

## THE ACCURACY OF PREDICTING EYE AND HAIR PIGMENTATION BASED ON GENETIC MARKERS IN RUSSIAN POPULATIONS

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Prediction of eye and hair color from DNA is being increasingly employed in forensic medicine and the studies of ancient populations. HlrisPlex-S is a prediction tool that was developed using the data collected from Dutch donors and verified for some other European populations. The accuracy of its predictions for other world populations has not been studied yet. Unlike the majority of other world populations, Russian populations are characterized not only by dark but also by light color eyes and hair and therefore pose a special interest in this respect. The aim of this work was to determine the accuracy of eye and hair color predictions for Russian populations. We studied 144 representatives of indigenous populations of Russia (Avars, Aleuts, Buryats, Itelmens, Karelans, Koryaks, Maris, Nanais, Russians, Rutulians, Chuvashes, Chukchi, Evenks, and Evens). Anthropological photos were taken of all individuals. Based on the photos, the anthropologists identified eye and hair color phenotypes. SNP-markers were genotyped using the HlrisPlex panel. Based on the genotypes, the phenotypes were predicted and subsequently compared to the actual phenotypes. We obtained a series of HlrisPlex accuracy indicators for the populations inhabiting the European part of Russia and Siberia. On the whole, prediction accuracy was satisfactory, although a bit lower than for West European populations. Further research could look for additional markers increasing the accuracy of predictions for Russian populations.

**Keywords:** eye color, hair color, genetic markers, prediction, gene pool, indigenous people, HlrisPlex-S

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**Compliance with ethical standards:** the study was approved by the Ethics Committee of the Research Centre for Medical Genetics (Protocol No 3/1 dated September 5, 2018). The samples used in this work were obtained from a population genetic study. Informed consent was obtained from all study participants.

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## ТОЧНОСТЬ ПРЕДИКЦИИ ПИГМЕНТАЦИИ ВОЛОС И ГЛАЗ ПО ГЕНЕТИЧЕСКИМ МАРКЕРАМ ДЛЯ ПОПУЛЯЦИЙ РОССИИ

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Предикция цвета глаз и волос по генотипу становится распространенным инструментом в судебно-медицинской экспертизе и в исследованиях древних популяций. Для этого широко используется панель HlrisPlex-S, разработанная на выборке голландцев и верифицированная для некоторых других популяций Западной Европы. Однако точность ее предсказаний для представителей других регионов мира не изучена. Особый интерес представляют популяции России, в которых (в отличие от большинства других популяций мира) присутствуют не только темные, но и светлые оттенки цвета волос и глаз. Целью работы было определить точность предикции цвета глаз и волос для популяций России. Мы изучили 144 представителя коренного населения России (аварцев, алеутов, бурят, ительменов, карел, коряков, марийцев, нанайцев, русских, рутульцев, чувашей, чукчей, эвенков, эвенов). Для всех индивидов были сделаны антропологические фотографии. На основании фотографий эксперты-антропологи проводили определение цвета глаз и волос. Для тех же индивидов проводили генотипирование SNP-маркеров панели HlrisPlex. На основании генотипов предсказывали фенотипы и предсказанные фенотипы сопоставляли с реальными. Получена серия показателей точности HlrisPlex для популяций Европейской части России и Сибири. В целом точность оказалась удовлетворительной, хотя и несколько сниженной по сравнению с точностью для популяций Западной Европы. В будущих исследованиях возможно провести поиск дополнительных маркеров, повышающих точность предикции для популяций России.

**Ключевые слова:** цвет глаз, цвет волос, генетические маркеры, предикция, генофонд, коренное население, HlrisPlex-S

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In the last decade, prediction of eye and hair color from DNA has paved its way into forensic medicine and population genetics. Today, it is possible to predict the physical appearance of an unknown person from their biological sample. Phenotype prediction is used to help crime investigations, identify disaster victims, study DNA samples of ancient populations, conduct genetic genealogy analysis, etc. So far, there have been abounding studies [1–10] that have identified a number of key genes and genetic sites involved in pigmentation. The most critical of them were included in the HlrisPlex panel and its expanded version HlrisPlex-S [8–11]. Genotyping of 25 DNA markers (SNPs and indels) included in HlrisPlex [10] helps to rapidly and reliably predict eye and hair color; HlrisPlex-S analyzes these 25 polymorphisms + 16 more predictive of skin color.

Original publications on HlrisPlex [8–11] demonstrate that the system generates reliable results for European populations. HlrisPlex was developed using European datasets, primarily Dutch, and verified in Polish, Greek and Irish populations. The accuracy of HlrisPlex-based prediction has not been tested yet in the populations inhabiting other parts of the world. Because the majority of non-Europeans have dark eyes and dark hair, such tests will not have any informative value in most other non-European continents. However, in some populations living on the border between Europe and Asia (Altai region, the Caucasus, regions to the East of the Ural Mountains), both dark and light hair/eye phenotypes are common. Genetically, such individuals can significantly differ from Western Europeans [12]; this means that the range of genetic markers determining their hair/eye pigmentation may also be different. Even populations of the Ural region, which are genetically closer to Western Europeans than to the inhabitants of the Caucasus and Western Siberia, are more genetically distant from the Dutch as compared to Irish, Polish and Greek populations, whose specimens were used for HlrisPlex verification.

The aim of this work was to evaluate predictive power of HlrisPlex-S for eye and hair color prediction for the populations of North Eurasia using biological samples and photos of indigenous peoples taken during our expedition fieldwork.

## METHODS

### Sample collection and phenotyping

As part of the field study of gene pools conducted by our team [13], we took images of the indigenous populations of Russia and bordering countries. The populations included in the study were examined during a few expeditions in 2015–2019. The following inclusion criteria were applied: 1) age over 18 years; 2) 4 ancestors (two grandfathers and 2 grandmothers) identifying themselves as belonging to the studied ethnic group; 3) the anthropological image of a participant; 4) written informed consent to participate. Exclusion criteria were as follows: 1) the lack of enough images preventing reliable identification of eye and hair color; 2) incomplete profile of the genotyped markers.

The study was carried out in 144 individuals representing the following populations:

- 1) European Russia — Russian, Mari, Chuvash, Karelian, Rutulian, Avar ( $n = 66$ , 65 males and 1 female);
- 2) Siberia and Far East — Buryat, Evenk, Even, Nanai, Koryak, Itelmen, Chukchi, Aleut ( $n = 78$ , 45 males and 33 females).

Eye/hair color was identified from the obtained photos by 3 experts; 2 of the experts were physical anthropologists with extensive experience in phenotyping; the other one was a

geneticist specially trained in phenotyping. The experts worked independently. If the results were inconsistent with each other, the phenotyping procedure was repeated: this time, the experts worked together in order to reach a consensus. Eye color (dark, blue or intermediate) was successfully determined for 144 study participants. Hair color identification was successful in fewer cases because it was impossible to tell the natural hair color of most women from the photos and because some men had grey hair or were bald. Phenotyping results are shown in Table 1.

### Genotyping and prediction of eye and hair color from genotypes

DNA was isolated from blood/saliva samples by classic phenol-chloroform extraction [14]. Genotyping was done using an Infinium Omni5Exome-4 v1.3 BeadChip kit (Illumina; USA) and an iScan array scanner. The quality of genotyping data was analyzed in GenomeStudio v2.0 (Illumina; USA). For all samples, the call rate (CR) was over 0.99, suggesting that the obtained data were suitable for further analysis. The BeadChip array can genotype over 4 million SNPs; the data it generates can be used in a variety of different studies. Genotypes matching 29 markers for eye/hair/skin color prediction included in the HlrisPlex panel were extracted from the obtained array of genotyping data. The HlrisPlex-S panel contains a total of 25 predictive DNA markers for eye/hair color and 16 DNA markers for skin color. Of them, we successfully genotyped 19 markers of eye/hair color and 10 markers of skin color. The HlrisPlex panel allows prediction from a partial genotyping profile (a few obligatory markers are critical, others merely improve the accuracy of prediction), therefore a set of 19 out of 25 markers is sufficient to achieve good quality of prediction with HlrisPlex (predictive markers of skin color were not accounted for in our study). Nevertheless, clarification should be provided about the excluded marker rs312262906. Without it, predictions were generated only for eye color but not for hair color. The rs312262906 polymorphism causes a reading frame shift in the MC1R gene and is associated with red hair color. According to ExAC, the frequency of this polymorphism reaches 0.0038 in European populations and is 0.0000 (< 0.0001) in Asian populations; therefore, the probability of occurrence of at least 2 alternative alleles in our sample was negligible. This allowed us to assign the 0/0 genotype to this marker for all samples in order to predict hair color.

Genotypes were shortlisted using PLINK 1.9 [15]. The obtained genotypes are presented in Table 2.

HlrisPlex-S and the online webtool of the Department of Genetic Identification (Erasmus MC) [16] generated predictions for eye color (light, intermediate, or dark) and hair color (red, light, intermediate, or dark) for all the samples.

### Evaluation of eye/hair color prediction accuracy

Phenotypes predicted by HlrisPlex from the obtained genotypes were compared to the actual phenotypes identified by the anthropologists from the images taken during our expeditions; quality metrics were calculated for all 144 samples. The constructed 5-grade scales for eye/hair pigmentation were converted into conventional 3-grade scales in order to make phenotyping results suitable for comparison with HlrisPlex-S data.

To analyze the accuracy of HlrisPlex-S-based predictions, the following quality metrics were calculated:

- precision (the ratio of true positives to the total number of positive predictions);

**Table 1.** Phenotypes (eye and hair color) identified from anthropological images

Sample	Metapopulation	Ethnicity	Sex	Age at the time of sample	Hair color	Eye color
FES-0001	Siberia	evenk	m	63	not analyzed	dark
FES-0002	Siberia	nanai	m	33	dark	dark
FES-0003	Siberia	nanai	m	42	dark	dark
FES-0004	Siberia	nanai	m	29	dark	dark
FES-0005	Siberia	nanai	m	58	dark	dark
FES-0006	Siberia	nanai	m	62	dark	light
FES-0007	Siberia	nanai	m	68	dark	dark
FES-0008	Siberia	nanai	m	64	not analyzed	dark
FES-0009	Siberia	nanai	m	52	dark	dark
FES-0010	Siberia	nanai	m	55	dark	dark
FES-0011	Siberia	nanai	m	46	not analyzed	dark
FES-0012	Siberia	nanai	m	51	dark	dark
FES-0013	Siberia	even	m	52	intermediate	light
FES-0014	Siberia	even	m	21	dark	light
FES-0015	Siberia	even	m	39	dark	dark
FES-0016	Siberia	even	m	21	dark	dark
FES-0017	Siberia	even	m	20	dark	dark
FES-0018	European Russia	bashkir	m	64	not analyzed	dark
FES-0019	Siberia	buryat	m	76	not analyzed	dark
FES-0020	Siberia	buryat	m	68	not analyzed	dark
FES-0021	Siberia	buryat	m	50	dark	dark
FES-0022	Siberia	buryat	m	68	dark	dark
FES-0023	European Russia	chuvas	m	33	intermediate	light
FES-0024	European Russia	chuvas	m	51	blond	light
FES-0025	European Russia	chuvas	m	53	intermediate	light
FES-0026	European Russia	chuvas	m	42	dark	light
FES-0027	European Russia	chuvas	m	41	dark	dark
FES-0028	European Russia	chuvas	m	36	red	light
FES-0029	European Russia	chuvas	m	55	not analyzed	dark
FES-0030	European Russia	chuvas	m	45	dark	dark
FES-0031	European Russia	chuvas	m	33	red	dark
FES-0032	European Russia	chuvas	m	46	intermediate	dark
FES-0033	European Russia	chuvas	m	32	intermediate	dark
FES-0034	European Russia	chuvas	m	41	not analyzed	light
FES-0035	European Russia	chuvas	m	49	intermediate	dark
FES-0036	European Russia	chuvas	m	53	not analyzed	dark
FES-0037	European Russia	chuvas	m	46	intermediate	dark
FES-0038	European Russia	chuvas	m	57	dark	dark
FES-0039	European Russia	chuvas	m	42	not analyzed	light
FES-0040	European Russia	chuvas	m	47	red	light
FES-0041	European Russia	chuvas	m	23	intermediate	light
FES-0042	European Russia	avar	m	52	not analyzed	dark
FES-0043	European Russia	avar	m	55	not analyzed	light
FES-0044	European Russia	avar	m	20	intermediate	light
FES-0045	European Russia	rutulian	m	36	not analyzed	dark
FES-0046	European Russia	rutulian	m	38	dark	dark
FES-0047	European Russia	rutulian	m	83	not analyzed	dark
FES-0048	European Russia	rutulian	m	57	not analyzed	dark
FES-0049	European Russia	rutulian	m	55	dark	dark
FES-0050	European Russia	rutulian	m	56	dark	dark
FES-0051	European Russia	rutulian	m	65	not analyzed	dark
FES-0052	Siberia	even	f	46	not analyzed	dark

## ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ | ГЕНЕТИКА

Continuation of Table 1

Sample	Metapopulation	Ethnicity	Sex	Age at the time of sample	Hair color	Eye color
FES-0053	Siberia	koryak	f	74	not analyzed	dark
FES-0054	Siberia	even	m	50	dark	dark
FES-0055	Siberia	even	f	18	not analyzed	dark
FES-0056	Siberia	even	f	56	not analyzed	dark
FES-0057	Siberia	even	f	51	not analyzed	dark
FES-0058	Siberia	chukchi	f	47	not analyzed	dark
FES-0059	Siberia	koryak	f	68	not analyzed	dark
FES-0060	Siberia	itelmen	f	56	not analyzed	dark
FES-0061	Siberia	koryak	f	56	not analyzed	dark
FES-0062	Siberia	koryak	f	34	not analyzed	dark
FES-0063	Siberia	even	m	63	not analyzed	dark
FES-0064	Siberia	even	f	66	not analyzed	dark
FES-0065	Siberia	kamchadal	f	82	not analyzed	dark
FES-0066	Siberia	itelmen	m	62	intermediate	dark
FES-0067	Siberia	itelmen	f	53	not analyzed	dark
FES-0068	Siberia	aleut	f	66	not analyzed	dark
FES-0069	Siberia	aleut	f	35	not analyzed	dark
FES-0070	Siberia	aleut	m	42	dark	dark
FES-0071	Siberia	aleut	f	72	not analyzed	dark
FES-0072	Siberia	aleut	f	69	not analyzed	dark
FES-0073	Siberia	aleut	m	63	dark	dark
FES-0074	Siberia	aleut	f	53	not analyzed	dark
FES-0075	Siberia	koryak	m	59	not analyzed	dark
FES-0076	Siberia	koryak	m	62	dark	dark
FES-0077	Siberia	chukchi	f	69	not analyzed	dark
FES-0078	Siberia	koryak	m	69	dark	dark
FES-0079	Siberia	koryak	m	43	dark	light
FES-0080	Siberia	koryak	m	55	dark	dark
FES-0081	Siberia	koryak	f	52	not analyzed	dark
FES-0082	Siberia	koryak	f	55	not analyzed	dark
FES-0083	Siberia	even	f	55	not analyzed	dark
FES-0084	Siberia	koryak	f	57	not analyzed	dark
FES-0085	Siberia	chukchi	m	27	dark	dark
FES-0086	Siberia	even	m	48	not analyzed	light
FES-0087	Siberia	chukchi	m	58	dark	dark
FES-0088	Siberia	chukchi	m	58	dark	dark
FES-0089	Siberia	chukchi	m	56	not analyzed	dark
FES-0090	Siberia	koryak	f	31	not analyzed	dark
FES-0091	Siberia	even	m	35	dark	dark
FES-0092	Siberia	chukchi	m	34	dark	dark
FES-0093	Siberia	itelmen	f	59	not analyzed	dark
FES-0094	Siberia	itelmen	f	58	not analyzed	dark
FES-0095	Siberia	itelmen	m	49	dark	dark
FES-0096	Siberia	itelmen	f	70	not analyzed	dark
FES-0097	Siberia	itelmen	m	38	dark	dark
FES-0098	Siberia	itelmen	f	60	not analyzed	dark
FES-0099	Siberia	itelmen	f	60	not analyzed	dark
FES-0100	Siberia	itelmen	m	20	dark	dark
FES-0101	Siberia	itelmen	f	55	not analyzed	dark
FES-0102	Siberia	itelmen	f	40	not analyzed	dark
FES-0103	Siberia	itelmen	m	39	dark	dark
FES-0104	Siberia	itelmen	f	56	not analyzed	dark

End of Table 1

Sample	Metapopulation	Ethnicity	Sex	Age at the time of sample	Hair color	Eye color
FES-0105	Siberia	itelmen	f	71	not analyzed	dark
FES-0106	Siberia	itelmen	m	59	not analyzed	dark
FES-0107	Siberia	itelmen	f	47	not analyzed	dark
FES-0108	Siberia	itelmen	m	58	dark	dark
FES-0109	European Russia	mari	m	64	not analyzed	dark
FES-0110	European Russia	mari	m	56	dark	light
FES-0111	European Russia	mari	m	59	not analyzed	dark
FES-0112	European Russia	mari	m	38	dark	light
FES-0113	European Russia	mari	m	49	intermediate	light
FES-0114	European Russia	mari	m	58	dark	dark
FES-0115	European Russia	mari	m	50	dark	light
FES-0116	European Russia	mari	m	54	dark	dark
FES-0117	European Russia	mari	m	46	dark	intermediate
FES-0118	European Russia	mari	m	45	dark	light
FES-0119	European Russia	mari	m	70	not analyzed	dark
FES-0120	European Russia	mari	m	66	dark	intermediate
FES-0121	European Russia	mari	m	66	not analyzed	light
FES-0122	European Russia	mari	m	23	red	intermediate
FES-0123	European Russia	mari	m	51	blond	intermediate
FES-0124	European Russia	mari	m	37	red	light
FES-0125	European Russia	mari	m	58	not analyzed	dark
FES-0126	European Russia	mari	m	64	intermediate	light
FES-0127	European Russia	mari	m	61	not analyzed	dark
FES-0128	European Russia	mari	m	57	intermediate	dark
FES-0129	European Russia	russian	m	59	dark	light
FES-0130	European Russia	russian	m	34	intermediate	light
FES-0131	European Russia	russian	m	34	dark	light
FES-0132	European Russia	russian	m	40	dark	dark
FES-0133	European Russia	russian	m	32	dark	light
FES-0134	European Russia	russian	m	52	intermediate	light
FES-0135	European Russia	russian	m	30	dark	dark
FES-0136	European Russia	russian	m	41	dark	light
FES-0137	European Russia	karelian	m	75	intermediate	light
FES-0138	European Russia	karelian	m	79	not analyzed	light
FES-0139	European Russia	karelian	m	70	not analyzed	light
FES-0140	European Russia	karelian	m	66	intermediate	light
FES-0141	European Russia	karelian	f	79	not analyzed	light
FES-0142	European Russia	karelian	m	68	dark	light
FES-0143	European Russia	karelian	m	59	not analyzed	light
FES-0144	European Russia	karelian	m	62	dark	light

– recall (the ratio of true positives to the sum of true positives and false negatives in the class);

– accuracy (the proportion of correct predictions);

–  $F_1$  score (the harmonic mean of precision and recall),

– AUC (area under curve) for ROC-curves (the true positive rate plotted against the false-positive rate at various threshold settings).

Quality metrics values are provided in Tables 3 and 4.

## RESULTS

We photographed 144 representatives of the indigenous populations inhabiting European Russia and Siberia. Their

DNA samples were genotyped for the markers included in the HirisPlex panel. Phenotyping and genotyping data obtained for each study participant were saved to a combined database.

To evaluate the quality of eye/hair color prediction by HirisPlex-S in new populations phenotyped in advance, we predicted eye and hair color from their genotypes using the online webtool [16]. Results of eye color prediction for each individual case are shown in Table 5. Tables 1 and 5 allow comparing the observed and predicted phenotypes for each individual sample. Prediction quality metrics for the entire dataset are provided in Table 3.

In our study, AUC values, the most widely used quality metric, ranged between 0.89 and 0.59 for different phenotypic classes, averaging 0.79. For Russian populations, AUC values

## ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ | ГЕНЕТИКА

**Table 2.** Genotypes of markers included in the HlrisPlex panel for eye and hair color prediction

RS	rs11547464	rs1805005	rs1805006	rs1805007	rs2228479	rs1110400	rs28777	rs12821256	rs4959270	rs12203592	rs1042602	rs1800407	rs2402130	rs12913832
Color prediction	Eyes and hair													
CHROM	16	16	16	16	16	16	5	12	6	6	11	15	14	15
POS	89986091	89985844	89985918	89986117	89985940	89986130	33958959	89328335	457748	396321	88911696	28230318	92801203	28365618
REF	G	C	C	G	G	A	C	A	C	G	C	G	A	A
ALT	A	A	A	A	G	A	G	A	A	A	A	G	G	G
FES-0001	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0002	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0
FES-0003	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0004	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0005	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/1
FES-0006	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1
FES-0007	./.	0/1	0/0	./.	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0008	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0009	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0010	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0011	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0
FES-0012	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0
FES-0013	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0
FES-0014	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/1	0/0
FES-0015	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/1	0/0
FES-0016	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0
FES-0017	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0
FES-0018	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	1/1	0/0	0/0	0/1
FES-0019	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/1	0/0	0/0	0/0	0/0	0/0
FES-0020	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	1/1	0/1	0/0	0/0	0/0	0/0
FES-0021	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0
FES-0022	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/1	0/0	0/0	0/0	0/0	0/0
FES-0023	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	1/1	0/1	0/1	0/0	0/1	0/1
FES-0024	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/1	0/0	0/0	0/1	0/0	0/0	1/1
FES-0025	0/0	0/0	0/0	0/1	0/0	0/0	1/1	0/0	0/1	0/1	0/0	0/0	0/0	0/1
FES-0026	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/1	0/0	0/1	1/1
FES-0027	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/1	0/0	0/0	0/0	./.	0/0	0/0
FES-0028	0/0	0/0	0/0	0/1	0/0	0/0	0/1	0/0	0/1	0/0	0/0	0/1	0/0	1/1
FES-0029	0/0	0/1	0/0	0/1	./.	0/0	./.	0/0	1/1	0/0	0/0	0/0	0/0	0/1
FES-0030	0/0	0/0	0/0	0/1	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/1
FES-0031	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/1	0/0
FES-0032	0/0	0/0	0/0	0/0	0/1	0/0	1/1	0/0	0/1	1/1	0/1	0/0	0/1	0/1
FES-0033	0/0	0/0	0/0	0/0	0/1	0/0	1/1	0/0	0/1	0/0	0/1	0/0	0/1	0/1
FES-0034	0/0	0/0	0/0	0/0	0/1	0/0	1/1	0/0	0/1	0/0	0/0	0/0	0/0	0/1
FES-0035	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	1/1	0/0	0/1	0/0	0/0	0/1
FES-0036	0/0	0/0	0/0	0/0	0/1	0/0	1/1	0/0	0/1	0/0	0/1	0/0	0/0	0/1
FES-0037	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	1/1	0/1	0/0	0/0	0/0	0/1
FES-0038	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/1	0/0	0/1
FES-0039	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/1	0/1	0/0	0/1	0/0	0/1	1/1
FES-0040	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/1	0/0	0/0	0/0	1/1
FES-0041	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/1	0/1	0/0	0/0	0/1	1/1
FES-0042	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/0	1/1	0/0	0/1	0/0	0/0	0/1
FES-0043	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/1	0/1	0/1
FES-0044	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/1	0/0	0/0	1/1
FES-0045	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/1	0/0	0/1	0/0
FES-0046	0/0	0/0	0/0	0/0	0/1	0/0	0/1	0/1	1/1	0/0	0/1	0/0	0/0	0/0
FES-0047	0/0	0/1	0/0	0/0	0/0	0/0	1/1	0/0	0/1	0/0	0/0	0/0	0/1	0/1
FES-0048	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/1	0/1	0/0	0/0	0/1	0/1
FES-0049	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/0	0/1	0/0	0/0	0/0	0/0	0/0
FES-0050	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/1	0/0	0/1	0/0
FES-0051	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/1	0/0	0/0	0/1	0/0	0/1
FES-0052	0/0	0/0	0/0	0/0	0/0	0/0	0/1	./.	0/0	0/0	0/0	0/0	0/0	0/0
FES-0053	0/0	0/0	0/0	0/0	0/0	0/0	0/1	./.	0/0	0/0	0/0	0/0	0/1	0/0

## Continuation of Table 2

## ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ | ГЕНЕТИКА

Continuation of Table 2

RS	rs11547464	rs1805005	rs1805006	rs1805007	rs2228479	rs1110400	rs28777	rs12821256	rs4959270	rs12203592	rs1042602	rs1800407	rs2402130	rs12913832
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REF	G	C	C	G	G	A	C	A	C	G	C	G	A	A
ALT	A	A	A	A	G	A	G	A	A	A	A	G	G	G
FES-0054	0/0	0/0	0/0	0/0	0/1	0/0	0/0	./.	0/0	0/0	0/0	0/0	0/0	0/0
FES-0055	0/0	0/0	0/0	0/0	0/1	0/0	0/1	./.	0/1	0/1	0/0	0/0	0/1	0/0
FES-0056	0/0	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/1	0/0	0/0	0/0	0/0	0/0
FES-0057	0/0	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/1	0/0	0/0	0/0	0/0	0/0
FES-0058	0/0	0/0	0/0	0/0	0/0	0/0	0/1	./.	0/0	0/0	0/0	0/0	0/1	0/0
FES-0059	0/0	0/0	0/0	0/0	0/1	0/0	0/0	./.	1/1	0/0	0/0	0/0	0/0	0/0
FES-0060	0/0	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/0	0/0	0/0	0/0	0/0	0/0
FES-0061	0/0	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/0	0/0	0/0	0/0	0/0	0/0
FES-0062	0/0	0/0	0/0	0/0	0/1	0/0	0/0	./.	0/1	0/0	0/0	0/0	0/0	0/0
FES-0063	0/0	0/0	0/0	0/0	0/1	0/0	0/0	./.	0/0	0/0	0/0	0/0	0/0	0/0
FES-0064	0/0	0/0	0/0	0/0	0/1	0/0	0/0	./.	0/0	0/0	0/0	0/0	0/0	0/0
FES-0065	0/0	0/0	0/0	0/0	0/1	0/0	0/1	./.	0/0	0/0	0/0	0/0	0/0	0/1
FES-0066	0/0	0/0	0/0	0/0	0/0	0/0	1/1	./.	1/1	0/0	0/0	0/0	0/0	0/0
FES-0067	0/0	0/0	0/0	0/0	0/1	0/0	0/1	./.	0/0	0/0	0/0	0/0	0/0	0/0
FES-0068	0/0	0/0	0/0	0/0	0/0	0/0	1/1	./.	0/0	0/0	0/0	0/0	0/0	0/0
FES-0069	0/0	0/0	0/0	0/0	0/0	0/0	0/1	./.	1/1	0/0	0/1	0/0	0/0	1/1
FES-0070	0/0	0/0	0/0	0/0	0/1	0/0	0/0	./.	0/1	0/0	0/0	0/0	0/0	0/1
FES-0071	0/0	0/0	0/0	0/0	0/1	0/0	1/1	./.	0/1	0/0	0/0	0/0	0/1	0/1
FES-0072	0/0	0/0	0/0	0/0	0/0	0/0	1/1	./.	0/1	0/0	0/0	0/0	0/0	0/0
FES-0073	0/0	0/0	0/0	0/0	0/0	0/0	0/1	./.	0/1	0/0	0/0	0/0	0/1	0/1
FES-0074	0/0	0/0	0/0	0/0	0/0	0/0	1/1	./.	1/1	0/0	0/0	0/0	0/0	0/1
FES-0075	0/0	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/0	0/0	0/0	0/0	0/0	0/0
FES-0076	0/0	0/0	0/0	0/0	0/1	0/0	0/0	./.	0/0	0/0	0/0	0/0	0/0	0/0
FES-0077	0/0	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/1	0/0	0/0	0/0	0/0	0/0
FES-0078	0/0	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/0	0/0	0/0	0/0	0/0	0/0
FES-0079	0/0	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/0	0/0	0/0	0/0	0/1	0/0
FES-0080	0/0	./.	0/0	0/0	0/0	0/0	0/0	./.	0/1	0/0	0/0	0/0	0/0	0/0
FES-0081	0/0	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/0	0/0	0/0	0/0	0/0	0/0
FES-0082	0/0	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/1	0/0	0/0	0/0	0/0	0/0
FES-0083	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/1	0/0
FES-0084	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/1	0/0	0/0
FES-0085	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/1	0/0	0/0	0/0	0/0	0/0
FES-0086	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0
FES-0087	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	1/1	0/0	0/0	0/0	0/0
FES-0088	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0089	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0090	0/0	0/0	0/0	0/0	./.	0/0	0/0	0/0	0/0	0/0	0/0	./.	0/0	0/0
FES-0091	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	1/1	0/1	0/0	0/0	0/1
FES-0092	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0093	0/0	0/0	0/0	0/0	0/1	0/0	0/1	0/0	0/0	1/1	0/0	0/1	0/0	0/0
FES-0094	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0
FES-0095	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0096	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0
FES-0097	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0098	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0
FES-0099	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0100	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/1
FES-0101	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0
FES-0102	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0
FES-0103	0/0	0/0	0/0	0/0	0/1	0/0	0/1	0/0	0/0	0/1	0/0	0/0	0/1	0/0
FES-0104	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0105	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0106	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

## Continuation of Table 2

Continuation of Table 2

RS	rs11547464	rs1805005	rs1805006	rs1805007	rs2228479	rs1110400	rs28777	rs12821256	rs4959270	rs12203592	rs1042602	rs1800407	rs2402130	rs12913832
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REF	G	C	C	G	G	A	C	A	C	G	C	G	A	A
ALT	A	A	A	A	G	A	G	A	A	A	A	G	G	G
FES-0107	0/0	0/0	0/0	0/0	0/1	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0108	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/1	0/0
FES-0109	0/0	0/0	0/0	0/1	0/0	0/0	0/1	0/0	0/1	0/0	0/1	0/0	0/0	0/0
FES-0110	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/1	0/0	0/1	0/0	0/0	0/1
FES-0111	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/1	0/1	0/0	0/0	0/0	0/1
FES-0112	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/1
FES-0113	0/0	0/0	0/0	0/0	0/1	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/1
FES-0114	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/1	0/0	0/1	0/0	0/0	0/1
FES-0115	0/0	0/0	0/0	0/0	0/1	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/1
FES-0116	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/1	0/1	0/1	0/1	0/0	0/0	0/0
FES-0117	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	1/1	0/0	0/0	0/0	0/0	0/1
FES-0118	0/0	0/0	0/0	0/0	0/1	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	1/1
FES-0119	0/0	0/1	0/0	0/0	0/1	0/0	1/1	0/0	0/1	0/1	0/1	0/1	0/0	0/0
FES-0120	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	1/1
FES-0121	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	1/1	0/0	0/0	0/0	0/0	1/1
FES-0122	0/0	0/0	0/0	0/0	1/1	0/0	1/1	0/0	0/1	0/0	0/0	0/0	0/0	0/1
FES-0123	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/1	0/0	1/1	0/0	0/0	0/1
FES-0124	0/0	0/0	0/0	0/0	0/1	0/0	1/1	0/0	0/0	0/0	0/1	0/0	0/0	1/1
FES-0125	0/0	0/0	0/0	0/0	0/1	0/0	1/1	0/0	0/1	0/0	0/1	0/0	0/0	0/1
FES-0126	0/1	0/1	./.	./.	0/1	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/1
FES-0127	0/0	0/0	0/0	0/0	0/1	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FES-0128	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	1/1	0/0	0/0	0/1
FES-0129	0/0	0/0	0/0	0/0	0/0	0/1	0/1	./.	1/1	0/1	0/1	0/0	./.	1/1
FES-0130	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	1/1	0/0	0/0	0/0	0/0	1/1
FES-0131	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/1	0/0	0/1	0/0	0/0	1/1
FES-0132	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/1
FES-0133	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/1	0/0	0/0	1/1
FES-0134	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/1	0/0	0/1	1/1
FES-0135	1/1	1/1	1/1	0/1	./.	0/0	./.	0/0	./.	0/1	0/1	0/1	0/1	0/1
FES-0136	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	1/1	0/1	0/0	0/0	0/1	1/1
FES-0137	0/0	0/0	0/0	0/0	0/1	0/0	1/1	0/0	0/1	0/0	1/1	0/0	0/1	1/1
FES-0138	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/1	0/0	0/1	0/0	0/1	1/1
FES-0139	0/0	./.	0/0	0/0	0/0	0/0	1/1	0/0	0/1	0/0	0/0	0/0	0/0	1/1
FES-0140	0/0	0/0	0/0	0/0	0/1	0/0	1/1	0/0	0/1	0/0	0/1	0/0	0/0	1/1
FES-0141	0/0	0/0	0/0	0/0	0/1	0/0	1/1	0/1	0/1	0/1	0/1	0/1	0/0	1/1
FES-0142	0/0	0/0	0/0	0/1	0/0	0/0	1/1	0/0	0/1	0/1	0/1	0/1	0/0	1/1
FES-0143	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	1/1
FES-0144	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/1	0/0	0/0	0/0	0/0	1/1

are a bit lower than those observed in Western Europeans (0.89). For example, AUC for light eye color is 0.94 for Western Europeans vs 0.89 for Russians. A decrease in AUC values can be observed for all eye and hair color phenotypes represented in our study. Of note, prediction quality metrics for intermediate eye color and light hair color are not provided in this article because these 2 phenotypes were underrepresented in our sample. If necessary, they can be calculated using the data from Tables 1 and 5. Their values turned out to be even lower than in Western European populations, but due to a very small sample size of these 2 phenotypic classes (< 5 individuals), the obtained results cannot be considered reliable.

Russian populations are very heterogeneous genetically. We purposefully included genetically contrasting groups of indigenous populations of European Russia and Siberia in the

sample. Table 4 describes the quality of eye color prediction by HlrisPlex for these 2 metapopulations (the quality of hair color prediction was not evaluated due to a small sample size, see Methods). The accuracy of eye color prediction for the populations of European Russia was close to the prediction accuracy for the pooled sample. There was some decline in accuracy, in comparison with Western European samples, but, on the whole, the accuracy of prediction was satisfactory (AUC about 0.8). For Siberian populations, prediction quality was much poorer (AUC = 0.6).

## DISCUSSION

The collection of anthropological images of the indigenous peoples of Russia laid the foundation for our study. The photos

End of Table 2

taken in 3 planes in accordance with anthropological standards are a valuable resource for research into the associations between phenotypic traits and genotypes. In this study, such images were used to identify eye and hair color. The fact that phenotyping was independently conducted by 3 different experts and the availability of photos for verification render the results of our study reliable and reproducible.

For genotyping, we used the most comprehensive, state-of-the-art, popular HirisPlex-S system that has proven its accuracy in the studies of modern and ancient Western European populations [8, 11, 17]. HirisPlex-S prediction accuracy for the populations outside Western Europe was evaluated by comparing the observed phenotypes identified from the photos to the phenotypes predicted from DNA. Of all quality metrics (Table 3), AUC posed the greatest interest

because AUC values characterizing HlrisPlex performance are available for Western European populations [16]. So, we were able to directly compare the accuracy of HlrisPlex predictions between Western European and Russian populations.

On the whole, the values of prediction quality metrics obtained for the majority of phenotypic classes (Table 3) were quite high (0.6–0.9), suggesting that use of HlrisPlex in Russian populations is justified. None of the systems predicting phenotypes from DNA is 100% accurate; for some classes, HlrisPlex prediction accuracy is below 0.9 even for Western European populations. In our opinion, this study has demonstrated the fitness of HlrisPlex for use in Russian populations and its satisfactory accuracy of prediction. However, HlrisPlex prediction accuracy is lower for Russian populations than for Western Europeans (0.8 vs 0.9 on average). Therefore,

**Table 3.** Parameters of prediction accuracy of HlrisPlex for Western European populations and Russia

	Western European populations	Russia			
	AUC	AUC	Precision	Accuracy	Recall
Light eye color	0.94	0.89	0.89	0.88	0.63
Intermediate eye color	0.74	–	–	–	–
Dark eye color	0.95	0.89	0.85	0.87	0.98
Red hair color	0.93	0.59	0.33	0.92	0.2
Blond hair color	0.81	–	–	–	–
Intermediate hair color	0.74	0.72	0.32	0.66	0.56
Dark hair color	0.86	0.84	0.94	0.68	0.57

**Table 4.** Prediction accuracy of HlrisPlex for Russian regions

	European Russia	Siberia
Light eyes	0.89	0.57
Dark eyes	0.86	0.56

we believe that HlrisPlex can be used in Russian populations but still recommend to account for the detected decline in accuracy when interpreting the obtained data.

In our study, Russian populations were divided into 2 datasets: European Russia and Siberia. Previous population genetic studies [18, 19] revealed that these metapopulations are contrasting in terms of their genetic origin. They also turned out to be contrasting in terms of phenotype prediction quality, which was considerably lower for Siberia (Table 4). The data in Tables 1 and 5 demonstrate that HlrisPlex predicts dark eyes for almost all Siberian samples, although some representatives of Siberian populations have light color eyes; the division into light and intermediate shades is arbitrary, but even so, the color of their eyes is not dark as predicted by HlrisPlex. Perhaps, light color eyes sometimes seen in indigenous Siberian populations is associated with other alleles or other genes, as compared to Europeans,

meaning that the system based on Western European datasets cannot correctly predict light (not dark) color of eyes in those populations. A decrease in the prediction accuracy for the inhabitants of European Russia may have the same nature, but because this population is genetically closer to the populations of Western Europe, the differences in the allele spectrum and the decrease in prediction accuracy are not so pronounced. This can inspire new research aimed at identifying additional genetic markers that could improve the accuracy of prediction of pigmentation phenotypes from genotypes.

## CONCLUSIONS

The analysis of correlations between genotypes and eye/hair pigmentation phenotypes of Russian populations aided by the widely used HlrisPlex-S panel confirmed its fitness for use in these

**Table 5.** Phenotypes (eye and hair color) predicted from genotypes included in the HlrisPlex panel

Sample	Predicted eye color	Predicted hair color
FES-0001	dark	not analyzed
FES-0002	dark	dark
FES-0003	dark	dark
FES-0004	dark	dark
FES-0005	dark	dark
FES-0006	dark	dark
FES-0007	dark	not predicted
FES-0008	dark	not analyzed
FES-0009	dark	dark
FES-0010	dark	dark
FES-0011	dark	not analyzed
FES-0012	dark	dark
FES-0013	dark	dark
FES-0014	dark	dark
FES-0015	dark	dark
FES-0016	dark	dark
FES-0017	dark	dark
FES-0018	dark	not analyzed
FES-0019	dark	not analyzed
FES-0020	dark	not analyzed

Continuation of Table 5

Sample	Predicted eye color	Predicted hair color
FES-0021	dark	dark
FES-0022	dark	dark
FES-0023	dark	intermediate
FES-0024	light	blond
FES-0025	dark	intermediate
FES-0026	light	blond
FES-0027	dark	intermediate
FES-0028	light	red
FES-0029	dark	not analyzed
FES-0030	dark	intermediate
FES-0031	dark	blond
FES-0032	dark	intermediate
FES-0033	dark	intermediate
FES-0034	dark	not analyzed
FES-0035	dark	intermediate
FES-0036	dark	not analyzed
FES-0037	dark	intermediate
FES-0038	dark	blond
FES-0039	light	not analyzed
FES-0040	light	intermediate
FES-0041	light	intermediate
FES-0042	dark	not analyzed
FES-0043	dark	not analyzed
FES-0044	light	blond
FES-0045	dark	not analyzed
FES-0046	dark	intermediate
FES-0047	dark	not analyzed
FES-0048	dark	not analyzed
FES-0049	dark	dark
FES-0050	dark	intermediate
FES-0051	dark	not analyzed
FES-0052	dark	not analyzed
FES-0053	dark	not analyzed
FES-0054	dark	dark
FES-0055	dark	not analyzed
FES-0056	dark	not analyzed
FES-0057	dark	not analyzed
FES-0058	dark	not analyzed
FES-0059	dark	not analyzed
FES-0060	dark	not analyzed
FES-0061	dark	not analyzed
FES-0062	dark	not analyzed
FES-0063	dark	not analyzed
FES-0064	dark	not analyzed
FES-0065	dark	not analyzed
FES-0066	dark	intermediate
FES-0067	dark	not analyzed
FES-0068	dark	not analyzed
FES-0069	light	not analyzed
FES-0070	dark	dark
FES-0071	dark	not analyzed

Continuation of Table 5

Sample	Predicted eye color	Predicted hair color
FES-0072	dark	not analyzed
FES-0073	dark	intermediate
FES-0074	dark	not analyzed
FES-0075	dark	not analyzed
FES-0076	dark	dark
FES-0077	dark	not analyzed
FES-0078	dark	dark
FES-0079	dark	dark
FES-0080	dark	dark
FES-0081	dark	not analyzed
FES-0082	dark	not analyzed
FES-0083	dark	not analyzed
FES-0084	dark	not analyzed
FES-0085	dark	dark
FES-0086	dark	not analyzed
FES-0087	dark	dark
FES-0088	dark	dark
FES-0089	dark	not analyzed
FES-0090	dark	not analyzed
FES-0091	dark	dark
FES-0092	dark	dark
FES-0093	dark	not analyzed
FES-0094	dark	not analyzed
FES-0095	dark	dark
FES-0096	dark	not analyzed
FES-0097	dark	dark
FES-0098	dark	not analyzed
FES-0099	dark	not analyzed
FES-0100	dark	intermediate
FES-0101	dark	not analyzed
FES-0102	dark	not analyzed
FES-0103	dark	dark
FES-0104	dark	not analyzed
FES-0105	dark	not analyzed
FES-0106	dark	not analyzed
FES-0107	dark	not analyzed
FES-0108	dark	dark
FES-0109	dark	not analyzed
FES-0110	dark	intermediate
FES-0111	dark	not analyzed
FES-0112	dark	intermediate
FES-0113	dark	dark
FES-0114	dark	intermediate
FES-0115	dark	dark
FES-0116	dark	intermediate
FES-0117	dark	intermediate
FES-0118	light	intermediate
FES-0119	dark	not analyzed
FES-0120	light	intermediate
FES-0121	light	not analyzed
FES-0122	dark	blond

End of Table 5

Sample	Predicted eye color	Predicted hair color
FES-0123	dark	intermediate
FES-0124	light	blond
FES-0125	dark	not analyzed
FES-0126	dark	not predicted
FES-0127	dark	not analyzed
FES-0128	dark	intermediate
FES-0129	light	intermediate
FES-0130	light	blond
FES-0131	light	blond
FES-0132	dark	intermediate
FES-0133	light	intermediate
FES-0134	light	blond
FES-0135	light	red
FES-0136	light	intermediate
FES-0137	light	blond
FES-0138	light	not analyzed
FES-0139	light	not analyzed
FES-0140	light	blond
FES-0141	light	not analyzed
FES-0142	light	red
FES-0143	light	not analyzed
FES-0144	light	blond

previously unstudied populations, although its prediction accuracy was lower than in Western European datasets that had served as a basis for this classifier. A decrease in accuracy (from 0.94 to 0.89) is not that dramatic for the populations of European Russia, as

compared to Siberian samples. This decrease might be associated with an impact of population-specific SNPs well-represented in the populations of North Eurasia but rarely found in Western Europe and, therefore, not included in the HlrisPlex-S panel.

## References

- Bouakaze C, Keyser C, Crubezy E, Montagnon D, Ludes B. Pigment phenotype and biogeographical ancestry from ancient skeletal remains: inferences from multiplexed autosomal SNP analysis. *Int J Legal Med.* 2009; 123 (4): 315–25.
- Branicki W, Brudnik U, Kupiec T, Wolanska-Nowak P, Szczerbinska A, Wojas-Pelc A. Association of polymorphic sites in the OCA2 gene with eye colour using the tree scanning method. *Ann Hum Genet.* 2008; 72 (Pt 2): 184–92.
- Candille SI, Absher DM, Beleza S, Bauchet M, McEvoy B, Garrison NA, et al. Genome-wide association studies of quantitatively measured skin, hair, and eye pigmentation in four European populations. *PLoS One.* 2012; 7 (10): e48294.
- Han J, Kraft P, Nan H, Guo Q, Chen C, Qureshi A, et al. A genome-wide association study identifies novel alleles associated with hair color and skin pigmentation. *PLoS Genet.* 2008; 4 (5): e1000074.
- Lippert C, Sabatini R, Maher MC, Kang EY, Lee S, Arikan O, et al. Identification of individuals by trait prediction using whole-genome sequencing data. *Proc Natl Acad Sci USA.* 2017; 114 (38): 10166–71.
- Liu F, van Duijn K, Vingerling JR, Hofman A, Uitterlinden AG, Janssens AC, et al. Eye color and the prediction of complex phenotypes from genotypes. *Curr Biol.* 2009; 19 (5): R192–3.
- Maronas O, Sochtig J, Ruiz Y, Phillips C, Carracedo A, Lareu MV. The genetics of skin, hair, and eye color variation and its relevance to forensic pigmentation predictive tests. *Forensic Sci Rev.* 2015; 27 (1): 13–40.
- Walsh S, Chaitanya L, Clarisse L, Wirken L, Draus-Barini J, Kovatsi L, et al. Developmental validation of the HlrisPlex system: DNA-based eye and hair colour prediction for forensic and anthropological usage. *Forensic Sci Int Genet.* 2014; 9: 150–61.
- Walsh S, Kayser M. A Practical Guide to the HlrisPlex System: Simultaneous Prediction of Eye and Hair Color from DNA. *Methods Mol Biol.* 2016; (1420): 213–31.
- Walsh S, Liu F, Wollstein A, Kovatsi L, Ralf A, Kosiniak-Kamysz A, et al. The HlrisPlex system for simultaneous prediction of hair and eye colour from DNA. *Forensic Sci Int Genet.* 2013; 7 (1): 98–115.
- Chaitanya L, Breslin K, Zuñiga S, Wirken L, Pośpiech E, Kukla-Bartoszek M, et al. The HlrisPlex-S system for eye, hair and skin colour prediction from DNA: Introduction and forensic developmental validation. *Forensic Sci Int Genet.* 2018; (35): 123–35.
- Pagani L, Lawson DJ, Jagoda E, Mörseburg A, Eriksson A, Mitt M, et al. Genomic analyses inform on migration events during the peopling of Eurasia. *Nature.* 2016 Oct 13; 538 (7624): 238–42. DOI: 10.1038/nature19792.
- Balanovska EV, Zhabagin MK, Agdzhoyan AT, Chukhryayeva MI, Markina NV, Balaganskaya OA, et al. Populyatsionnye biobanki: printsipy organizatsii i perspektivy primeneniya v genogeografi i personalizirovannoy meditsine. *Genetika.* 2016; (12): 1371–87. Russian.
- Powell R, Gannon F. Purification of DNA by phenolextraction and ethanol precipitation. *Practical Approach Series.* Oxford: Oxford University Press, 2002.
- Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience.* 2015 December; 4 (1):

- s13742-015-0047-8. DOI: 10.1186/s13742-015-0047-8.
16. Department of Genetic Identification of Erasmus MC. HlrisPlex-S Eye, Hair and Skin Colour DNA Phenotyping Webtool. [software]. Available from: <https://hirisplex.erasmusmc.nl/>.
  17. Draus-Barini J, Walsh S, Pospiech E, Kupiec T, Glab H, Branicki W, et al. Bona fide colour: DNA prediction of human eye and hair colour from ancient and contemporary skeletal remains. *Investigative Genetics*. 2013 January; (4): 3. DOI: 10.1186/2041-2223-4-3.
  18. Jeong C, Balanovsky O, Lukianova E, Kahbatkyzy N, Flegontov P, Zaporozhchenko V, et al. The genetic history of admixture across inner Eurasia. *Nat Ecol Evol*. 2019 Jun; 3 (6): 966–76. DOI: 10.1038/s41559-019-0878-2.
  19. Triska P, Chekanov N, Stepanov V, Khusnutdinova EK, Kumar GPA, Akhmetova V, et al. Between Lake Baikal and the Baltic Sea: genomic history of the gateway to Europe. *BMC Genet*. 2017 Dec 28; 18 (Suppl 1): 110. DOI: 10.1186/s12863-017-0578-3.

**Литература**

1. Bouakaze C, Keyser C, Crubézy E, Montagnon D, Ludes B. Pigment phenotype and biogeographical ancestry from ancient skeletal remains: inferences from multiplexed autosomal SNP analysis. *Int J Legal Med*. 2009; 123 (4): 315–25.
2. Branicki W, Brudnik U, Kupiec T, Wolanska-Nowak P, Szczerbinska A, Wojas-Pelc A. Association of polymorphic sites in the OCA2 gene with eye colour using the tree scanning method. *Ann Hum Genet*. 2008; 72 (Pt 2): 184–92.
3. Candille SI, Absher DM, Beleza S, Bauchet M, McEvoy B, Garrison NA, et al. Genome-wide association studies of quantitatively measured skin, hair, and eye pigmentation in four European populations. *PLoS One*. 2012; 7 (10): e48294.
4. Han J, Kraft P, Nan H, Guo Q, Chen C, Qureshi A, et al. A genome-wide association study identifies novel alleles associated with hair color and skin pigmentation. *PLoS Genet*. 2008; 4 (5): e1000074.
5. Lippert C, Sabatini R, Maher MC, Kang EY, Lee S, Arikan O, et al. Identification of individuals by trait prediction using whole-genome sequencing data. *Proc Natl Acad Sci USA*. 2017; 114 (38): 10166–71.
6. Liu F, van Duijn K, Vingerling JR, Hofman A, Uitterlinden AG, Janssens AC, et al. Eye color and the prediction of complex phenotypes from genotypes. *Curr Biol*. 2009; 19 (5): R192–3.
7. Maronas O, Sochtig J, Ruiz Y, Phillips C, Carracedo A, Lareu MV. The genetics of skin, hair, and eye color variation and its relevance to forensic pigmentation predictive tests. *Forensic Sci Rev*. 2015; 27 (1): 13–40.
8. Walsh S, Chaitanya L, Clarsisse L, Wirken L, Draus-Barini J, Kovatsi L, et al. Developmental validation of the HlrisPlex system: DNA-based eye and hair colour prediction for forensic and anthropological usage. *Forensic Sci Int Genet*. 2014; 9: 150–61.
9. Walsh S, Kayser M. A Practical Guide to the HlrisPlex System: Simultaneous Prediction of Eye and Hair Color from DNA. *Methods Mol Biol*. 2016; (1420): 213–31.
10. Walsh S, Liu F, Wollstein A, Kovatsi L, Ralf A, Kosiniak-Kamysz A, et al. The HlrisPlex system for simultaneous prediction of hair and eye colour from DNA. *Forensic Sci Int Genet*. 2013; 7 (1): 98–115.
11. Chaitanya L, Breslin K, Zuñiga S, Wirken L, Pośpiech E, Kukla-Bartoszek M, et al. The HlrisPlex-S system for eye, hair and skin colour prediction from DNA: Introduction and forensic developmental validation. *Forensic Sci Int Genet*. 2018; (35): 123–35.
12. Pagani L, Lawson DJ, Jagoda E, Mörseburg A, Eriksson A, Mitt M, et al. Genomic analyses inform on migration events during the repopulation of Eurasia. *Nature*. 2016 Oct 13; 538 (7624): 238–42. DOI: 10.1038/nature19792.
13. Балановская Е. В., Жабагян М. К., Агджоян А. Т., Чухряева М. И., Маркина Н. В., Балаганская О. А. и др. Популяционные биобанки: принципы организации и перспективы применения в геногеографии и персонализированной медицине. *Генетика*. 2016; (12): 1371–87.
14. Powell R, Gannon F. Purification of DNA by phenolextraction and ethanol precipitation. *Practical Approach Series*. Oxford: Oxford University Press, 2002.
15. Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience*. 2015 December; 4 (1): s13742-015-0047-8. DOI: 10.1186/s13742-015-0047-8.
16. Department of Genetic Identification of Erasmus MC. HlrisPlex-S Eye, Hair and Skin Colour DNA Phenotyping Webtool. [software]. Available from: <https://hirisplex.erasmusmc.nl/>.
17. Draus-Barini J, Walsh S, Pospiech E, Kupiec T, Glab H, Branicki W, et al. Bona fide colour: DNA prediction of human eye and hair colour from ancient and contemporary skeletal remains. *Investigative Genetics*. 2013 January; (4): 3. DOI: 10.1186/2041-2223-4-3.
18. Jeong C, Balanovsky O, Lukianova E, Kahbatkyzy N, Flegontov P, Zaporozhchenko V, et al. The genetic history of admixture across inner Eurasia. *Nat Ecol Evol*. 2019 Jun; 3 (6): 966–76. DOI: 10.1038/s41559-019-0878-2.
19. Triska P, Chekanov N, Stepanov V, Khusnutdinova EK, Kumar GPA, Akhmetova V, et al. Between Lake Baikal and the Baltic Sea: genomic history of the gateway to Europe. *BMC Genet*. 2017 Dec 28; 18 (Suppl 1): 110. DOI: 10.1186/s12863-017-0578-3.