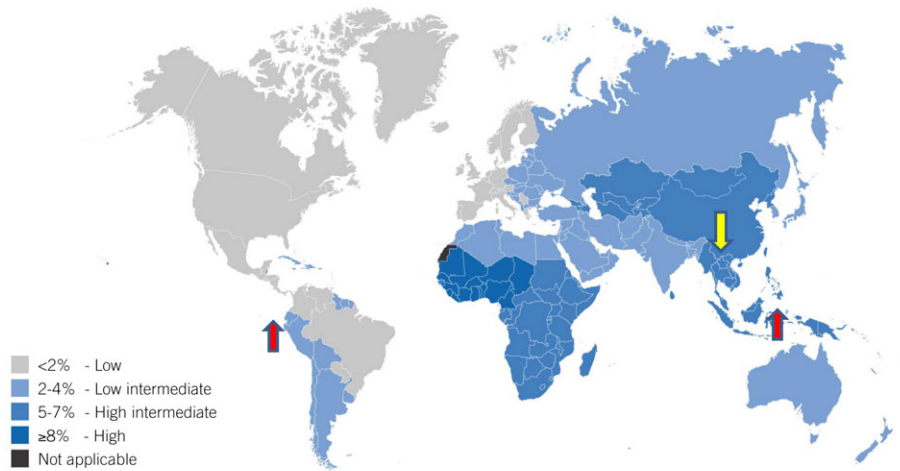


Figure 1. The map shows the prevalence of HBsAg-positive adults in the world. The arrows indicate where the prevalence in children does not coincide with that in adults. The red arrows indicate higher levels of HBsAg-positivity in children compared to adults, whereas the yellow arrows indicate lower levels in children compared to adult. Reprinted with permission from: Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection, WHO, page 12, © World Health Organization 2015 [<http://www.who.int/hiv/pub/hepatitis/hepatitis-b-guidelines/en/>]

countries, the prevalence in children is higher than in adults, whereas the opposite is true in the Asian countries, except for Indonesia and East Timor, where it is higher [3]. Between 1990 and 2005 there was an increase in chronic HBV infections among younger age groups in southern-eastern sub-Saharan Africa, whereas in central sub-Saharan Africa there was a corresponding decrease [3]. In central and eastern Europe, the most affected age group was younger than 9 years old, with a minimal decrease between 1990 and 2005. Between 1990 and 2005, the strongest reduction in prevalence of HBsAg in children was recorded in south east Asia [3]. This decrease is attributable to the introduction of effective vaccination programs.

MOLECULAR BIOLOGY OF HBV AND HBeAg

HBV, the smallest DNA virus infecting man, belongs to the family *Hepadnaviridae* and is the prototype member of the genus *Orthohepadnavirus*. HBV has a partially double-stranded, circular DNA genome of ~3 200 base pairs. This compact genome contains four partly or completely overlapping open reading frames (ORFs): *precore/core* (*preC/C*) that encodes the e antigen (HBeAg) and core protein (HBcAg); *P* for polymerase (reverse transcriptase), *PreS1/PreS2/S* for surface proteins (three forms of HBsAg, small (S), middle (M) and large (L)) and X for a transcriptional trans-activator protein

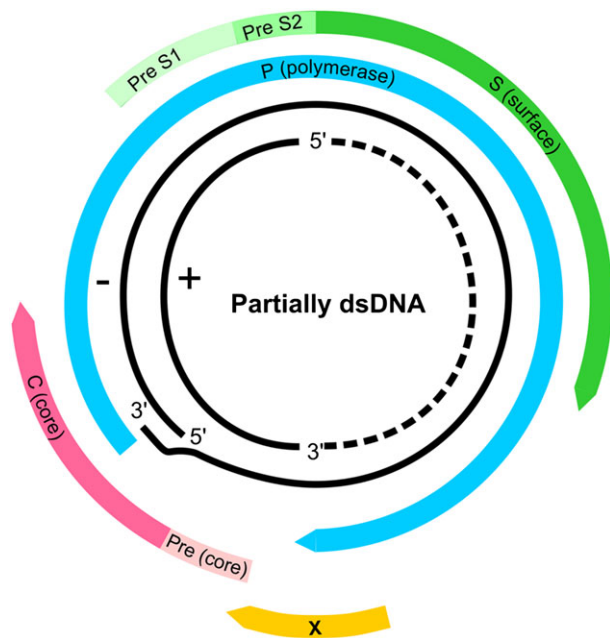


Figure 2. The genome of hepatitis B virus (HBV). The partially double stranded DNA (dsDNA) with the complete minus (-) strand and the incomplete (+) strand. The four open reading frames (ORFs) are shown: *precore/core* (*preC/C*) that encodes the e antigen (HBeAg) and core protein (HBcAg); *P* for polymerase (reverse transcriptase), *PreS1/PreS2/S* for surface proteins (three forms of HBsAg, small (S), middle (M) and large (L)) and X for a transcriptional trans-activator protein

large (L)) and X for a transcriptional trans-activator protein [5] (Figure 2).

HBV replicates by reverse transcription of the pregenomic RNA (pgRNA), a 3.5 kb RNA intermediate [6], which is transcribed, by the cellular RNA polymerase II, from the covalently closed circular form of HBV DNA in the hepatocyte nucleus [7]. In order to be reverse transcribed the pgRNA has to be folded into a secondary structure, known as the encapsidation signal (ϵ) [8] (reviewed in [9]) and enclosed in the viral capsid, which is comprised of HBcAg. Anti-HBc antibodies are a measure of exposure to HBV but not necessarily immunity. Surrounding the capsid is an envelope made up of lipid membrane from the host, in which are embedded HBsAg proteins coded by the virus (Figure 3). The presence of anti-HBs signals immunity to HBV. In addition to the structural proteins, HBc and HBs antigens, HBV codes for two non-particulate proteins X (a transcriptional transactivator protein) and HBeAg [10].

HBeAg is encoded by the *preC/C* ORF (1814 – 2452/2488 from the *EcoRI* site, [11]) and the basic core promoter (BCP) of HBV controls the transcription of the *preC/C* region [12,13] (reviewed in [14]). The *preC/C* fusion protein, which is the precursor of HBeAg has a signal peptide on its amino end that targets it to the endoplasmic reticulum, where it is post-translationally modified [15]. The amino end is truncated at amino acid 19, whereas the carboxyl end is cleaved at

variable sites. The mature HBeAg is secreted and is soluble in serum [16] (Figure 4).

Although HBeAg is not required for viral assembly or replication it is conserved in all orthohepadnaviruses [17] and is important for natural infection *in vivo* [18]. Clinically, HBeAg is an index of viral replication, infectivity, inflammation, severity of disease and response to antiviral therapy. Seroconversion from HBeAg-positive to HBeAg-negative/anti-HBeAg-positive phase usually heralds resolution of infection [19]. However, the emergence of BCP and *precore* mutations can lead to HBeAg-negative infection, where viral replication and inflammation remain high. While the exact function of HBeAg has not been elucidated, it has been shown to be an immunoregulatory protein, which acts as a tolerogen [20] and an immunogen [21], triggers an interleukin-1 response [22] and regulates toll-like receptor 2 (TLR-2) expression [23]. Immune regulation mediated by HBeAg may lead to chronicity and persistence following perinatal infection and in adults prevent severe liver injury as result of infection [18]. Chronic infection will result following perinatal transmission from HBeAg-positive mothers. On the other hand, transmission from HBeAg-negative mothers can result in acute hepatitis or acute liver failure [24]. In addition to being an important milestone of chronic HBV infection, HBeAg status (HBeAg-positivity

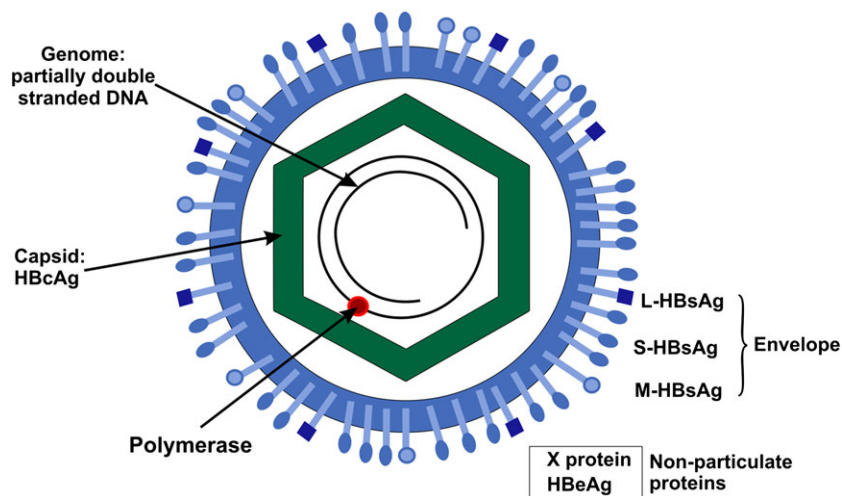


Figure 3. Schematic representation of hepatitis B virus (HBV), showing the structure of the virion, composed of a partially double stranded DNA genome, enclosed by a capsid, comprised of HBcAg and surrounded by a lipid envelope containing large (L)-HBsAg, middle (M)-HBsAg and small (S)-HBsAg. The virus also expresses two non-particulate proteins X protein and HBeAg

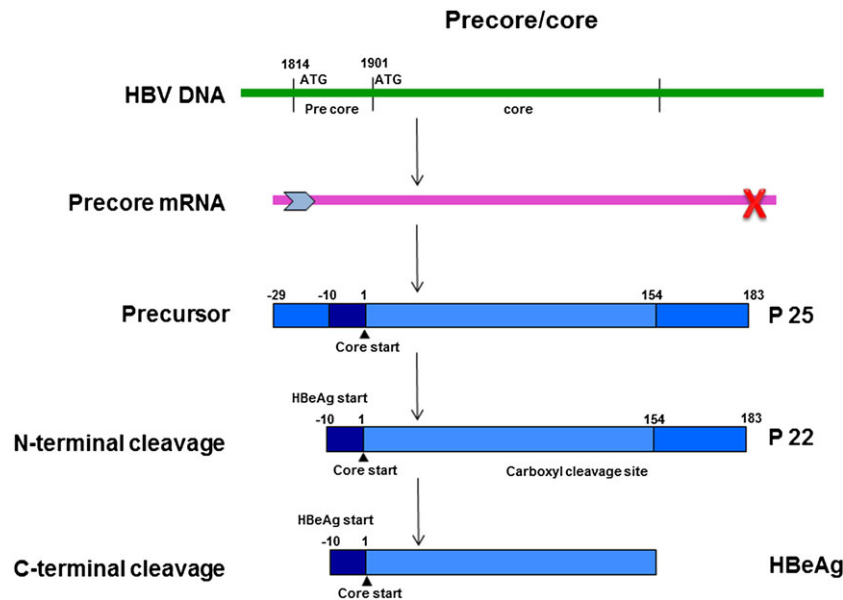


Figure 4. Schematic representation of the expression of HBeAg in hepatitis B virus (HBV). Nucleotide numbering is from the *EcoRI* cleavage site on the DNA genome and amino acid numbering is from position 1 of the core protein

versus HBeAg-negativity) is also a determinant of the mode of transmission of the virus.

NATURAL HISTORY OF HBV INFECTION IN CHILDREN

Development of chronic infection, following acute hepatitis B, requires the expression of HBeAg [25] and thus the risk of developing chronic hepatitis is:

- >90% in newborns of HBeAg-positive mothers [26,27]. This is probably because of the immature immune system of the infant [28] and/or the foetus developing immune tolerance as a result of the transplacental crossing of either the virion or HBeAg, the only HBV antigen that can cross the placenta [18]. Transplacental crossing of HBeAg induces a specific unresponsiveness of helper T cells to both HBcAg and HBeAg [29].
- 25%–35% in infants and children under the age of 5 years
- 6% in children 5 to 15 years
- <5% in adults [30]

Differentiated by the level of viral replication and the host immune response, chronic HBV infection acquired perinatally or in early infancy is broadly divided into four phases in children (Figure 5):

(1) The **high replicative, low inflammatory** phase (previously immune tolerant phase)

[31] is HBsAg-positive, HBeAg-positive, with high HBV DNA levels ($>2 \times 10^5$ IU/ml) [32], with minimal liver inflammation, normal aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels and asymptomatic [33]. The duration of this phase can be up to 3 decades if transmission occurred perinatally, whereas following horizontal transmission it may be very short and undetected [34].

- (2) The **immune clearance or reactive** phase is characterized by fluctuating ALT and HBV DNA levels and ending with spontaneous HBeAg loss [32,35]. HBeAg seroconversion is accompanied by elevated ALT and decreased HBV DNA levels [32]. Spontaneous HBeAg seroconversion occurs at a lower rate in children born to HBsAg-positive mothers compared to HBsAg-negative mothers [36] and less frequently in children, who acquired their infection perinatally compared those infected horizontally [37]. In south east Asia, the annual HBeAg seroconversion rate is 4%–5% in children older than 3 years, but only 2% in those younger than 3 years [38]. In contrast, in Euro-Mediterranean and African countries, HBeAg seroconversion is more frequent, occurring at an annual rate of 14%–16% [25,39]. Thus close to 90% of the individuals in Euro-Mediterranean and African countries are

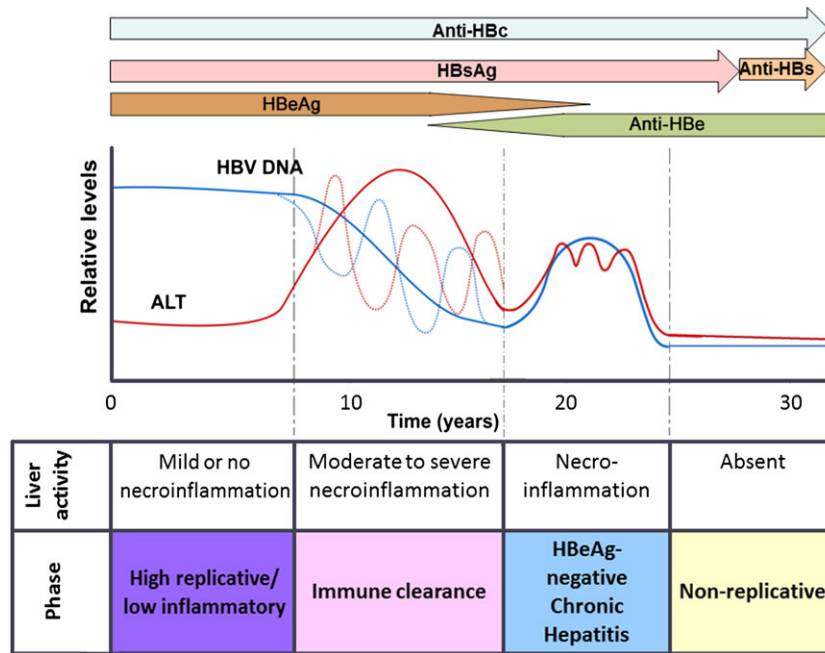


Figure 5. Natural history of infection of hepatitis B virus (HBV) in children over time showing the four phases, the liver histology and activity, the expression of serological markers (antigens: HBsAg and HBeAg; antibodies: anti-HBc, anti-HBe and anti-HBs) and the relative levels of HBV DNA and ALT. The recently suggested nomenclature is used [31]

HBeAg-negative/anti-HBeAg positive by the age of 20 years [39–41], compared to only 5% in south east Asia [25]. On average, HBeAg seroconversion usually occurs in children younger than 15 years [42]. Prognosis for these children is generally good [27].

The duration of this phase is variable. The shorter duration of the HBeAg-positive phase in Euro-Mediterranean and African regions compared to that in South-East Asia leads to significantly lower rates of development of advance liver disease in these regions [25]. Delayed HBeAg seroconversion may prolong the inflammatory response and lead to more severe liver disease [43]. Then again, if HBeAg seroconversion occurs very early, before the age of 3 years, this can also lead to increased ALT levels, with severe liver injury and the rapid development of HCC before the age of 10 years [38,44]. The genotype or subgenotype of HBV can affect the age at which HBeAg loss occurs as well as its frequency in a population (see below).

(3) The **HBeAg-negative chronic hepatitis phase** [31], where necroinflammation persists with

high or fluctuating ALT levels and immune clearance is ineffective. Viral loads are moderate to high and liver disease is progressive.

(4) The **low replicative phase** (previously “inactive HBsAg-positive carrier” phase) [31] (post-HBeAg seroconversion) is the phase characterized by the absence of HBeAg, anti-HBe positivity, normal ALT and low or undetectable HBV DNA levels ($<2 \times 10^3$ IU/ml).

Two additional phases, the **reactivation** phase and the **HBsAg loss or occult** phase [45], have been described in the natural history of HBV infection [31] but these are infrequent in children. The reactivation phase is characterized by the recurrence of viremia, reversion to HBeAg-positivity and hepatic flares [46]. During the HBsAg loss phase, the entire, episomal, replication-competent genomes can persist intrahepatically, in the presence or absence of serological markers (occult infection) [45]. However, spontaneous HBsAg loss is rare in children, occurs at 0.6%–1% per annum, especially if children were infected perinatally and have minimal liver injury [47–49]. This seroconversion rate is lower than that seen in individuals infected as adolescents or in adulthood [50]. More than 90% of patients, infected

in childhood, remain HBsAg carriers in adulthood [50]. Acquisition of infection horizontally is associated with a higher HBsAg clearance rate, as is being born to a HBsAg-negative mother [46]. The levels of anti-HBs after loss of HBsAg are higher in children born to HBsAg-positive mothers compared to those born to HBsAg-negative mothers. [46]

Albeit that liver damage is minimal in the majority of children, some can manifest with mild inflammation and acute hepatitis [49], as well serious complications of HBV infection, including cirrhosis and HCC, 2 to 7 years after infection [44]. Risk factors for early HCC development include cirrhosis and HBeAg seroconversion before 3 years [44]. Normal ALT levels and anti-HBeAg seroconversion are no guarantee against cirrhosis and HCC, especially in untreated individuals [44]. In a European study, children developed HBeAg-negative chronic hepatitis and/or HCC after an average 5-year follow-up [50]. It is possible that there are different mechanisms for the development of HCC in adults and children. The former require higher viral loads and liver inflammation, whereas integration of HBV in the human genome may trigger HCC in children [51].

Although genetic and environmental factors, including socioeconomic and hygiene levels, can play a role in the natural history of HBV infection and the development of advanced liver disease, it is becoming increasingly evident that the genotypes/subgenotypes of HBV and specific mutations can play a leading role [25,52].

GENOTYPES AND SUBGENOTYPES OF HBV

Sequence heterogeneity is a feature of HBV, because the viral encoded polymerase lacks proof-reading ability. To date, based on an intergroup divergence of greater than 7.5% across the complete genome, HBV has been classified phylogenetically into 9 genotypes, A to I [11,53–55], with a putative 10th genotype, "J", isolated from a single individual [56], which is a recombinant of genotype C and gibbon HBV in the S region [57]. With between ~4% and 8% intergroup nucleotide difference across the complete genome and good bootstrap support, genotypes A – D, F, H and I are classified further into at least 35 subgenotypes [55]. The genotypes differ in genome length, the size of ORFs and the proteins translated [11], as well as the development of various mutations [52]. Based on HBsAg heterogeneity [11], nine serological subtypes, *ayw1*, *ayw2*,

ayw3, *ayw4*, *ayr*, *adw2*, *adw4*, *adwq*, *adr* and *adrq*, have been identified. A broad, highly statistically significant correlation exists between serological subtypes and genotypes: *adw* is associated with genotypes A, B, F, G and H, *adr* with C and *ayw* with D and E [58] but many exceptions exist (Figure 6).

The genotype of HBV can influence the outcome of HBV infection in children because it can affect the frequency of HBeAg-positivity, the age at which HBeAg loss occurs and the mode of transmission. Therefore the natural history of HBV infection can differ in different geographical regions ([52,55,59,60] and references cited therein).

GEOGRAPHIC DISTRIBUTION OF GENOTYPES

Globally and locally the various genotypes, and in some cases the subgenotypes, have distinct geographical distributions [11,55] (Figure 7). Genotype A is found in Africa, Europe and the Americas whereas genotypes B and C predominate in south east Asia. Although genotype D is distributed in all continents and is referred to as the cosmopolitan genotype, its subgenotypes can be geographically distributed [61]. Genotype E is confined to western Africa and distributed to other regions of the world following emigration from Africa. Central and South America are the regions where genotype F and H originate. Although genotype G was originally described in Georgia, United States of America, it has been isolated in the United Kingdom, Germany and Italy. Subgenotype A1 predominates in Africa and in regions outside Africa where there has been mass migration from Africa [62]. The subgenotype of A found outside Africa is subgenotype A2. Subgenotype B1 prevails in Japan and B2 in south east Asia, with B5 (formerly B6) found in Alaska. Thus in the two regions of the world where HBV occurs at high endemicity different genotypes prevail. Subgenotype A1, genotypes D and E circulate in sub-Saharan Africa and genotypes B and C in south eastern Asia.

RECOMBINATION BETWEEN GENOTYPES

In geographical regions where a number of genotypes co-circulate, recombination between genotypes can occur, and this provides a mechanism of variation within individuals and in the population in general [11]. Genotype A and D recombinants have been found in Africa [63],

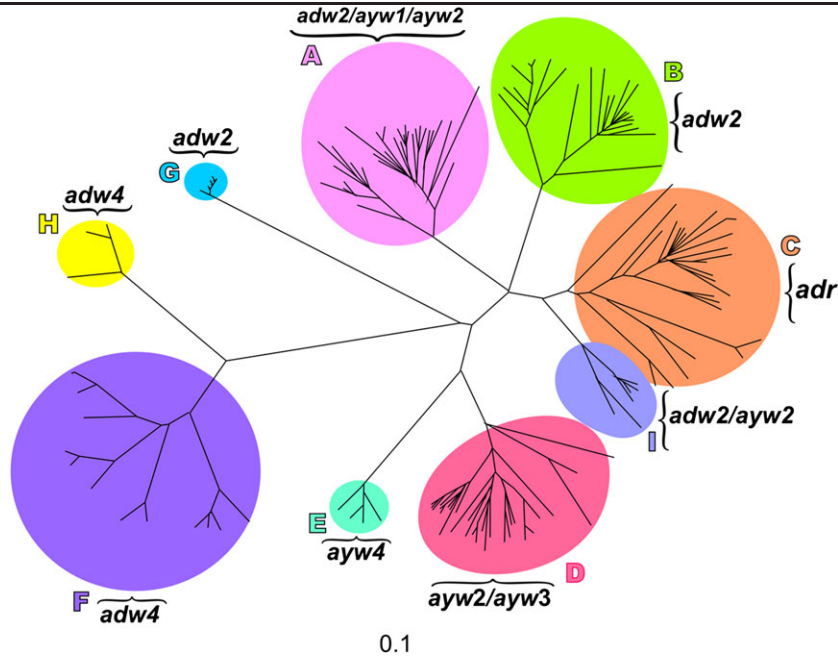


Figure 6. An unrooted phylogenetic tree of full genome sequences of hepatitis B virus (HBV) representative of the nine major genotypes, established using neighbor-joining. Letters A to I represent the nine genotypes and the associated serological subtypes are indicated

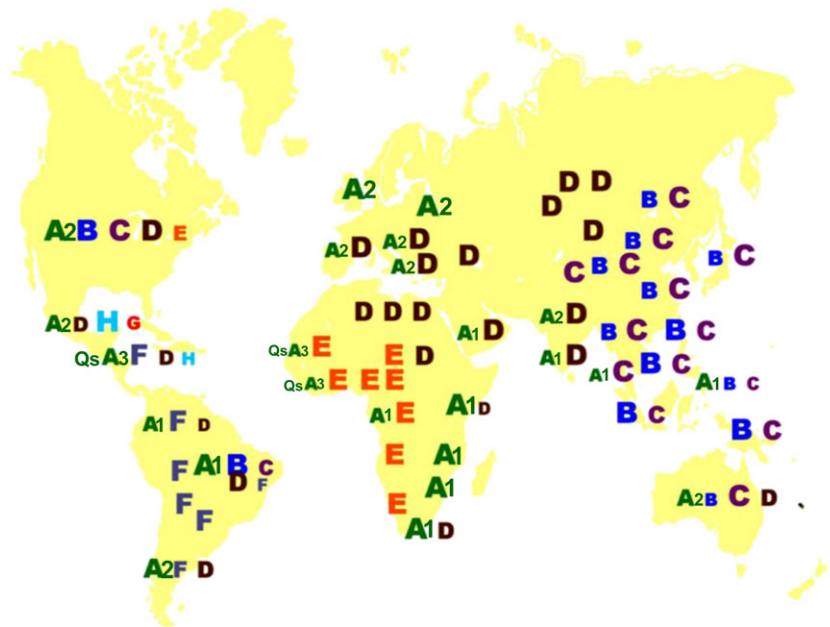


Figure 7. The geographic distribution of the hepatitis B virus (HBV) genotypes and subgenotypes of A (A1, A2, QsA3: Quasi-subgenotype A3) [55]. The relative size of the letter/number indicates the relative prevalence of the genotype or subgenotype.

whereas in Asia genotype B/C recombinants occur [64,65]. Tibet has a 26.2% HBV carrier rate, and 96% of the isolates sequenced were C/D recombinants [66]. The breakpoints occurred most frequently in

the BCP/PC region, with breakpoints found in the small *S* and *core* regions [67,68]. The recombination may provide a selection advantage to the viral strains, and the recombinants may become the

dominant strains of a quasispecies and persist in a population. Genotype I is composed entirely of recombinants, and 93% of genotype B strains represent recombinants [68]. Thus, four of the six subgenotypes of genotype B (B2–B4) represent genotype B recombined with genotype C in the *precore/core* region, whereas only subgenotypes B1 found mainly in Japan [65] and B5 (previously B6) from a Canadian Inuit population [69] represent genotype B without this recombination. Subgenotypes B2–B4 show a higher risk of serious complications of HBV infection including cirrhosis and development of HCC, compared to B1 and B5 [60].

GENOTYPING AND SUBGENOTYPING METHODS

Although the HBV *S* gene sequence is generally adequate to assign genotypes [58], the complete sequence of the HBV genome provides additional information with respect to phylogenetic relatedness [70,71]. Moreover, recombinants may not be identified when using a single region of the HBV genome for phylogenetic analysis. Nevertheless, although complete genome sequencing, followed by phylogenetic analysis, provides the gold standard for genotyping, it does not allow for rapid and direct analysis on a large scale basis [11] and requires expertise and thus capacity development in computer processing coupled with phylogenetic analyses. In order to expedite and facilitate genotyping a number of methods have been developed [11,72,73]. A number of commercially available assays are available, for example, genotype-specific probes assay (Smitest HBV Genotyping Kit, Genome Science, Fukushima, Japan), reverse hybridization of PCR products to probes on nitrocellulose strips (the line probe assay, LiPa™, Innogenetic Inc, Gent, Belgium) and enzyme linked immunosorbent assay (ELISA) (HBV Genotype EIA, Institute of Immunology, Tokyo, Japan). Each one has its advantages and disadvantages [11,72,73], which should be taken into account, when selecting the genotyping method appropriate for a particular study or application [74].

THE EFFECT OF GENOTYPE/SUBGENOTYPE ON HBeAg EXPRESSION

HBeAg, discovered in 1972 [10], has been the classical marker for HBV replication, and its presence for longer than 10 weeks can herald the transition to

chronic infection [35]. Transition from the immune clearance phase to the HBeAg-negative chronic hepatitis phase is accompanied by HBeAg seroconversion, the movement of HBcAg from the nucleus to the cytoplasm [75] and an increase in the frequency of HBV strains with BCP and precore mutations [76,77]. Mutations in the BCP and *preC* can influence the expression of HBeAg at the transcriptional, translational and post-translational levels. Transcription of the *preC* mRNA is affected by A1762T/G1764A [78]. At the translational level, mutations in the Kozak sequence preceding the *preC* start codon (1814 from the *EcoRI* site) can affect the expression of HBeAg by a leaky scanning mechanism [79] whereas G1896A introduces a stop codon that leads to the truncation of the HBeAg precursor [80]. The G1862T mutation in the *preC* affects expression at a post-translational level [81].

The propensity to develop precore mutations can be influenced by the HBV genotype or subgenotype. As described previously, the *preC/C* region is transcribed into the precore mRNA that is translated into the precursor of HBeAg (Figure 4). The *preC/C* region also overlaps with the region that is transcribed into the pgRNA, which is the RNA intermediate of HBV replication. In order to be successfully encapsidated, the pgRNA has to be folded into a secondary structure known as ϵ , which has to be stable, for viral replication to proceed. The sequence of the *preC/C* region differs in the different genotypes, and in some cases, the subgenotypes [17]. For example, 1858C in the *preC/C* region is positively associated with genotypes A, F and H and 1858T with genotypes B, D and E [58]. Subgenotype C2 has 1858T as opposed to C1 that has 1858C and F1/F4 can be differentiated from F2/F3 by having 1858T instead of 1858C [58]. 1888A is positively associated with subgenotype A1 [58].

Subgenotypes A1, A2 and genotype D, which circulate in southern Africa, will be used to explain and illustrate how nucleotide differences can influence HBeAg expression. Comparison of the sequences of BCP/*PreC* region of the different (sub) genotypes A1, A2 and D reveals variations that can account for the differences in HBeAg expression (Figure 8A). Furthermore, the frequency of these mutations/variations also differs between these (sub) genotypes (Figure 8B) [82]. The presence of 1858C in genotype A precludes the G1896A mutation found in genotype D because

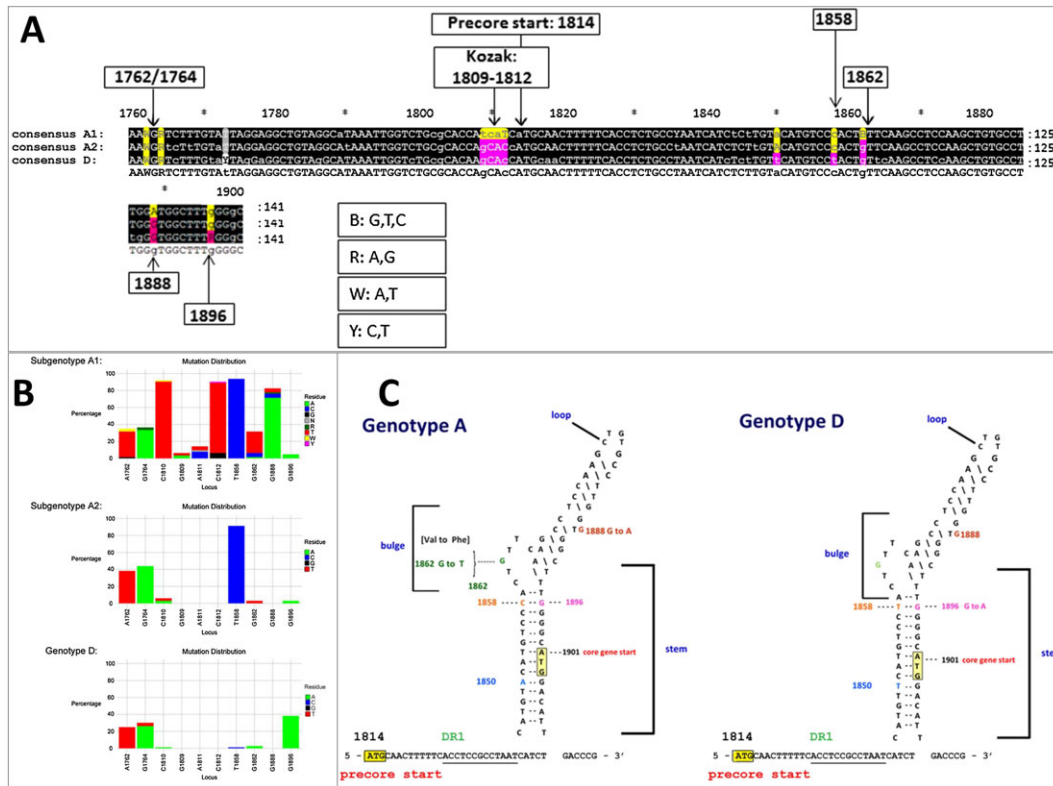


Figure 8. A comparison of (sub) genotypes A1, A2 and D. (A) Comparison of the consensus sequence of the basic core promoter/precure (BCP/PC) region of (sub) genotypes A1, A2 and D. Numbering from the *EcoRI* site. (B) Mutation distribution graphs generated using the Mutation Reporter Tool [82] showing the percentage of mutant residues relative to the reference motif found at 10 loci of interest specified (1762, 1764, 1809–1812, 1858, 1862, 1888, 1896). Three data sets were submitted to the tool to produce the three graphs showing the mutation distribution for 63 subgenotype A1 samples, 34 subgenotype A2 samples and 76 genotype D samples. The reference motif used was AGGCACTGGG. This is also shown by the letter preceding each locus on the X-axis. To facilitate direct comparisons between the graphs, conserved loci were not suppressed, and the Y-axis was scaled to 100% by selecting the appropriate controls on the input page. (C) Nucleotide sequence and predicted secondary structure of the hepatitis B virus (HBV) pregenome encapsidation signal (ϵ) [8]. The DNA instead of the RNA sequence is shown for ease of interpretation. The sequence for genotypes A and D is shown. The two sequences differ in nucleotides 1850 and 1858 in this region. The initiation codons of the precure (1814) and core (1901) are labeled. Nucleotide substitutions found in the two genotypes are shown at the sides of the stem-loop structure. Please note 1862T and 1888A only occur in subgenotype A1 and not subgenotype A2 of genotype A. Numbering from the *EcoRI* site

the presence of 1858C and 1896A would destabilize ϵ and compromise viral replication (Figure 8C) [83,84]. 1896A converts the codon for tryptophan to a stop codon, leading to the truncation of the HBeAg precursor and abrogation of HBeAg expression (Figure 9A) [80]. This is the mutation that prevails in isolates obtained from HBeAg-negative individuals infected with genotype D. The only mutations that can influence HBeAg expression in strains isolated from individuals infected with subgenotype A2, which has 1858C, are 1762T/1764A (Figure 9B). On the other hand, in addition to the 1762T/1764A, subgenotype A1 strains, which also have 1858C, can develop

mutations in the Kozak sequence (1809–1812) and the G1862T (Figure 9C) [85], accounting for the higher frequency of HBeAg-negativity observed in individuals infected with this subgenotype. In a case control study, comparing individuals infected with subgenotypes A1, A2 and genotype D, respectively, it was shown, regardless of the age group, the frequency of HBeAg-positivity was lower in individuals infected with subgenotype A1 compared to the other (sub) genotypes [86]. This difference reached statistical significance in individuals younger than 30 years of age [86].

As a result of the distinct geographic distributions of the (sub) genotypes globally, the

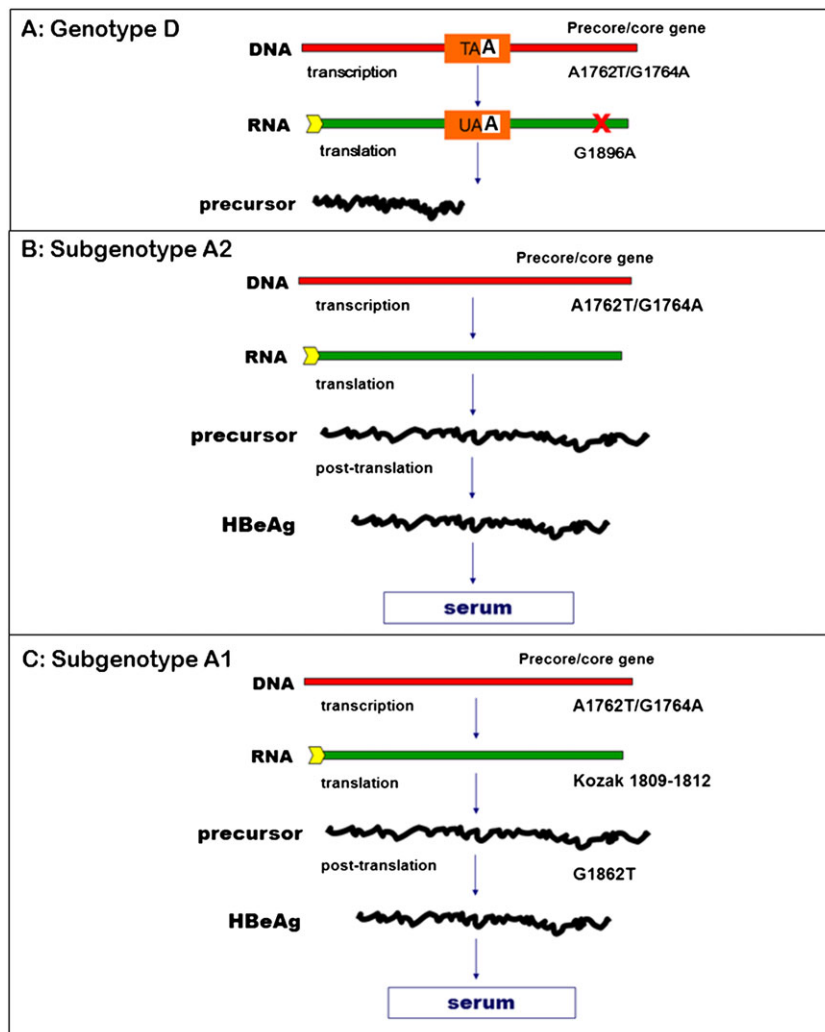


Figure 9. Schematic representation of the expression of HBeAg in hepatitis B virus (HBV) (sub) genotype D, A2 and A1. A1762T/G1764A, which decreases the expression of transcription of precore mRNA and therefore HBeAg expression can occur in (sub) genotypes A1, A2 and D. (A) Genotype D can develop the G1896A mutation, converting the codon TAG for tryptophan to a stop codon TAA, leading to the truncation of the precursor, abrogating HBeAg, expression. (B) Subgenotype A2 can only develop A1762T/G1764A. (C) Subgenotype A1 has unique characteristics and can develop mutations in the Kozak (1809 – 1812), which affects the translation of HBeAg and G1862T that interferes with HBeAg expression at the post-translational level. Nucleotide numbering is from the *EcoRI* cleavage site on the DNA genome

percentage of HBeAg-negative chronic hepatitis in the world also varies. Thus in the Mediterranean areas and Maghreb, where genotype D, which can develop G1896A, prevails, 80% to 90% of chronic hepatitis patients are HBeAg-negative. This percentage is 30%–50% in southern east Asia where genotypes B and C circulate. In Chinese patients infected with genotype B, loss of HBeAg occurs earlier and more frequently than in those infected with genotype C [87]. On the other hand, in regions where subgenotype A2 is found, such as northern

America and Europe only 10% of the chronic HBV carriers are HBeAg-negative. HBeAg loss occurs in native Alaskans infected with subgenotype A2, genotypes B, D and F before the age of 20, whereas in those infected with genotype C it occurs at 40 years or older [88]. Ghanaian blood donors, infected with genotype E with normal ALT, had lower frequency of HBeAg-positivity (25%) [89] compared to Taiwanese blood donors infected with genotype B (30%) or C (41%) [90]. The HBeAg-positive blood donors infected with genotype E

had a median age of 22 years [89] and were 10 years younger than those infected with either genotype B or C [90]. The frequency of HBeAg-positivity in genotype E infected blood donors, with normal ALT, remained stable between the ages 16 and 52 years, whereas it decreased in the blood donors with elevated ALT [89]. Likewise, chronic hepatitis B Chinese patients showed higher HBeAg-positivity when infected with genotype B (53%) or genotype C (69%) compared to Ghanaians chronically infected with genotype E (34%) [89]. This difference in frequency of HBeAg-positivity was highly significant in individuals younger than 30 years [89]. Similarly, as already stated, relatively earlier seroconversion occurs in patients infected with subgenotype A1 compared to those infected with other (sub) genotypes A2 and D [86].

In a comprehensive analysis, Ott *et al.* (2012) estimated the prevalence of HBeAg-positivity in females from 21 regions of the world in 1990 and 2005 [91]. In 1990, the highest prevalence was seen in girls aged 0 to 9 years (55%–91%) and decreased to 12%–16% in the 60–69 year age group. In the childbearing age group of 20–29 years the levels ranged from 30% to 43%. The highest levels in all age groups were found in Oceania (91% in the 0–9 age group), with the lowest levels in southern and western sub-Saharan Africa (55%–62%) [91]. Although the trends were similar in 2005, there were some changes. The prevalence dropped most dramatically in the birth to 9 year olds in Oceania (~23%) with minimal or no decrease in southern (7%) and western (0.2%) sub-Saharan Africa, respectively. The reduction evident in some geographical regions was a result of the good implementation of successful vaccination programs [91].

The expression of HBeAg is one of the major factors influencing the frequency and mode of transmission of HBV [92].

FREQUENCY OF TRANSMISSION

In a study carried out in Iran [93], where a data-mining approach was used, the overall transmission rate was 15.7% (5.4% and 27.3% for male and female index cases, respectively). The frequency of transmission was more than double for HBeAg-positive females (49%) compared to HBeAg-negative females (23.4%). There was a lower intrafamilial transmission rate of HBV in patients with hepatitis D virus (HDV) co-infection, being

statistically significant between patients positive and negative for anti-HDV antibody. This was most pronounced for HBeAg-negative female index cases, where the frequency of transmission was only 5% in those who were anti-HDV-positive compared to 25% in anti-HDV-negative females [93]. A number of factors can influence the interaction of HBV and HDV including HBV genotype. Interference of HBV replication by HDV was more apparent in patients infected with HBV genotype A compared to those infected with genotypes D or E [94]. The prevalence and characteristics of HDV infection in the pediatric population have not been widely researched.

MODES OF TRANSMISSION OF HBV IN CHILDREN

Children can be infected ante-natally, peri-natally or horizontally. Mother-to-child transmission (MTCT) can occur by all three modes:

- (1) **Ante-natal transmission** or infection *in utero* occurs in the third trimester of pregnancy possibly when HBV, from maternal blood, traverses the placenta or as a result of placental leakages, infects the foetus [27,95]. The presence of HBV in placental villous capillary endothelial cells [96] and trophoblastic cells [97] implicates “cellular transfer” of HBV from mother to foetus. This intrauterine transmission, occurs infrequently and accounts for <5% of MTCTs from HBsAg-positive, HBeAg-positive mothers [29]. Maternal blood levels of HBV DNA of $>10^8$ copies/ml [97] and HBeAg-positivity [96] were shown to be a risk factors for intrauterine transmission of HBV. Moreover, a direct correlation between maternal HBV DNA levels and those in cord blood exists [97]. The administration of hepatitis B immune globulin (HBIG) from 28 weeks gestation onwards reduced this risk of transmission [98]. However, this mode of transmission cannot be prevented by the administration of both HBIG and vaccine after birth [99].
- (2) **Perinatal transmission** occurs at or near the time of delivery (28 weeks of gestation up to 7 days after birth) by percutaneous and/or permucosal exposure to maternally infected fluids in the birth canal [100,101]. HBsAg has been detected in 33% of amniotic fluid samples, 50% of cord blood samples, 98% of vaginal fluid samples, 71% of breast milk samples and

95.3% of samples of gastric contents from newborns [102]. However, only a minority of amniotic fluid and cord blood samples were shown to be HBV DNA-positive [103]. Perinatal transmission is the major mode of transmission in geographical regions where there is a high frequency of HBeAg-positivity in females at gestational age. HBeAg is associated with higher viral loads and acts as a tolerogen and immunogen [20,21]; thus, 70% to 90% of babies born to HBeAg-positive mothers will become chronic carriers of the virus within 3 months of birth [104–110]. In contrast, chronic infection occurs in only 10% of babies born to mothers who are HBeAg-negative [111].

- (3) **Post-natal or horizontal transmission** occurs parenterally via apparent or inapparent percutaneous or permucosal exposure to bodily fluids [35]. In addition to blood, saliva, urine and seminal fluid have also been implicated in transmission of HBV [112]. Horizontal transmission can be intra- and inter-familial [113], iatrogenic, by the indiscriminate use of injections with non-sterilized equipment and as a result of cultural practices, including scarification and tattooing [40,114,115]. MTCT can occur during childcare activities including breastfeeding [116], although no difference was noted between transmission from HBsAg-positive mothers to infants that were breast-fed compared to those that were bottle-fed [102]. Children infected perinatally can be a source of infection for siblings and playmates [116–119]. The risk of infection among children increases with age [113]. The behaviors implicated in intra-familial transmission include sharing of bath towels, sharing of chewing gum or partially eaten sweets, sharing of toothbrushes and biting of fingernails in conjunction with scratching the backs of carriers [113]. HBV exposure during childhood can lead to a large proportion of adolescents being infected by the time they reach the age of sexual maturity, when sexual transmission becomes the dominant route of transmission [120,121]. In low incidence regions of HBV, transmission mainly occurs horizontally, iatrogenically in health care personnel, drug addicts and by sexual contact in heterosexual couples or men-who-have-sex-with men [116].

THE INFLUENCE OF GENOTYPES AND SUBGENOTYPES ON MODE OF TRANSMISSION

HBV is classified in at least nine genotypes and at least 35 subgenotypes, which can have distinct geographical distributions. The different (sub) genotypes can develop various mutations that can affect HBeAg expression, which can determine the dominant mode of transmission and natural history of infection depending on the frequency of HBeAg-positivity in a population. Even before (sub) genotypes were defined and their geographical distribution well known, it was recognized that the frequency and mode of transmission of HBV varied in the different ethnic groups around the world and that these were influenced by the frequency of HBeAg-positivity [107].

A German study showed that three times as many west Asian HBsAg-positive carriers were HBeAg-positive compared to those of European descent [122]. Similarly, Chinese mothers in Singapore and Malaysia were more likely to be HBeAg-positive compared to either Indian or Malay mothers [123,124]. A higher percentage of HBsAg-positive mothers were HBeAg-positive and had higher viraemia in south east Asia (40%) compared to mothers in Africa (5%) [125]. MTCT occurs in 40%–64% Chinese mother–infant pairs, but only in 30% and 10% of their African and European counterparts, respectively [25,126]. Differences can also be observed within a single locale and is dependent on ethnicity. In a study carried out in Thrace, Greece, children of Turkish descent were more likely to be infected peri-natally (61.8%) compared to native Greek children (39%) and immigrant children from the former Union of Soviet Socialist Republic (USSR) (22%), who were infected by percutaneous exposure [127]. Horizontal MTCT occurs frequently in the Chinese at 6 weeks to 3 months after birth [128,129], whereas in other regions such as Senegal [130] and Saudi Arabia [131] transmission occurs later in infancy and childhood. Thus perinatal MTCT can account for 50% of chronic infections in endemic regions of Asia and the Pacific Islands [46] whereas it is less frequent in Africa, the other geographical region, where HBV is endemic and horizontal transmission dominates [40].

Today we know that these differences can be accounted for because of the distribution of different (sub) genotypes in the various ethnic groups

and geographical regions (Figure 7). In south east Asia, the prevalent genotypes are B and C and HBeAg to anti-HBe seroconversion, in 90% of carriers of these genotypes, occurs at the mean age of 30–35 years [132–134]. Thus women of gestational age are frequently HBeAg-positive, have high viral loads and consequently MTCT is frequent [87,90]. On the other hand, in Africa where the prevalent genotypes are subgenotype A1, genotypes D and E [40], carriers of these genotypes seroconvert earlier than the average gestational age. Thus MTCT occurs infrequently in less than 10% of the cases [135–139], but also associated with HBeAg-positivity and high viral loads [136].

THE INFLUENCE OF GENOTYPES AND SUBGENOTYPES ON THE NATURAL HISTORY OF HBV INFECTION IN CHILDREN

The few studies that have looked at the natural history of HBV infection in children have demonstrated geographical variations that can also be attributed to the different distributions of (sub) genotypes and their influence of HBeAg expression.

Taiwanese children of mothers, who were older than 40 years when they lost HBeAg, had delayed HBeAg seroconversion [140]. The presence of maternal HBeAg but not HBsAg at birth, delayed the children's HBeAg seroconversion significantly and this delay was enhanced when maternal HBeAg persisted [140].

HBeAg expression also determines whether acute HBV infection develops into a chronic infection; thus, a necessary prerequisite is that the strains of HBV infecting an individual express HBeAg and thus have a wild-type *BCP/PC* region [25,141,142]. Wild-type at position 1896 of the precore region is the strongest predictor of MTCT in mothers infected with genotype E [136]. However, transmission of strains carrying G1896A can occur perinatally [143]. Genotype C patients, compared to genotype B patients, have a delayed HBeAg seroconversion in the immune clearance phase of chronic HBV infection, which may contribute to a more progressive liver disease and more refractory response to antiviral therapy [43].

PREVENTION OF HBV INFECTION AND ERADICATION OF HBV

Since the early 1980s, there has been a successful vaccine against HBV, which can prevent HBV infection by eliciting an immune response in 95% of

vaccinees [144]. The success of this vaccine was first demonstrated in Taiwan, which was one of the first countries to implement universal vaccination. HBsAg carrier rates in children decreased from 10% in 1984 to <1% in 2004, with a concomitant 68% decrease of acute liver failure in infants younger than 12 months, and a 75% reduction of HCC in children aged 6 to 14 years [42,145]. However, despite the effectiveness of this vaccine and the implementation of universal vaccination, following a recommendation by the World Health Organization (WHO) in the early 1990s, in over 184 countries, eradication of HBV is still not on the horizon.

To reduce MTCT the WHO recommends administration of both the vaccine and HBIG within 24 h of birth, which reduce transmission by 90% – 98% [1]. Children of HBeAg-positive mothers are at greater risk of developing chronic hepatitis even after immunization [146], and although administration of HBIG born to HBeAg-negative mothers did not reduce the rate of chronic hepatitis, it can prevent infantile acute liver failure [146].

Active and passive immunoprophylaxis in children cannot prevent MTCT in 10% of children [147]. The reasons for this outcome include:

- (1) Vaccination failure, often as a result of
 - (a) incomplete or delayed vaccination [148]
 - (b) high HBV DNA levels in mothers leading to *in utero* transmission.
 - (c) Vaccine escape mutations [148]
 - (d) Low response to vaccine and/or waning anti-HBs levels [149,150]
- (2) Occult infection

Occult HBV infection is defined as the presence of HBV DNA in the liver (with and without HBV DNA detected in the serum) in HBsAg-negative individuals [151]

- (3) Reactivation of HBV infection, often as a result of immunosuppression.
- (4) Immigration into countries with low HBsAg prevalence, where universal vaccination has not been implemented. This has led to a change in the epidemiological profile, with the prevalence of HBsAg, HBeAg and the genotype of HBV in immigrant children reflecting that of their country of origin [62,152].

The unprecedented human migrations, which the world is currently facing as a result of the

refugee crisis, will result in the change of the geographical distribution of HBV genotypes and subgenotypes and a concomitant change in the natural history of HBV infection in different regions. Considering that the (sub) genotypes can affect the frequency and rate of HBeAg seroconversion, knowledge of the (sub) genotypes circulating in a region, as well as the mutations found in strains from infected children, can inform on better prevention, management and treatment options, which can be customized to a certain degree. In order to effectively manage HBV infection and to respond to the call of the inaugural World Hepatitis Summit of September 2015 [153], for the global eradication of HBV by 2030, it is necessary that pediatricians understand the natural history of the infection, particularly the course of spontaneous HBeAg seroconversion.

A priority for all pediatricians and maternity service providers should be the implementation of the prevention recommendations of the WHO for all neonates to receive the first dose of hepatitis B vaccine within 24 h of birth. This is essential in all regions of the world, especially regions such as Africa, where HBV is still hyperendemic and vaccine coverage is not optimal.

CONFLICT OF INTEREST

The authors have no competing interest

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