
EXPERIMENTAL WORKS

The Prevalence of HBsAg Subtypes and HBV Genotypes in Native Population Groups of Siberia

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Abstract—The prevalence of HBsAg and its subtypes and hepatitis B virus (HBV) genotypes in native population groups of five Siberian regions—the Republic of Altai, Kemerovo oblast, Irkutsk oblast, Yamalo-Nenets autonomous oblast (the YaNAO), and Krasnoyarsk krai (a total of 5657 samples)—was studied. Statistically significant differences were found in these groups for the studied HBV markers and types. HBsAg was the most prevalent in Altaians in Republic of Altai (13.4% of samples); Dolgans and Nganasans in Krasnoyarsk krai (13.2%); Teleuts in Kemerovo oblast (10.2%); and Buryats in Irkutsk oblast (5.4–8.2%). HBsAg prevalence was minor in Khants, Komi, Nenets and Selkups in YaNAO (0.7–1.7%). The study of the partial HBsAg-gene sequences of 143 HBV isolates from different groups of the five Siberian regions revealed that 130 (90.9%) belonged to genotype D (subtypes ayw2 and ayw3), 10 (7%) to genotype C (subtype adrq+), and 3 (2.1%) to genotype A (subtype adw2). Of the 130 genotype D isolates, 120 belonged to subgenotypes D1 (34.3%), D2 (22.4%), and D3 (27.3%), while the genotype was not determined in 10 (7%) isolates. Subgenotype D1 (HBsAg subtype ayw2) was prevalent among Kazakhs in the Republic of Altai (85.7% of the strains), Teleuts in Kemerovo oblast (60.0%), Russians in Irkutsk oblast (87.5%), and Dolgans and Nganasans in Krasnoyarsk krai (68.8%). Subgenotype D2 (subtype ayw3) was prevalent among Khants and Komi (75%) and Nentsi (57.1%) from the YaNAO. Subgenotype D3 (subtype ayw2) was prevalent in Altaians in the Republic of Altai (76.2%) and Buryats from Irkutsk oblast (50%). Significant prevalence of genotype C (subgenotype C1, 18.8%) was first found in the Far North of Siberia (Taimyr Peninsula, Krasnoyarsk krai). On the basis of the obtained data, we suggested that there exist several epidemiologically different sources of HBV expansion in native Siberian population groups.

Keywords: hepatitis, hepatitis B, *Hepadnaviridae*, native population, subtype, genotype

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INTRODUCTION

Hepatitis B virus (HBV) is a member of *Hepadnaviridae*. The circular genome of HBV is DNA having a size of 3.2 kb. Differences in the structure of HBsAg surface antigen (hepatitis B surface antigen, which is the main protein diagnostic marker of hepatitis B) allow nine antigen subtypes of this protein to be distinguished: ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq+, and adrq- [16, 20, 28, 29]. Amino acid substitutions determining belonging in each subtype have

been described [36, 37, 42]. Based on differences in the sequences of genomic DNA of virus isolates, phylogenetic classification of human HBV isolates allows them to be divided into eight genotypes (A–H) [14, 33, 36, 38, 39, 43, 48] and not less than 24 HBV subgenotypes (A1, A2, etc.) [24, 40, 41, 44]. New works reporting the discovery of new HBV subgenotypes are regularly published [17, 31, 32, 46, 51]. However, according to some specialists [47], the taxonomic classification of some of them needs to be confirmed.

Serological and phylogenetic classification of HBV are complementary for researchers and used if the antigenic characteristics of this pathogen are studied (e.g., for the purpose of issues of preventive vaccination and immune diagnostics of HBV) or genetic (including research on the issues of HBV evolution or epidemic chains in cases of contamination). Furthermore, the same HBV genotype can be attributable to several subtypes [41].

The prevalences of genotypes, HBV subgenotypes, and HBsAg subtypes in different geographic regions are variable [19, 20, 41]. Genotype A (HBsAg subtypes ayw1 and adw2) is most common for the countries of Northwestern Europe, North America (subgenotype A1 prevails), and Africa (subgenotype A2). Genotypes B (ayw1 and adw2) and C (mostly adr, adrq+/-, ayr, and adw2) prevail in Southeast Asia and Oceania. At the same time, subgenotype B1 is the most common for Japan; B2 and B4 for China and Vietnam; B3 for Indonesia and Polynesia; C1 and C2 for East Asia; and C3 and C4 for Oceania, Australia, and New Zealand. Genotype D (ayw2, ayw3, and adw2) is the most common on the global scale and predominates in the countries of the Mediterranean Basin, the Middle East, India, and Russia. Genotype E (ayw4) is the most common for the countries of West Africa, and genotype F (ayw4 and adw4) for Central and South America [41], and also cited by 27]. Several isolates of genotype G (adw2) have been identified in North America and Western Europe [48], while sporadic prevalence of genotype H (adw4) has been described for Central America and California [14].

Genotype D (HBsAg subtypes ayw2 and ayw3) predominates in the Russian Federation. It includes 85% of HBV isolates from Moscow [13], 100% of HBV isolates from Samara [23], 98% of isolates from Novosibirsk oblast (Koltsovo and Novosibirsk) and Barnaul [2–4], 100% of isolates from the YaNAO [8], and 95% of isolates from Irkutsk oblast [9]. Genotype D is also observed in Belarus (up to 89%) [45], Estonia (81%) [49], Uzbekistan (according to the data of different authors, from 69 to 87%) [15, 25], and Tajikistan (94%) [26]. Subgenotype D2 is predominant in Russia and neighboring countries [50]. However, subgenotypes D1 and D3 also circulate on this territory [9, 45].

In our previous research [8–11, 34], significant differences were noted in the prevalence of HBsAg and HBsAg subtypes and HBV subgenotypes in different groups of the native Siberian population. Research on the molecular genetic HBV variety circulating in almost completely isolated native populations that may have specific phylogenetic branches of this pathogen allow evolutionary differences in HBV populations in Siberia to be found. In the present scientific paper, we summarize the results of previous research and new data on studied groups of native populations of the Far North and southern, and eastern areas of Siberia.

MATERIALS AND METHODS

Samples. 5657 samples of blood plasma (hereinafter, samples) from native Siberian populations were studied (Table 1). Samples were collected in 1993–2006 during ethnogenetic expeditions to the regions listed in the tables. At the same time, we filled out and collected forms with name, gender, ethnicity, ethnicity of parents (if they were different), and current place of residence as described earlier in [8]. All samples were divided into groups according to the territorial and ethnic backgrounds of donors (Tables 1, 2). Data on the age and gender composition of groups are in Table 2. All persons studied gave prior written consent for their participation in the research. The research was accepted by the State Research Center for Virology and Biotechnology “Vector.”

ELISA. For HBsAg detection, we used JSC Vektor-Best kits (Koltsovo, Novosibirsk oblast).

PCR and sequencing. Total DNA was detected from 50 µL of blood plasma as described in [2]. *hep75b*, *hep73*, *hep3*, *hep33*, *hep4*, *hep34*, *HB2452S*, *hep38*, and *hep36M* primers were used for amplification of HBV genome regions together overlapping the *Pre-S1/PreS2/S* regions of the HBV genome followed by sequencing of the obtained fragments [35]. Nucleotide sequences of DNA fragments were detected via genetic analyzers and reagents from Applied Biosystems (United States).

Analysis of sequences. Obtained sequences were processed via the DNA STAR SeqMan program (United States) (<http://www.dnastar.com>) and aligned with corresponding regions of prototype sequences deposited in the GenBank database using the MegAlign program from the same manufacturer. Phylogenetic analysis was carried out in the PHYLIP (v. 3.53) package [22]. To construct a matrix of genetic distances in accordance with the algorithm of a 2-parametric program of the Kimura model, we used the DNADIST program; topology of phylogenetic trees was reconstructed via the UPGMA program using the NEIGHBOR program.

The studied DNA sequence of the HBV genome obtained during analysis of the sample (hereinafter, this sequence is referred to as “isolate”) was added to the concrete genotype and subgenotype when this isolate was placed on the branch of a phylogenetic tree including prototype isolates of only one genotype (subgenotype) and excluding isolates of other genetic groups. The HBsAg subtype of the isolate was determined from the results of comparison of a reconstructed amino acid sequence with descriptions of each of the known subtypes [36, 37, 42].

The nucleotide sequences obtained in the present research were deposited in the GenBank database with the unique codes JX090605–JX090724 and JX125364–JX125386.

Statistical data processing. All samples were separated into 5 groups by regions (oblast, republic, krai, and okrug) of donors and 12 groups of raions (Table 1).

Table 1. Prevalence of different HBsAg genotypes and subtypes in groups from Western Siberia. Summary data on every group and groups as a whole are indicated in black type

Repub- lic/oblast/ okrug/krai	Raion	Years of col- lection	Codes of samples	Main ethnicity	Sample numbers	HBSAO (+)	Number of iso- lates	HBV genotypes/subgenotypes						HBsAg subtypes		
								A	C	D1	D2	D3	D*	ayw2	ayw3	others
Republic of Altai	Kosh-Agach raion	1999– 2000	A-ZH	Kazakhs	194	10 5.2%	7	0	0	6 85.7%	0	0	1 14.3%	7 100%	0	0
	Ust-Kan raion	1993, 1995, 1997	A-MS	Altaians	231	31 13.4%	21	0	0	4 19.0%	1 4.8%	16 76.2%	0	20 95.2%	1 4.8%	0
All of Republic of Altai					425	41 9.6%	28	0	0	10 35.7%	1 3.6%	16 57.1%	1 3.6%	27 96.4%	1 3.6%	0
Kemerovo oblast	Belov raion	2003	Ke-BEK	Teleuts	137	14 10.2%	5	0	0	3 60.0%	0	0	2 40.0%	4 80.0%	0	1 20.0%
	All of Kemerovo oblast				137	14 10.2%	5	0	0	3 60.0%	0	0	2 40.0%	4 80.0%	0	1 20.0%
Irkutsk oblast	Alar raion	2005	I-AL	Buryats	487	40 8.2%	24	0	2 8.3%	2 8.3%	52 0.8%	12 50.0%	3 12.5%	14 58.3%	7 29.2%	3 12.5%
	Nuket raion	2006	I-NU	Buryats	654	35 5.4%	11	1 9.1%	1 9.1%	3 27.3%	4 36.4%	2 18.2%	0	3 27.3%	6 54.5%	2 18.2%
	Irkutsk raion	2003– 2005	I-IR	Russians	250	9 3.6%	8	0	0	7 87.5%	0	0	1 12.5%	8 100%	0	0
All of Irkutsk oblast					1391	84 6.0%	43	1 2.3%	3 7.0%	12 27.9%	9 20.9%	14 32.6%	4 9.3%	25 58.1%	13 30.2%	5 11.6%
The YaNAO	Shuryshkar raion	1999, 2000– 2002	Y-SH, Y-SHUR, Y-PIT	Khants, Komi	932	27 2.9%	20	0	0	1 5.0%	15 75.0%	2 10.0%	2 10.0%	5 25.0%	14 70.0%	1 5.0%
	Priural raion	2003– 2005	Y-PR	Nenets	1263	10 0.8%	5	0	0	1 20.0%	1 20.0%	2 40.0%	1 20.0%	3 60.0%	1 20.0%	1 20.0%
	Krasnosedkup raion	2006	Y-KR	Selkups	284	2 0.7%	1	0	1 100%	0	0	0	0	0	0	1 100%
	Purov raion	1992– 1993	Y-SAM	Nenets	704	12 1.7%	7	2 28.65%	0	0	0	4 57.1%	1 14.3%	2 28.6%	3 42.9%	2 28.6%
All of the YaNAO					3183	51 1.6%	33	2 6.1%	1 3.0%	2 6.1%	20 60.6%	5 15.2%	3 9.1%	18 54.5%	5 15.2%	7 21.9%
Krasno- yarsk krai	Dudin raion	2000	Kr-DU	Dolgans, Nganasans	408	54 13.2%	32	0	6 18.8%	22 68.8%	0	4 12.5%	0	5 78.1%	0	7 21.9%
	Turukhan raion	2000	Kr-TH	Kethes	113	7 6.2%	2	0	0	0	2 100%	0	0	0	2 100%	0
All of AO Krasnoyarsk krai					521	61 11.7%	34	0	6 17.6%	22 64.7%	2 5.9%	4 11.8%	0	25 73.5%	2 5.9%	7 20.6%
All in all					5657	251 4.4%	143	3 2.1%	10 7.0%	49 34.3%	32 22.4%	39 27.3%	10 7.0%	91 63.6%	34 23.8%	18 12.6%

Table 2. HBsAg carriers in age and gender groups

Region	Raion	Men	Women	<than 35	≥than 35	Comments
Republic of Altai	Kosh-Agach raion	6/106 5.7%	4/88 4.5%	7/139 5.0%	3/55 5.5%	There are no significant differences
	Ust-Kan raion	15/85 17.6%	16/146 11.0%	19/114 16.7%	12/117 10.3%	The same
All of Republic of Altai		21/191 11.0%	20/234 8.5%	26/253 10.3%	15/172 8.7%	There are no significant differences
Kemerovooblast	Belov raion	4/56 7.1%	10/81 12.3%	1/45 2.2%	13/92 14.1%	There are more people infected in the group of people older than 35 than in the group of people younger than 35 ($p > 0.95$)
All of Kemerovo oblast		4/56 7.1%	10/81 12.3%	1/45 2.2%	13/92 14.1%	The same
Irkutsk oblast	Alar raion	13/157 8.3%	27/330 8.2%	17/187 9.1%	23/300 7.7%	There are no significant differences
	Nukut raion	11/202 5.4%	24/452 5.3%	15/272 5.5%	20/382 5.2%	The same
	Irkutsk raion	2/96 2.1%	7/154 4.5%	6/138 4.3%	3/112 2.7%	The same
All of Irkutsk oblast		26/455 5.7%	58/936 6.2%	38/597 6.4%	46/794 5.8%	There are no significant differences
the YaNAO	Shuryshkar raion	7/245 2.9%	20/687 2.9%	14/425 3.3%	13/507 2.6%	There are no significant differences
	Priural raion	4/482 0.8%	6/781 0.8%	6/845 0.7%	4/418 1.0%	The same
	Krasnoselkup raion	2/113 1.8%	0/171 0%	1/206 0.5%	1/78 1.3%	The same
	Purov raion	8/269 3.0%	4/435 0.9%	9/427 2.1%	3/277 1.1%	The same
All of the YaNAO		21/1109 1.9%	30/2074 1.4%	30/1903 1.6%	21/1280 1.6%	The same
Krasnoyarsk krai	Dudin raion	23/170 13.5%	31/238 13.0%	37/325 11.4%	17/83 20.5%	There are more people infected in the group of people older than 35 than in the group of people younger than 35 ($p > 0.95$)
	Turukhan raion	5/54 9.3%	2/59 3.4%	0/63 0%	7/50 14.0%	The same ($p > 0.99$)
All of Krasnoyarsk krai		28/224 12.5%	33/297 11.1%	37/388 9.5%	24/133 18.0%	The same ($p > 0.975$)
All in all		100/2035 4.9%	151/3622 4.2%	132/3186 4.1%	119/2471 4.8%	There are no significant differences

Groups were compared by ten parameters: (1) prevalence of HBsAg (ratio of HBsAg-positive samples to total samples of group), (2) prevalence of genotype A, (3) genotype C, (4) subgenotype D1, (5) D2, (6) D3, (7) genotype D of unidentified subgenotypes, (8) HBsAg subtype ayw2, (9) ayw3, and (10) other subtypes (in the cases of 2–10, prevalence was detected as the ratio of isolates of this type in the group to all isolates of the group) (Table 1). At the same time, groups from regions were compared to each other (every group); groups of raions were compared to each other inside the region. The prevalence of HBsAg was compared in age and gender groups inside every group specifically (Table 2). To determine the accuracy of different numerical data obtained in pairwise comparisons, we

used Fisher's exact test or criteria χ^2 with Yates' correction according to the sample's characteristic. Probability value $p > 0.95$ was used as the accuracy cutoff of differences. The SEQBOOT and CONSENSE programs of the PHYLIP package (v. 3.53) were used for calculation of the indices of statistical support of phylogenetic tree nodes in a bootstrap test with 500 replicates [22].

RESULTS AND DISCUSSION

The obtained data from all studied samples are listed in Table 1. It seems that the prevalence of HBsAg in the total group of the native population (4.4%) is close to the rate of chronic carriers of HBV among the

Siberian urban population [2, 11] and in Russia as a whole (4–5%) [1, 6, 13]. Furthermore, it was three high endemic groups were excluded due to the occurrence of HBV with a high prevalence of HBsAg: Altaians in the Republic of Altai (13.4%), Dolgans and Nganasans in Krasnoyarsk krai (13.2%), and Teleuts in Kemerovo oblast (10.2%). In comparing the prevalence of HBsAg in the age and gender groups in all the studied regions and raions (Table 2), it was shown that infection is unrelated to gender or age in the group of people who are older than 35 among the studied people. For several raions, an increase was shown in the risk of infection with age in the group of people older than 35. This allows it to be supposed that, among the native population, there are HBV transmission mechanisms that are not related to the main risk factors that are typical for the urban communities of Siberia: intravenous drug abuse and risky sexual behavior [2–4].

Phylogenetic relations between studied samples are shown in the figure. Among virus-positive samples, genotype D was prevalent (Table 1). Among subgenotypes, there were no dominant types: all three subgenotypes (D1, D2, and D3) were in the same proportions (Table 1). Among HBsAg subtypes, ayw2 was prevalent; ayw3 was also expressed (Table 1). The obtained picture of HBV variety in the group does not contradict the results of earlier Russian researchers [2, 3, 8–11, 23, 34, 49, 50]. At the same time, the ratio of HBV subgenotypes and HBsAg subtypes are very different in certain territories (Table 1).

*Republic of Altai (Southwestern Siberia;
Kazakhs and Altaians)*

In samples from this group, the prevalence of HBsAg was 9.6%, which is evidence of high endemic occurrence of HBV [30]. The rate of HBsAg carriers in the Republic of Altai was more than in Irkutsk oblast ($p > 0.975$) and the YaNAO ($p > 0.999$), but was close to the values obtained for high endemic groups of Kemerovo and Krasnoyarsk krai (Table 1). The most prevalent HBV subgenotypes in isolates were D3 (57.1%) and D1 (35.7%). The prevalence of subgenotype D3 was higher than the prevalence of isolates in Kemerovo oblast ($p > 0.95$), the YaNAO ($p > 0.99$), and Krasnoyarsk krai ($p > 0.999$). Subgenotype D1 occurred more often than in isolates from the YaNAO ($p > 0.99$), but less than in isolates from Krasnoyarsk krai ($p > 0.95$). Subgenotype D2 was minor (3.6%). Among isolates of the Republic of Altai, its prevalence was less than in the YaNAO ($p > 0.999$). The dominant HBsAg subtype was ayw2 in the group of the samples from the Republic of Altai. Its prevalence was 96.4%, which was more than in the samples from Irkutsk oblast ($p > 0.995$) and the YaNAO ($p > 0.999$). The detected prevalence of HBsAg subtype ayw3 (3.6%) was less than in samples of the group from Irkutsk oblast ($p > 0.975$) and the YaNAO ($p > 0.999$) (Table 1).

Two raions of Republic of Altai differed greatly in their studied characteristics. In comparison of groups from these raions (Kosh-Agach and Ust-Kan), differences were detected in the prevalence of HBsAg (5.2 and 13.4%, respectively; $p > 0.99$), D1 subgenotypes (85.7 and 19%, respectively; $p > 0.99$), and D3 (0 and 76.2%, respectively; $p > 0.999$) (Table 1). These data confirm that there is no, or almost no, HBV transmission in the groups of the raions. Based on the relative geographic proximity of the raions of the republic, this could be explained as the result of limited contacts between different ethnic groups: the donors for the samples from Kosh-Agach raion were Kazakhs, while those in Ust-Kan were Altaians.

Kemerovo Oblast (Southwestern Siberia; Teleuts)

The small size of this group did not allow significant differences to be obtained in most comparisons with other groups. However, the group from Kemerovo oblast showed differences from the group from the YaNAO in a range of values: in the samples from Kemerovo oblast, a higher value was detected of prevalence of HBsAg (10.2%; $p > 0.999$) and subgenotype D1 (60%; $p > 0.95$). In the samples from Kemerovo oblast, DNA of subgenotype D2 or subtype ayw3 was not detected, which is the reason why the rates of isolates of these types (0%) were smaller than their analogues in the YaNAO ($p > 0.95$ in both cases). The prevalence of subgenotype D3 in the group from Kemerovo oblast was also 0%, less than in the group from the Republic of Altai ($p > 0.95$; hereinafter, some results of comparison will duplicate previous results for the convenience of readers) (Table 1).

*Irkutsk Oblast (Southeastern Siberia;
Buryats and Russians)*

This group could be associated with HBV with prevalence of a midendemic character [30]; the rate of HBsAg-positive carriers is 6% in it, which is more than in the YaNAO ($p > 0.999$), but less than in the Republic of Altai ($p > 0.975$) and Krasnoyarsk krai ($p > 0.999$) (Table 1). No HBV subgenotypes were dominant in the group from Irkutsk oblast: D1, D2, and D3 were introduced at almost the same rate (Table 1). The prevalence of subgenotype D1 (27.9%) was higher than in the YaNAO ($p > 0.95$), but less than in Krasnoyarsk krai ($p > 0.995$). Subgenotype D2 (20.9%) was discovered in the group from Irkutsk oblast less than in the group from the YaNAO ($p > 0.999$). The prevalence of subgenotype D3 (32.6%) was more or less the same in the group from Irkutsk oblast and the groups from other regions. Ayw2 was predominant among HBsAg subtypes in the group from Irkutsk oblast. Its prevalence was 58.1%, which was more than in the YaNAO ($p > 0.95$), but less than in the group from the Republic of Altai ($p > 0.995$). The rate of subtype ayw3 in the group from Irkutsk oblast (30.2%) was more than in

the group from the Republic of Altai ($p > 0.975$) and Krasnoyarsk krai ($p > 0.975$) (Table 1).

Three raions of in Irkutsk oblast were incorporated into the research. Comparison of groups of Alar and Nukut raions (both of these raions are populated by Buryats) had no significant differences in all studied parameters. Groups of Alar and Irkutsk raions (the last group was composed of Russians) differed in the prevalence of HBsAg (8.2 and 3.6%, respectively; $p > 0.95$). Moreover, subgenotype D3 was prevalent in Alar raion (50 versus 0% in Irkutsk oblast; $p > 0.95$), while D1 was prevalent in Irkutsk oblast (87.5% in comparison with 8.3% in Alar raion; $p > 0.999$). Differences in prevalence of subgenotype D1 (27.3 and 87.5% respectively; $p > 0.95$), subtypes ayw2 (27.3 and 100% respectively; $p > 0.99$), and ayw3 (54.5 and 0% respectively; $p > 0.95$) were obtained in comparing Nukut and Irkutsk raions (Table 1). It seems that the significant differences in the HBV parameters in groups represented generally by Buryats and Russians are the result of rare HBV transmission between these groups. It should be noted that three isolates of HBV of genotype C and 1 isolate of genotype A (Nukut raion) were found in the groups from Alar and Nukut raions (Buryats); at the same time, all eight isolates from Irkutsk raion were genotype D (Fig. 1, Table 1). This means that the groups from Alar and Nukut raions have other sources of HBV transmission than do the residents of Irkutsk oblast.

*The YaNAO (Northwestern Siberia;
Khants, Komi, Nenets, and Selkups)*

The YaNAO group had many differences from the other groups studied. As was noted earlier, a significantly lower level of prevalence of HBsAg (1.6%) was found in this group in comparison with all groups from other regions (Table 1). A low (around 2%) prevalence of HBV markers among Nenets and Komi in neighboring regions has been detected by other researchers as well [21]. The YaNAO group was the only one with prevalence of subgenotype D2 and subtype ayw3. Prevalence of subgenotype D2 in the YaNAO (60.6%) was higher than in other groups ($p > 0.999$ for the Republic of Altai, Irkutsk oblast, and Krasnoyarsk krai; $p > 0.95$ for Kemerovo oblast). Conversely, the prevalence of subgenotype D1 in the YaNAO (6.1%) was less than in other groups ($p > 0.99$ for the Republic of Altai; $p > 0.95$ for Kemerovo and Irkutsk oblast; $p > 0.999$ for Krasnoyarsk krai). The rate of subgenotype D3 (15.2%) in the YaNAO was less than in the Republic of Altai ($p > 0.995$). Subtype ayw3 in the YaNAO occurred more often (54.5%) than in groups from the Republic of Altai ($p > 0.999$) and Kemerovo ($p > 0.95$) and Krasnoyarsk krai ($p > 0.999$). At the same time, subtype ayw2 occurred in the YaNAO less often (30.3%) than in groups from the Republic of Altai ($p > 0.999$) and Irkutsk ($p > 0.95$) and Krasnoyarsk krai ($p > 0.999$) (Table 1). It is interesting that the

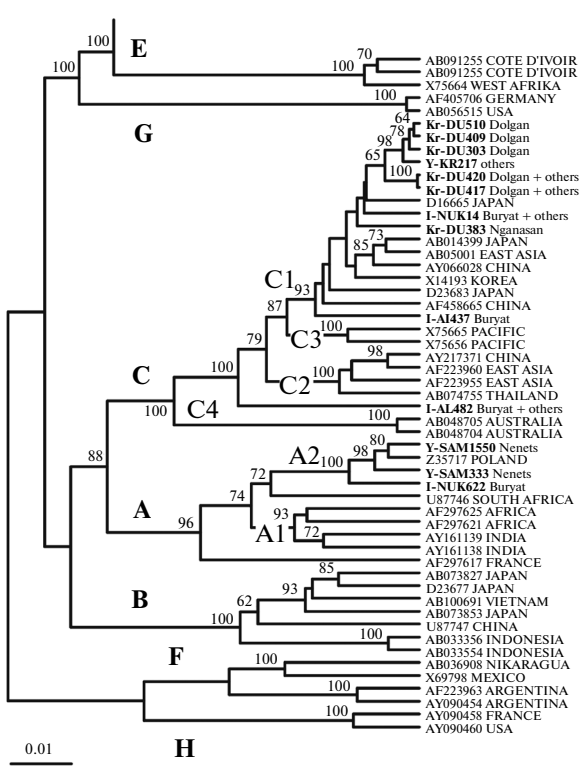
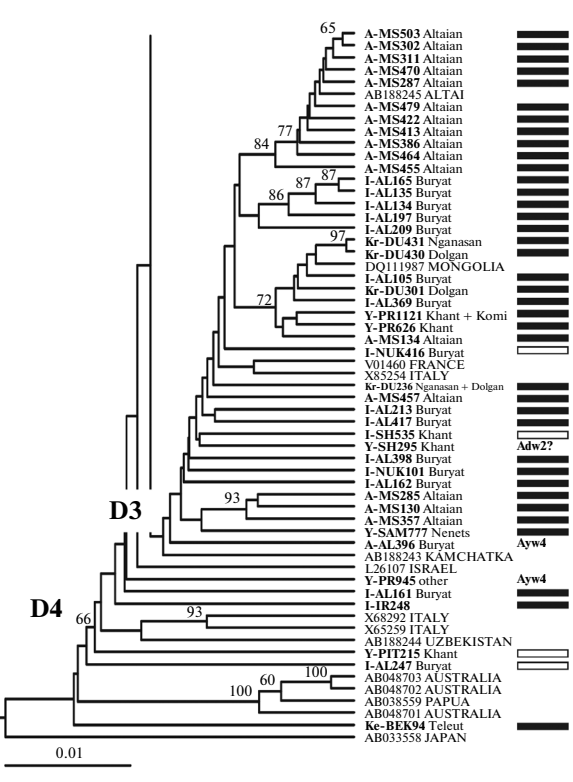
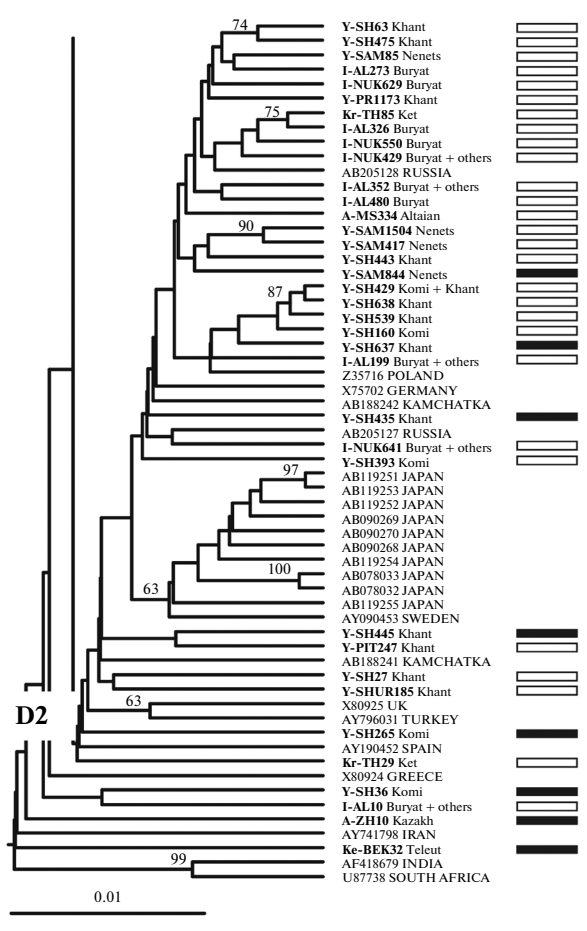
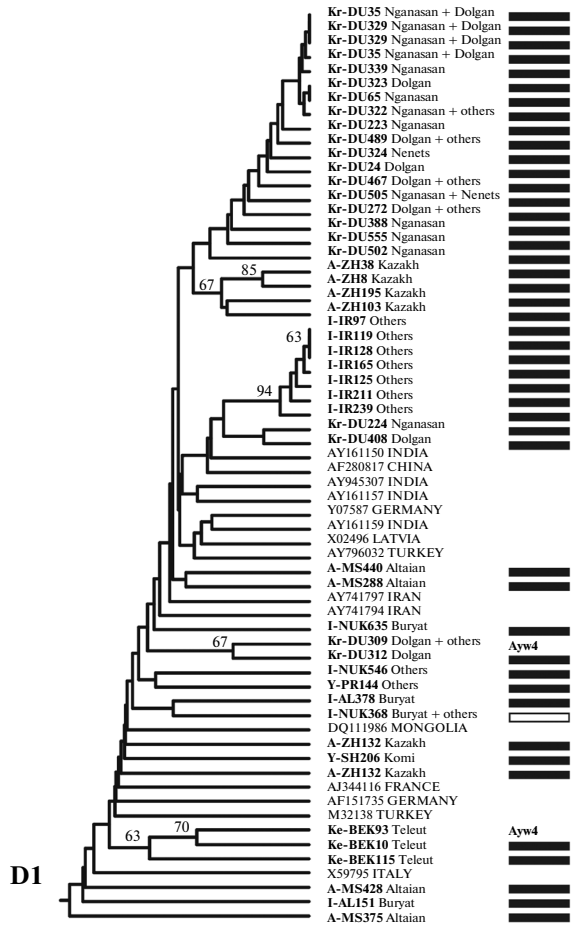
only the HBV isolate from Krasnoselkup raion had genotype C.

The groups from four raions of the YaNAO were almost the same in terms of parameters of HBV infection (Table 1). The exceptions were Shuryshkar and Priural raion, where differences were reported by rates of HBsAg-positive carriers (2.9 and 0.8% respectively; $p > 0.999$) and isolates of subgenotype D2 (75 and 20% respectively; $p > 0.95$). In all other paired comparisons of raions of the YaNAO, no differences were found in the studied parameters. Thus, we can propose that, in spite of the different ethnic backgrounds in the YaNAO, the huge territory of region, and the low population density, there is a common HBV population without epidemiological barriers. At the same time, it seems that the YaNAO generally is isolated from HBV transmissions from groups in other studied regions.

*Krasnoyarsk Krai (Northern Siberia;
Dolgans, Nganasans, and Keths)*

This group was considered to have high HBV endemicity. The prevalence of HBsAg was 11.7% in this group, which is more than in the groups from Irkutsk oblast ($p > 0.999$) and the YaNAO ($p > 0.999$) (Table 1). Such a high level of prevalence of HBsAg was not detected earlier in the northern areas of central and western Siberia. The authors of some previous papers [8–10, 34] believe that HBV prevalence decrease from the south to the north of Siberia. In the group from Krasnoyarsk krai, subgenotype D1 (–64.7%) prevailed in comparison with groups from the Republic of Altai ($p > 0.95$), Irkutsk oblast ($p > 0.995$), and the YaNAO ($p > 0.999$). The prevalence of subgenotype D2 in the group from Krasnoyarsk krai (5.9%), as in other groups, was less than in the YaNAO group ($p > 0.999$). The rate of subgenotype D3, which was also small for this group (11.8%), was less than in the case of the Republic of Altai ($p > 0.999$). The majority of isolates from Krasnoyarsk krai was belonged to genotype C of HBV (17.6%), while, for example, genotype C of HBV was not detected in any of the 28 isolates from the Republic of Altai (Table 1; for further information, please see below). All these isolates belonged to subgenotype C1 (subtype adrq+) (Fig. 1). As in all other groups (excluding the YaNAO), subtype ayw2 predominated in the group from Krasnoyarsk krai (73.5%; $p > 0.999$ in comparison with the YaNAO). The rate of subtype ayw3 (5.9%) was less than in the YaNAO group ($p > 0.999$).

Two studied raions of Krasnoyarsk krai had no significant differences in any parameter (Table 1). The reason for this is likely not so much the epidemiological homogeneity of these raions, as it is the small population size of the group from Turukhan raion, which was not enough for correct statistical analysis (Table 1). Thus, the main contribution to the characteristics of the group from Krasnoyarsk krai, which have been described earlier, was from the very large



Phylogenetic tree of studied HBV isolates. Numbers of studied isolates (which are the same as in Table 1) are indicated in black type. Donor ethnicity including metis is marked by italic type. The HBsAg subtype of the corresponding isolate is marked by a square to the right side: black—ayw2, white—ayw3, others—alphabetical symbols. For prototype sequences, the number in the GenBank database and the name of territory of the isolate are indicated. Branches of genotypes and subtypes are marked by a corresponding letter. Indices of support of nodes (more than 60) and scale range of genetic length are reported.

group from Dudin raion. For example, all the features usual for the group from Krasnoyarsk krai were found in it: high prevalence of HBsAg, predominance of subgenotype D1 and subtype ayw2, and significant contribution of isolates of genotype C. Two HBV isolates extracted from blood samples of Kethos of Turukhan raion were assigned to subgenotype D2 and subtype ayw3 from blood samples of Dolgans and Nganasans of Dudin raion (Table 1).

Discovery of the very large rate of isolates of HBV genotype C (17.6%) in the north of Krasnoyarsk krai (Dudin raion located on the Taimyr Peninsula), taken together with data from other researchers, could confirm the current or former existence of HBV transmission from the east to the west of northern Siberia. Thus, in the neighboring Republic of Sakha (Yakutia), a high level of prevalence was detected of HBV genotype C isolates (24.1%) and mixed infections of genotypes C and D (13.8%) [7], as well as a general high endemic of HBV (the prevalence of HBsAg is 10.8–23.8%) [5]; it is close to the data from the Dudin raion of Krasnoyarsk krai. Earlier, HBsAg subtype adr_q+ and HBV genotype C were detected via ELISA using high-avidity monoclonal antibodies in 8 (33%) out of 24 blood samples from residents of Anadyr of the Chukotka AO [12]. The same rate of HBV isolates of genotype C (25%) in the Chukotka AO has been reported in the papers of other authors using PCR and HBV DNA sequencing for these purposes [18]. Moreover, only one isolate of genotype C in our research in Krasnoselkup raion of the YaNAO (located to the south and the west of Taimyr) was phylogenetically the same as isolates of subgenotypes C1 of the Dudin and Krasnoyarsk districts (Fig. 1). It is likely that the population of the western areas of Siberia played a part in the northern route of HBV transmission that was found.

Thus, the results of the present research on HBsAg detection in five studied regions of Siberia confirm the presence of three groups that have highly endemic prevalence of HBV: Altaians in the Republic of Altai (13.4%), Dolgans and Nganasans in Krasnoyarsk krai (13.2%), and Teleuts in Kemerovo oblast (10.2%), in which public HBV preventative measure need to be repeated. The detection of HBV genotypes in 143 isolates showed that 130 (90.9%) of them are genotype D, 10 (7%) are genotype C, and only 3 (2.1%) are genotype A. Differing prevalence of HBV subgenotypes was detected in isolates of studied groups: subgenotype D1 (subtype ayw2) was predominant in isolates from Kazakhs in the Republic of Altai (85.7%), Teleuts in Kemerovo oblast (60.0%), Russians in Irkutsk oblast (87.5%), and Dolgans and Nganasans in Krasnoyarsk

krai (68.8%); D2 (ayw3) was prevalent in Khants and Komi (75%) and Nentsi (57.1%) from Purov raion and the YaNAO; D3 (ayw2) was prevalent in isolates of Altaians in the Republic of Altai (76.2%) and Buryats from Alar raion in Irkutsk oblast (50%). Genotype C (adr_q+) of HBV was found in isolates of groups of Dudin raion in Krasnoyarsk krai, Alar and Nukut raion in Irkutsk oblast, and Krasnoselkup raion in the YaNAO; genotype A was found in isolates of groups from Purov raion, the YaNAO, and Nukut raion in Irkutsk oblast. The data that have been obtained confirm the former existence of several different vectors of HBV infection in the native populations of population of Siberia, as well as their epidemiological difference from each other.

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