To the editor.

Chronic liver diseases such as chronic hepatitis B, cirrhosis, and liver cancer caused by hepatitis B virus (HBV) infection pose a serious threat to public health. Mother-to-child transmission is one of the main routes of HBV dissemination. So far, immunoprophylaxis is an important strategy for preventing and controlling HBV infection.

In China, the chronic HBV infection rate in children gradually decreased due to the universal implementation of the hepatitis B vaccine strategy since 1992. However, some studies have reported that mutations within the major hydrophilic region (MHR) region within the surface gene of HBV result in immune escape and contribute to immunoprophylaxis failure. Moreover, the genotype distribution is unequal in different areas in China.

The HBV genotypes and surface gene mutation among children born after the universal HBV vaccination program have not been investigated in Huzhou, China. Thus, the present study was made up to delineate the molecular characteristics in vaccinated children with HBsAg positive in this area.

Materials and Methods. In the present study, 58 children vaccinated with HBsAg positive from the serum HBsAg screening were enrolled among 7342 children in the Huzhou Central Hospital. The study was approved by the ethics committee of Huzhou Central Hospital in accordance with the ethical guidelines of the Declaration of Helsinki.

Routine HBV serological markers, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were detected in the Department of Laboratory Medicine. HBV DNA was quantified, and the HBV surface gene region was amplified and sequenced as previously described methods. Genotyping of HBV was performed by using an online tool (https://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi). Serotype was determined as previously described. In addition, the amino acid (AA) substitutions in the surface gene region were analyzed in comparison to the standard reference HBV isolates obtained from GenBank (Genotype B: AB073846, AB602818, D00329; Genotype C: AB014381, KY123041, X04615) by using MEGA 7.0 software.

Results. Fifty-six samples were sequenced successfully. The characteristics of the HBV-infected children are described in Table 1. Among these children, 45 cases were infected with HBV genotype B, and 11 were infected with HBV genotype C. There were no significant differences between children HBV infected with genotypes B and C at demographic and virological characteristics. Furthermore, three serotypes were found in the present study: adw (42), adr (11), and ayw (3).

Eighteen AA substitution sites in the MHR region were identified in 21 of 56 children (37.5%). Among these sites, 12 AA substitutions were found within the 'a' determinant. The mutation rate in the 'a' determinant region was 28.6% (16/56); these substitutions included K122R, I126T, Q129H, P142H, and T143M (Table 2). It’s worth noting that eight children HBsAb positive (>10 mIU/mL) were infected with HBV; seven of these eight children (87.5%) were >9 years old. Among these HBV isolates, four (50%, 4/8) showed AA substitutions (P127T, T140I, T143M, G145R) within the 'a' determinant.

When we compared mutations within MHR between genotype B and genotype C, we found mutations in 33.3% of (15/45) children infected with genotype B and in 27.2% (3/11) children infected with genotype C. However, no statistical difference was found (P=0.702). Furthermore, frequencies of mutations in different regions between these two genotypes had no statistical differences (P>0.05) (Table 2).

The characteristics were compared between the children with and without MHR mutations (Table 3). Most children in the group without MHR mutations...
Table 1. Characteristics of HBV infected children.

| Characteristic                        | Total (n=58) | Genotype B (n=45) | Genotype C (n=11) | P value |
|---------------------------------------|--------------|-------------------|-------------------|---------|
| Age (years), Mean±SD                 | 11.4±3.4     | 11.2±3.3          | 12.2±3.8          | 0.384   |
| Gender (Male/Female)                 | 36/22        | 27/18             | 8/3               | 0.508   |
| Anti-HBs antibody (<10IU/mL>/10IU/mL) | 50/8         | 38/7              | 10/1              | 1.000   |
| HBeAg status (Positive/Negative)     | 51/7         | 40/5              | 9/2               | 0.614   |
| HBV DNA (log10IU/mL), Mean±SD       | 7.5±1.5      | 7.4±1.5           | 7.7±1.7           | 0.666   |
| ALT(U/L), Median(P25, P75)           | 27.7(20.1,42.5) | 28.4(19.5,45.6) | 24.0(21.6,36.9) | 0.657   |
| AST(U/L), Median(P25, P75)           | 26.4(20.7,35.2) | 28.0(20.8,35.5) | 21.8(19.0,30.6) | 0.252   |
| Clinical diagnosis(ASC/CHB)          | 45/13        | 33/12             | 10/1              | 0.426   |

Abbreviations: ASC, asymptomatic HBV carrier; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen.

Table 2. Amino acid substitutions in S region.

| Region in S region | Amino acid substitutions sites (n) | Substitutions frequencies (%) | P value |
|--------------------|-----------------------------------|-------------------------------|---------|
|                    | Genotype B (n=45) | Genotype C (n=11) | Genotype B (n=45) | Genotype C (n=11) |
| N-terminal region(1-99) | N3D/E/I(4/3/1), I4C/T(4/1), ASC/G/S(1/3/1), S6A/C/D/T(6/2/2/1), G7A/R(3/14), L21S(1), I25V(1), N40S(2), G7R(3), R24K(1), T47V(1), S61L(1), P62*(1), T47E(2), S53L(1), I68T(1) | S3N(7), T5A(1), G7R(3), R24K(1), T47V(1), S61L(1), P62*(1), T47E(2), S53L(1), I68T(1) | 1.46 | 1.84 | 0.339 |
| MHR region(100-169) | I110L(1), K122R(3), P127T/S(1/1), Q129H(2), T131N(1), M133L(1), T140I(2), K141N(1), P142H(2), T143M(2), D144A(1), G145R(1), K160N(1), Y161S/F/T(1/1/1), L162Y(1), W163G(1), R169S(1), I126T(2), R160K(1), F161Y(1), V168F(1) | I126T(2), R160K(1), F161Y(1), V168F(1) | 0.83 | 0.65 | 0.820 |
| A-determinant(124-147) | K122R(3), P127T/S(1/1), Q129H(2), T131N(1), M133L(1), T140I(2), K141N(1), P142H(2), T143M(2), D144A(1), G145R(1), K160N(1), Y161S/F/T(1/1/1), L162Y(1), W163G(1), R169S(1), I126T(2), R160K(1), F161Y(1), V168F(1) | I126T(2), R160K(1), F161Y(1), V168F(1) | 0.83 | 0.65 | 0.820 |
| The first loop region(124-138) | K122R(3), P127T/S(1/1), Q129H(2), | | | | | 0.274 |

www.mjhid.org Mediterr J Hematol Infect Dis 2022; 14; e2022061 Pag. 2 / 5
were boys (71.4%, 35/35), whereas, in the group with MHR mutations, only 47.6% (10/21) of children were boys. Additionally, no statistical differences were observed in the other factors, including AST, ALT levels; HBeAg status; and proportion of genotype B between the two groups ($P > 0.05$).

**Discussion.** The present results showed that the predominant HBV serotypes and genotypes were adw and B among children in the Huzhou area. It differed from our previous study, which showed that the distribution of HBV genotype B was in 43.5% (78/179) of HBV-infected adults in the same area.\(^9\) Considering the children in this study were all vaccinated and adults in our previous study were all not vaccinated, we assume that the B genotype of HBV may be more able to infect children vaccinated. Zheng et al.\(^10\) reported that most HBV infections in vaccinated Chinese blood donors were genotype B, which supports this suppose. On the contrary, genotype C may lead to a higher rate of HBV breakthrough infection than genotype B, as reported in a Taiwan study.\(^11\) However, more evidence must be collected to clarify the correlation between HBV genotype and HBV infection after vaccination.

MHR region is the main B-cell epitope, which may affect antibody immunogenicity. The 'a' determinant within MHR is the determinant antigen, and the target for the neutralizing antibody produced after the vaccine, many AA substitutions in the 'a' determinant affect the binding of neutralizing antibodies.\(^12\) In the present study, 21 of 56 children (37.5%) were found to have AA substitutions in MHR. Furthermore, 28.6% (16/56) of children harbored mutations in the 'a' determinant. Many previous studies confirmed that mutations in MHR, especially within the 'a' determinant, contributed to immune escape of vaccine.\(^13,14\) Therefore, present findings suggest that the risk of transmission of mutant HBV still exists in the Huzhou area. However, to our

---

**Table 3. Relationship between MHR mutations and clinical characteristics**

|                          | Children with MHR mutations n=21 | Children without MHR mutations n=35 | $P$ value |
|--------------------------|----------------------------------|------------------------------------|-----------|
| Age (years), Mean±SD     | 10.8±3.3                         | 11.7±3.4                           | 0.299     |
| Gender (male/female)     | 10/11                            | 25/10                              | 0.075     |
| Anti-HBs antibody (positive/negative) | 4/17                             | 4/31                               | 0.456     |
| HBeAg status (positive/negative) | 18/3                             | 31/4                               | 1.000     |
| HBV DNA (log10IU/mL), Mean±SD      | 7.3±1.5                          | 7.6±1.6                            | 0.589     |
| Genotype(B/C)            | 18/3                             | 27/8                               | 0.664     |
| ALT(U/L), Median(Q1, Q3) | 22.0(18.5,57.6)                   | 29.0(21.6,38.1)                    | 0.393     |
| AST(U/L), Median(Q1, Q3) | 27.3(18.9,38.3)                   | 26.3(21.0,35.2)                    | 0.906     |

**Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e antigen.
knowledge, the K141N and P142H substitution found in this study was not mentioned previously; if these two mutations could lead to vaccine immune escape, further investigation needs.

The most common immune escape mutant G145R/A is in the second loop of the 'a' determinant. However, the G145R mutation was only found in one child in this study, indicating this mutation is not common in Huzhou. Of note, the proportion of girls (52.4%, 11/21) in children with MHR mutations was higher than that in children without MHR mutations (28.6%, 10/35). However, the difference was not statistically significant (P=0.075) due to the relative sample size. However, few studies focus on this issue. Whether girls are more susceptible to HBV infection with mutant HBV deserves further investigation.

Anti-HBs can neutralize the HBsAg and eliminate the HBV infection, a protective marker in vaccine recipients. But in the present study, eight children with positive level anti-HBs (<100mIU/mL) were infected with HBV, suggesting that presence of low-level anti-HBs could not completely prevent HBV infection. Furthermore, seven of eight children (87%) were older than nine years; this result was consistent with other studies, which revealed that the anti-HBs levels gradually decreased with age in some vaccinated children.15-17 Previous studies have shown that people with anti-HBs remain at risk of HBV infection.18-20 Another research reported that the incidence of occult HB infection (OBI) in infants with low anti-HBs (<100mIU/mL) was significantly higher than that in non-vaccinate infants, indicating the occurrence of OBI in infants may be due to the limited neutralizing capacity provided by low anti-HBs titers.21 Therefore, we recommend that it is necessary to monitor and strengthen immunization in children with low-level anti-HBs to reduce the risk of HBV infection.

In summary, the present findings suggest that genotype B is the predominant genotype in children. It may be associated with the threat of HBV infection in vaccinated children, MHR mutations, and decreased levels anti-HBs in Huzhou. Further long-term prospective observation and functional analysis of mutant HBV strains in vitro and in vivo experiments are needed to confirm the findings in the present study. Nevertheless, the results may help a different vaccine improvement strategy, prevention, and control of HBV infection in children.

Acknowledgments: This work was supported by Huzhou Municipal Science and Technology Bureau [grant number. 2020GY02].

Correspondence to: Prof. Fuchu Qian, Department of Precision Medicine, Huzhou Central Hospital, Affiliated Central Hospital Huzhou University, Huzhou, China. Tel: (+86)-572-2819062. E-mail: qfc313009@126.com

Competing interests: The authors declare no conflict of Interest.

References:

1. Pan, C. Q.; Duan, Z. P.; Bhamidimarri, K. R., et al. An algorithm for risk assessment and intervention of mother to child transmission of hepatitis B virus. Clinical Gastroenterology and Hepatology: The Official Clinical Practice Journal of the American Gastroenterological Association. 2012, 10, 452-459. https://doi.org/10.1016/j.cgh.2011.10.041 PMID:22079509

2. Jia, J. D.; Hou, J. L.; Wei, L.; Zhuang, H. [Highlights of the guidelines of prevention and treatment for chronic hepatitis B (2019 version)], Zhonghua gan zang bing za zhi = Zhonghua ganziang bang za zhi = Chinese Journal of Hepatology. 2020, 28, 21-23. https://doi.org/10.3760/cma.j.issn.1007-3418.2020.01.006 PMID:32024639

3. Cui, F.; Shen, L.; Li, L., et al. Prevention of Chronic Hepatitis B after 3 Decades of Escalating Vaccination Policy, China. Emerging Infectious Diseases. 2017, 23, 765-772. https://doi.org/10.3201/eid2305.161477 PMID:28418296 PMCID:PMC5403029

4. Coleman, P. F. Detecting hepatitis B surface antigen mutants. Emerging Infectious Diseases. 2006, 12, 198-203. https://doi.org/10.3201/eid1203.050038 PMID:16494742

5. Harrison, T. J.; Hopes, E. A.; Oon, C. J.; Zanetti, A. R.; Zuckerman, A. J. Independent emergence of a vaccine-induced escape mutant of hepatitis B virus. Journal of Hepatology. 1991, 13 Suppl 4, S105-S107. https://doi.org/10.1016/0168-8278(91)90037-c PMID:1726888.

6. Li, H. M.; Wang, J. Q.; Wang, R., et al. Hepatitis B virus genotypes and genome characteristics in China, World Journal of Gastroenterology. 2015, 21, 6684-6697. https://doi.org/10.3748/wjg.v21.i21.6684 PMID:26074707

7. Qian, F.; Zou, W.; Jin, F.; Li, D.; Shen, Y. Prevalence of Potential Resistance Related Variants Among Chinese Chronic Hepatitis B Patients Not Receiving Nucleos(T)ide Analogues. Infection and Drug Resistance. 2020, 13, 2407-2416. https://doi.org/10.2147/IDR.S249476 PMID:32765014; PMCID: PMC7381783.

8. Velkov, S.; Protzer, U.; Michier, T. Global Occurrence of Clinically Relevant Hepatitis B Virus Variants as Found by Analysis of Publicly

Fang Jin1,2*, Dongli Li1,2*, Chenxin Yan3, Weihua Zou4 and Fuchu Qian1,2.*

1. Department of Precision Medicine, Huzhou Central Hospital, Affiliated Central Hospital Huzhou University, Huzhou, China.
2. Department of Precision Medicine, Affiliated Huzhou Hospital Zhejiang University School of Medicine, Huzhou, China.
3. Shulan International Medicine College of Zhejiang Shuren University, Hangzhou, China.
4. Department of Laboratory Medicine, Huzhou Central Hospital, Affiliated Central Hospital Huzhou University, Huzhou, China.

# These authors contributed equally to this work.

References:

1. Pan, C. Q.; Duan, Z. P.; Bhamidimarri, K. R., et al. An algorithm for risk assessment and intervention of mother to child transmission of hepatitis B virus. Clinical Gastroenterology and Hepatology: The Official Clinical Practice Journal of the American Gastroenterological Association. 2012, 10, 452-459. https://doi.org/10.1016/j.cgh.2011.10.041 PMID:22079509

2. Jia, J. D.; Hou, J. L.; Wei, L.; Zhuang, H. [Highlights of the guidelines of prevention and treatment for chronic hepatitis B (2019 version)], Zhonghua gan zang bing za zhi = Zhonghua ganziang bang za zhi = Chinese Journal of Hepatology. 2020, 28, 21-23. https://doi.org/10.3760/cma.j.issn.1007-3418.2020.01.006 PMID:32024639

3. Cui, F.; Shen, L.; Li, L., et al. Prevention of Chronic Hepatitis B after 3 Decades of Escalating Vaccination Policy, China. Emerging Infectious Diseases. 2017, 23, 765-772. https://doi.org/10.3201/eid2305.161477 PMID:28418296 PMCID:PMC5403029

4. Coleman, P. F. Detecting hepatitis B surface antigen mutants. Emerging Infectious Diseases. 2006, 12, 198-203. https://doi.org/10.3201/eid1203.050038 PMID:16494742

5. Harrison, T. J.; Hopes, E. A.; Oon, C. J.; Zanetti, A. R.; Zuckerman, A. J. Independent emergence of a vaccine-induced escape mutant of hepatitis B virus. Journal of Hepatology. 1991, 13 Suppl 4, S105-S107. https://doi.org/10.1016/0168-8278(91)90037-c PMID:1726888.

6. Li, H. M.; Wang, J. Q.; Wang, R., et al. Hepatitis B virus genotypes and genome characteristics in China, World Journal of Gastroenterology. 2015, 21, 6684-6697. https://doi.org/10.3748/wjg.v21.i21.6684 PMID:26074707

7. Qian, F.; Zou, W.; Jin, F.; Li, D.; Shen, Y. Prevalence of Potential Resistance Related Variants Among Chinese Chronic Hepatitis B Patients Not Receiving Nucleos(T)ide Analogues. Infection and Drug Resistance. 2020, 13, 2407-2416. https://doi.org/10.2147/IDR.S249476 PMID:32765014; PMCID: PMC7381783.

8. Velkov, S.; Protzer, U.; Michier, T. Global Occurrence of Clinically Relevant Hepatitis B Virus Variants as Found by Analysis of Publicly

www.mjhid.org Mediterr J Hematol Infect Dis 2022; 14; e2022061
9. Qian, F.; Qin, J.; s., Y.; L., D. Distribution of Hepatitis B virus in the patients from Huzhou area. Zhejiang J Prev Med (chinese). 2009, 21(11), 6-14.

10. Zheng, X.; Ye, X.; Du, P., et al. High prevalence of anti-hepatitis B core antigen in hepatitis B virus-vaccinated Chinese blood donors suggests insufficient protection but little threat to the blood supply. Transfusion. 2015, 55, 890-897. https://doi.org/10.1111/trf.12902 PMID: 25363504

11. Wen, W. H.; Chen, H. L.; Ni, Y. H., et al. Secular trend of the viral genotype distribution in children with chronic hepatitis B virus infection after universal infant immunization. Hepatology (Baltimore, Md). 2011, 53, 429-436. https://doi.org/10.1002/hep.24061 PMID: 21274864.

12. Huang, Y.; Wang, B.; Peng, Z.; Tang, N.; Chen, W. Hepatitis B virus surface gene mutants in immunophrophylaxis-failed infants from Southern China. Journal of Medical Virology. 2019, 91, 1069-1075. https://doi.org/10.1002/jmv.25430 PMID: 30761578.

13. Coppola, N.; Onorato, L.; Minichini, C., et al. Clinical significance of hepatitis B surface antigen mutants. World Journal of Hepatology. 2015, 7, 2729-2739. https://doi.org/10.4240/wjh.v7.i27.2729 PMID: 2664816; PMCID: PMC4663392.

14. Hsu, H. Y.; Chang, M. H.; Ni, Y. H., et al. Chronologic changes in serum hepatitis B virus DNA, genotypes, surface antigen mutants and reverse transcriptase mutants during 25-year nationwide immunization in Taiwan. Journal of Viral Hepatitis. 2017, 24, 645-653. https://doi.org/10.1111/jvh.12987 PMID: 28182307.

15. Lin, X.; Yang, J.; Lu, H., et al. Minimization of hepatitis B infection among children in Jiangsu, China, 12years after integration of hepatitis B vaccine into the expanded program on immunization. Vaccine. 2016, 34, 6458-6463. https://doi.org/10.1016/j.vaccine.2016.11.022 PMID: 27866767.

16. Yue, X.; Ge, C.; Zhuge, S., et al. Changes and analysis of anti-HBs titres after primary immunization in 1- to 16-year-old Chinese children: A hospital-based study. Journal of Viral Hepatitis. 2018, 25, 373-380. https://doi.org/10.1111/jvh.12818 PMID: 29091317.

17. Zhu, Q.; Shao, X.; Chen, S., et al. Epidemiological serosurvey of hepatitis B virus among children aged 1-14 years in Guangdong Province, China. International Journal of Infectious Diseases: IJID: Official Publication of the International Society for Infectious Diseases. 2018, 71, 25-29. https://doi.org/10.1016/j.ijid.2018.01.027 PMID: 29408358.

18. Wang, Z.; Zeng, J.; Li, T., et al. Prevalence of hepatitis B surface antigen (HBsAg) in a blood donor population born prior to and after implementation of universal HBV vaccination in Shenzhen, China. BMC Infectious Diseases. 2016, 16, 498. https://doi.org/10.1186/s12879-016-1834-2 PMID: 27647214; PMCID: PMC5028969.

19. Ye, X.; Li, T.; Xu, X., et al. Characterisation and follow-up study of occult hepatitis B virus infection in anti-HBc-positive qualified blood donors in southern China. Blood Transfusion. 2017, 15, 6-12. https://doi.org/10.2450/2016.0268-15 PMID: 27416568; PMCID: PMC5269423.

20. Diarra B, Yonli AT, Sorgho PA, Compaore TR, Ouattara AK, Zongo WA, Tao I, Traore L, Soubeiga ST, Djigma FW, Obiri-Yeboah D, Nagalo BM, Pietra V, Sanogo R, Simpore J. Occult hepatitis B virus infection and associated genotypes among HBsAg-negative subjects in Burkina Faso. Mediter J Hematol Infect Dis. 2018 Jan 1;10(1):e2018007 https://doi.org/10.4084/MJHID.2018.007

21. Zhou, S.; Li, T.; Allam, J. P., et al. Low occurrence of HBsAg but high frequency of transient occult HBV infection in vaccinated and HBIG-administered infants born to HBsAg positive mothers, Journal of Medical Virology. 2017, 89, 2130-2137. https://doi.org/10.1002/jmv.24861 PMID: 28543299.