Mitochondrial DNA Variation in Old Believer and Ethnic Russian Populations of Northern Siberia

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ABSTRACT: In 1653, the Patriarch Nikon modified liturgical practices to bring the Russian Orthodox Church in line with those of the of the Eastern (Greek) Orthodox Church, from which it had split two hundred years earlier. 'Old Believers' (staroveri) rejected these changes, and continued to worship using the earlier practices. Their actions resulted in persecution by the Russian Orthodox Church, which forced them to disperse across Siberia, where they formed remote communities. For the next three centuries, they lived in relative isolation from other Russian groups. To assess genetic diversity within Old Believers, along with the biological consequences of their isolation, we surveyed ~200 unrelated individuals from several villages for mtDNA variation, including Burnyi in the Baikitsk Raion of the Krasnoyarsk Krai, and Isetsk and Kirsanovo in the Tyumen Oblast. We also surveyed mtDNA variation in ~200 ethnic Russians from different parts of Siberia to determine the genetic relationship between Old Believers and other Slavic groups. Initial results indicate that West Eurasian haplogroups H, J and T are the predominant mtDNA lineages present in Old Believer communities, with mostly West Eurasian haplogroups (I, W, X) comprising the remaining mtDNAs. Somewhat surprisingly, haplogroup C mtDNAs were also detected in the Old Believers. This finding implied that they had admixed with local indigenous groups, since haplogroup C mtDNAs are not usually found in ethnic Russians. The pattern of mtDNA diversity in Old Believers, and their genetic affinities with ethnic Russian and other Slavic populations, is discussed.

(A) (B) (C)

Figure 1. (A) Old Believer church in the Trans-Baikal region; (B) Great Lent ritual in Old Believer church; (C) Russian icon showing Jesus holding two fingers to make the sign of the cross.

INTRODUCTION:

During the reign of Patriarch Joseph (1642-1652), there arose a reformist group of clergy whose aims included the restoration of the purity of the service books and stricter observance of various matters of spiritual discipline among the clergy generally. This movement was headed by the priest and confessor to the Tsar Stephan Vonifatiev, and the Archbishop of Novgorod, Nikon (1605-1681). Patriarch Nikon introduced changes intended to make Russian practices conform to Greek usage. This was offensive to some Russian Orthodox who believed that the Russian Church should adopt its own traditions. In 1653, he sent a memorandum to the churches in the land that instructed them in various revisions of the services and the books, and these reforms were met with opposition from many of the clergy. Among the major points that were contested were: (1) how many fingers would be used to make the sign of the cross; (2) the spelling of Jesus' name; (3) whether "Alleluia" should be sung two or three times; (4) the retention of certain words and phrases in the Creed; (5) the number of hosts to be used in the liturgy; and (6) whether the priests should walk around the altar with or against the passage of the sun (**Figure 1**). Although seemingly unimportant in themselves, these matters of ritual nevertheless embodied certain theological precepts and ideological alliances, and, hence, stirred considerable controversy upon their implementation. Moreover, many of the clergy felt that strict observance of the most minute details of the dogmas and disciplines of the church were necessary to salvation.

Nikon acted forcefully against the dissenters. He had his opponents flogged, exiled and even burned at the stake. Among the exiles was the arch-priest Avvakum, who had been one of the more prominent among the younger members of the reformatory circle in pre-Nikonian days and had spearheaded the conservative opposition to Nikon's edicts. He was eventually burned at the stake in 1682 and until then continued to serve as a spiritual leader for many of the dissenters. His followers became known as Old Believers, or those who followed the old rituals before the reform. Some conservative dissenters believed that the age of the Anti-Christ had come and that the end of the world was near. In the years 1666-1668, numerous fields throughout Western Russia went untended while the faithful dressed themselves in burial clothes and awaited the end of the world in their cemeteries at night, singing hymns and sitting in wooden coffins. Others set buildings afire where they waited inside to be cleansed and to perish in the flames so that they might join Christ before the Judgment Day. Between these and the others who were burned to death by persecutors, it has been estimated that more than 20,000 Old Believers died between 1672 and 1691 alone.

The Old Believers, who at one time may have composed 10% of the Russian population, were harshly persecuted under the tsars. Many fled into Asia in the 18th century and others were forcefully exiled from European Russia. Many communities lived in almost complete isolation for centuries. Under Catherine II, Paul and Alexander I, they were tolerated and thrived in some areas, but under Peter the Great and Nicholas I, they often had to flee to outer regions of Russia or to other countries to avoid death or imprisonment. The Old Believers were also not spared the purges of Josef Stalin in the 1930s. Entire families were taken away. Old practices were banned from public display, forcing followers to perform their religious rituals in the woods or at night. Because of the political turmoil of the past century, the Old Believers are believed to have substantially declined in numbers after the 1917 Soviet revolution. However, it has been recently estimated that there are between 2,000,000 and 2,500,000 faithful in Russia today.

After May 3, 1905, when Tsar Nicholas II issued the Edict of Toleration, Old Believers were allowed to function freely in Russia. The situation of this community since the Bolshevik Revolution of 1917 is poorly known, but there were attempts to overcome the schism. Metropolitan Sergius and the Holy Synod took unsuccessful measures to heal the rift in 1929, and mMeetings took place in the period following World War II. Finally, in 1971, the Russian Orthodox Church recognized the validity of the old rites and restored the status of the Old Believers within the church. Yet, it took another 20 years, after the fall of communism, before the Old Believers gained real religious freedom. They have now achieved legal recognition, and churches have been reopening at a rapid rate, creating an acute shortage of clergy. Thus far, however, full communion between Old Believers and the Russian Orthodox Church has not been re-established.

Although enduring much hardship during these years, many Old Believers have chosen to remain in their local villages. Quite recently, they have also begun to communicate with the outside world, and some villages are now even open to tourists who want to experience the old ways of Russia, which have been preserved by the Old Believers (**Figures 2 and 3**). However, due to the effects of communist rule and now because of increased contact with outsiders, the younger generations are becoming more modernized and forgetting the old ways. The economic changes of post-Soviet Russia have also taken a toll on the Starovery. State farms have collapsed, and many jobs in the villages have disappeared. The stresses of these conditions have even driven some Old Believers to commit suicide.

(A) (B)

Figure 2. (A) Round logs, colorful shutters and fences distinguish the Old Believers' houses in Tarbagatay, a village in the Trans-Baikal region. The villagers customarily clean their houses thoroughly at least twice a year by washing the ceiling, walls, floors and exterior. (B) Old Believers are well known for their distinct style of singing in which they create moving harmonies. Their songs and carols are a mix of old Russian, Polish and Ukrainian.

OBJECTIVES OF THE PROJECT

The biological origins of Slavic peoples has become of considerable interest to many researchers, who have begun conducting molecular genetic studies of these populations. However, no one has yet examined genetic variation in the isolated Old Believer populations, who may represent a cross-section of ethnic Russians from various parts of Siberia. These study provides with an opportunity to assess the extent of the genetic similarities of Old Believer populations to Russians and other Slavic groups. The historical origins of the Russian state are chiefly those of the East Slavs, the ethnic group that evolved into the Russian, Ukrainian, and Belorussian peoples. The major pre-Soviet states of the East Slavs were, in chronological order, medieval Kievan Rus', Muscovy, and the Russian Empire. Three other states---Poland, Lithuania and the Mongol Empire---also played crucial roles in the historical development of Russia.

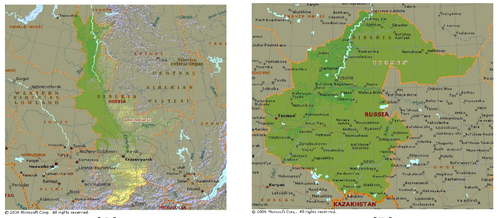
It is possible that Old Believers have become somewhat differentiated from other ethnic Russians because of the demographic consequences of religious persecution and geographic isolation from them. In other words, they may have undergone founder effects as a result of the population size fluctuations that they experienced during the past 300 years. It is known that different Old Believer communities sought refuge from this persecution in different regions of Russia, as well as in countries outside of their homeland. For example, the Old Believers living in the Tyumen region have been isolated from other ethnic Russian groups since the 17th century, and are thought to have moved to the Tyumen region from Pskov lands around 300 years ago. By contrast, those from the Krasnoyarsk Krai probably moved to this region in the 18th century upon their explusion from European Russia by Catherine the Great. Furthermore, the fragmentation of Staroveri into "Popovtsy" and "Bezpopovtsy" communties may have influenced patterns of biological variation within them. Therefore, by examining the mtDNA diversity in Old Believers, we may be able to detect genetic signatures of population reductions in these religious communities.

(A) (B)

Figure 3. (A) Old Believers marching in a Saint's Day parade in a local village. (B) Old Believer women performing a dance while wearing their traditional dress. This typically included a kichka, a glittering headpiece crowned with a jeweled brooch, a brilliantly colored scarf draped down to their waists, and strands of giant beads of amber coil.

SAMPLE COLLECTION

To characterize the genetic diversity within Old Believers, we surveyed ~200 unrelated individuals from several villages for mtDNA variation. We also surveyed mtDNA variation in ~200 ethnic Russians from different parts of Siberia to determine the genetic relationship between Old Believers and other Slavic groups. These samples included 24 individuals from Burnyi village in the Balkitsk Raion of the Krasnoyarsk Krai, and 59 and 99 individuals from the Isetsk and Kirsanovo villages, respectively, in the Tyumen Oblast (**Figure 4**). All samples were collected between 1999-2001. The ethnic Russian samples include 135 unrelated persons living across Siberia from whom samples were collected during studies of indigenous Siberian populations from 1999-2003. These included 53 individuals from the Novosibirsk region, and 82 individuals from the Krasnoselkup region of the Yamalo-Nenets Autonomous District. All bloods were fractionated after collection in local hospitals, and then transported to Institute of Cytology and Genetics, Novosibirsk for storage. For each set of samples, informed consent was obtained from individual participants using forms written in Russian and approved by the Institute of Cytology and Genetics (ICG). Genetic analysis of these samples was also conducted under approval of the Human Subjects Review Committee at the University of Pennsylvania.



(A) (B)

Figure 4. (A) A map of the Krasnoyarsk Krai. (B) A map of the Tyumen Oblast.

MITOCHONDRIAL DNA ANALYSIS

All Old Believer and ethnic Russian population samples were examined for mtDNA sequence variation by through SNP analysis. SNP analysis involved screening the samples for the diagnostic mutations of West Eurasian (H-K, N, R, T-X) (Torroni et al. 1994b, 1996, 1998; Macaulay et al. 1999) and East Eurasian (A-G, M, Y, Z) (Torroni et al. 1993b, 1994a; Schurr et al. 1999) haplogroups to determine the extent of genetic affinities that these ethnic groups have with populations from Europe, West and Central Asia, and Siberia. Haplogroup assignments of these mtDNAs were performed through RFLP analysis of PCR amplified segments contained informative phylogenetic markers using the following enzymes: +663 *Hae*III (00663); -3007 *Bsh*1236I (03010); +4643 *Rsa*I (04646); +4831 *Hae*II (04833); +4914 *Bfa*I (04911); -5176 *Alu*I (05178); +5301 *Bsm*FI (05301); -7025 *Alu*I (07028); -7598 *Hha*I (07600); +7933 *Mbo*I (07933); -8391 *Hae*III (08391); +8409 *Ssp*I (08414); +10397 *Alu*I (10400); +12308 *Hinf*I (12308); +13263 *Alu*I (13263); and +15907 *Rsa*I (15907), as described elsewhere (Torroni et al. 1993b, 1994a; Schurr et al. 1999; Forster et al. 2001; Yao et al. 2002).

RESULTS

The vast majority of Old Believer mtDNAs (~96%) belonged to West Eurasian haplogroups (**Figure 1, Table 1**). This was not a surprising result, given the predominance of these maternal lineages in Slavic groups. However, the frequencies of individual haplogroups varied considerably in the Staroveri communities. Those lineages that exhibited the greatest range of frequencies including haplogroups H, J, T and U. In fact, despite having the smallest sample size, the Burnyi sample showed the highest frequencies of haplogroups J and T, along with the lowest frequency of haplogroup H, of the three populations. These differences could have resulted from founder effects, lineal (familial) effects or some other stochastic influences, but require further elucidation.

Interestingly, each population sample (village) also exhibited low frequencies of haplogroup M*/C mtDNAs. This finding implied that the Staroveri have intermarried with local indigenous groups since moving to Siberia, since haplogroup C mtDNAs are not usually found in ethnic Russians. Alternatively, these East Eurasian mtDNAs could possibly have been contributed to ethnic Russian or Slavic groups during the Mongol invasion of Europe around 800 years ago, and maintained at low levels in Russians since this time. Additional analysis of HVS-I sequence data from these populations will be necessary to distinguish between these possibilities.

When compared with other Slavic populations, the Old Believers were distinctive from them. They had the lowest haplogroup H frequency of any Slavic group, generally lower frequencies of haplogroups I and K, and the highest frequency of U observed in Slavic peoples. Once their "unknown" samples are more fully characterized, it is likely that many of them will be shown to belong to these latter two haplogroups, and perhaps also pre-HV and HV. By contrast, the Siberian Russians whose mtDNAs that we analyzed exhibited haplogroup frequencies that were consistent with published data for ethnic Russians and other Slavic populations. All of these populations typically have ~40% haplogroup H mtDNAs, ~14% haplogroup U, and ~9% haplogroup T mtDNAs, with haplogroup I also being particular frequent in them.

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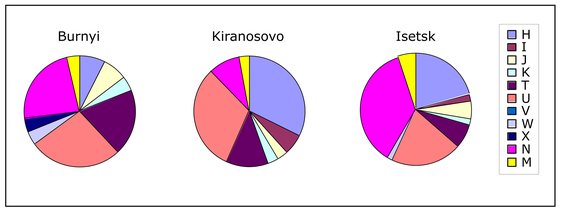

Figure 5. Haplogroup Frequencies in Old Believer Populations. Haplogroup N represents not only those mtDNAs belonging to this haplogroup but also all others that have not yet been assigned to a mtDNA lineage but which do not belong to haplogroup M

Table 1. Haplogroup Distributions (%) in Slavic Population of Different Ethnic Origins

Hg	OB (180)	Russians (135)	Bosnians (144)	Slovenians (104)	Poles (436)	Russians* (201)	Russians# (49)	Russians^ (69)	Ukrainians (36)
H	26.4	45.2	47.9	47.1	45.2	42.3	38.8	37.7	41.7
HV	-	-	0.7	0	0.9	2.0	4.1	2.9	0
pre-V	-	-	6.3	6.7	4.8	5.5	-	-	-
pre-HV	-	-	1.4	0	0	0.5	-	-	-
V	0	2.2	-	-	-	-	8.2	7.3	5.6
J	4.9	2.2	6.9	9.6	7.8	8.0	18.4	11.6	8.3
T	11.5	8.9	4.9	5.8	11.5	11.0	12.2	8.7	8.3
K	2.7	5.2	4.2	3.9	3.4	3.0	0	1.5	8.3
U	27.8	8.1	19.4	19.2	16.1	17.9	10.2	18.8	16.7
I	1.1	0.7	2.8	1.9	1.8	2.5	2.0	4.4	0
W	1.1	4.4	1.4	4.8	3.7	2.0	0	1.5	2.8
X	0.5	1.5	1.4	1.0	1.8	3.5	0	1.5	2.8
M	3.8	2.2	1.4	0	1.8	1.5	0	1.5	0
Other	-	-	1.4	0	1.2	0.5	-	-	-
UNK	23.3	19.3	-	-	-	-	6.1	2.9	5.6

Note: All published data are taken from Malyarchuk et al. (2001, 2002a, b, 2003). Russian population samples were obtained from these locations: * = Stavropol, Orel and Saratov; # = Krasnodar; and ^ = Belgorod. The dash, "-", means "not yet determined." "Other" = minor haplogroups such as N1 and R. "UNK" = not yet classified by SNP analysis. Haplogroup M includes all haplogroups subsumed within it, e.g., haplogroups C and Z.

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DISCUSSION

As noted above, the Old Believers have a mtDNA profile that is generally consistent with that of other Slavic populations. Based on these haplogroup frequencies, it is clear that they derive from a common ancestry with other Eastern Slavs. At the same time, they show some genetic differences from Slavic groups that may reflect a prolonged history of isolation and endogamy. To gain a clearer idea of the extent of genetic variation in these populations, it will be necessary to examine HVS-I sequence diversity, or perhaps even whole genome sequence data, from Staroveri and other Slavic groups. In this case, the extent of HVS-I sequence sharing will indicate the relative similarity of different Old Believer groups and the closeness of different Slavic ethnic groups. Here, we would anticipate seeing a closer relationship between Russians, Ukrainians and Belorussians, and a more distant one between these populations and Bosnians, Slovenians and Poles. The expanded sampling of Old Believer populations currently living in Russia might also reveal their geographic ties to different parts of Russia, and provide background information about possible admixture with indigenous groups as the Old Believers began settling the remoter areas of Siberia.

Furthermore, the data obtained in this study can be used to test hypotheses about the origins of Slavic populations. One such model, the hybridization theory, states that Russians, Ukrainians and Belorussians are a result of admixture between Slavonic tribes in central Europe and pre-Slavonic tribes of eastern Europe. An alternative model, the transformation theory, states that Russians emerged from ancient populations of eastern Europe, and Slavonic peoples only contributed culturally to this emergent ethnic group (Malyarchuk and Derenko 2001; Malyarchuk et al. 2001). The hybridization theory also predicts that diverse modern Slavonic populations will have certain combinations of genetic markers derived from the gene pool of the assumed ancestral Proto-Slavonic population. However, thus far, Slavonic populations sharing the same language group display a large amount of interpopulational genetic variation, and do not exhibit specific combinations of unique mtDNA types that clearly distinguish Russians from Germans and their neighboring Eastern European populations (Malyarchuk and Derenko 2001).

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