RESEARCH ARTICLE

The sequencing and interpretation of the genome obtained from a Serbian individual

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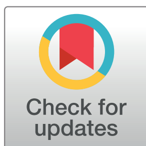

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Abstract

Recent genetic studies and whole-genome sequencing projects have greatly improved our understanding of human variation and clinically actionable genetic information. Smaller ethnic populations, however, remain underrepresented in both individual and large-scale sequencing efforts and hence present an opportunity to discover new variants of biomedical and demographic significance. This report describes the sequencing and analysis of a genome obtained from an individual of Serbian origin, introducing tens of thousands of previously unknown variants to the currently available pool. Ancestry analysis places this individual in close proximity to Central and Eastern European populations; i.e., closest to Croatian, Bulgarian and Hungarian individuals and, in terms of other Europeans, furthest from Ashkenazi Jewish, Spanish, Sicilian and Baltic individuals. Our analysis confirmed gene flow between Neanderthal and ancestral pan-European populations, with similar contributions to the Serbian genome as those observed in other European groups. Finally, to assess the burden of potentially disease-causing/clinically relevant variation in the sequenced genome, we utilized manually curated genotype-phenotype association databases and variant-effect predictors. We identified several variants that have previously been associated with severe early-onset disease that is not evident in the proband, as well as putatively impactful variants that could yet prove to be clinically relevant to the proband over the next decades. The presence of numerous private and low-frequency variants, along with the observed and predicted disease-causing mutations in this genome, exemplify some of the global challenges of genome interpretation, especially in the context of under-studied ethnic groups.

Introduction

The genetic variation between individuals accounts for much of observed human diversity and has the potential to provide information on phenotypic outcomes of clinical consequence. Studies of genetic variation provided by individual genome sequences have revealed that this

variation differs both within and between populations, and also varies considerably depending upon the population [1]. Moreover, characterization of genetic variation of individuals from multiple populations has revealed a correlation between genetic and geographic distances, and has become relevant for determining genetic ancestry and geographic origin [2–6]. Therefore, the characterization of genetic variation has been of major interest for diverse research fields, including medical, biological and anthropological sciences [2–10].

Sequencing of the first human genomes revealed that most genetic variation is derived from single nucleotide variants (SNVs), although insertions and deletions (indels) account for the majority of the variant nucleotides [11]. The increased accessibility of DNA sequencing has contributed to individual efforts from a range of distinct populations. To date, individual genomes from American [11, 12], Han Chinese [13], Russian [14], Khoisan [15], Bantu [15], Japanese [16], German [17], Gujarati Indian [18], Estonian [19], Pakistani [20] and Mongolian [21] populations have been sequenced and analyzed, among many others [1].

Larger-scale efforts to characterize human genetic variation have demonstrated that individuals from different populations carry particular combinations of rare and low-frequency variants. The 1000 Genomes Project Consortium has estimated that 86% of all variants are confined to a single continental group and that about 10% of variants observed in a population are private to that population [1]. Population-specific variants have the potential to be of both functional and biomedical importance [7, 22–24]. Furthermore, evidence of biologically meaningful population-specific variation [25] emphasizes the need for ethnically relevant reference genomes, as has been performed, for example, for the Korean population [26]. Although we are not claiming to have introduced a new reference genome here, it is nevertheless important to expand our sequencing efforts across diverse populations, particularly those that have not been previously studied [10, 27].

In this paper, we describe the sequencing of the first genome of an individual of Serbian origin, a member of a relatively small population in Central to Southeastern Europe. We identify tens of thousands of novel genetic variants in this individual, more than a hundred of which map to protein-coding regions and several hundred of which reside in close proximity to gene coding regions. The extent of observed genetic variation allowed comparisons with extant European populations and reaffirms support for the hypothesis of close correspondence between genetic and geographic distances [2]. These results contribute to ongoing efforts to understand human genetic variation and its geographic distribution, as well as placing the Serbian genome within the context of the broader European population structure. Testing for Neanderthal introgression in the genome, we find evidence to suggest gene flow from Neanderthal to an ancestral pan-European genome, with the Serbian genome being placed within the range of other European populations. After variant annotation, we assess the burden of potentially pathogenic variation present in this genome and identify variants of putative clinical and pharmacogenetic relevance. Finally, we draw conclusions pertaining to the phenotypic consequences and biomedical interpretation of individually sequenced genomes.

Materials and methods

Donor information

The individual whose genome was sequenced and analyzed is a male of Serbian descent. The data, both derived and raw, are publicly available through the Personal Genome Project website [28], participant ID: hu3BDC4B.

Sample collection and DNA sequencing

Two milliliters of saliva were self-collected by the donor and stored using the DNA Genotek Oragene DISCOVER (OGR-500) sample collection kit. Extraction of DNA from the sample and subsequent sequencing were performed at the BGI (Shenzhen, China) on an Illumina HiSeq 2000 sequencer, using standard protocols. To minimize the likelihood of systematic bias in sampling, two libraries were prepared with an insert size of 500 bp each, with paired-end reads of length 90 bp. Sequencing was then carried out in four lanes for each library to ensure at least 30-fold coverage.

Read mapping and variant calling

Single Nucleotide Variants (SNVs) and indels were called using four different pipelines through a combination of two read mappers and two variant callers. The GRCh37 human genome was used as the reference genome to map the paired-end reads. The two read mappers used were BWA-MEM [29] and Bowtie2 [30]. The two variant callers were GATK [31] and Platypus [32]. The GATK pipeline included additional read and variant processing steps such as duplicate removal using Picard tools [33], base quality score recalibration, indel realignment, and genotyping and variant quality score recalibration using GATK, all used according to GATK best practice recommendations [34, 35].

As described later in the Results, variants identified using the BWA+ GATK pipeline were used for all downstream analysis. Variants in the intersection of all four pipelines (two read mappers and two variant callers) were considered to be confidently identified, where the intersection is defined as variant calls for which the chromosome, position, reference, and alternate fields in the VCF files were identical. All variant calls were subsequently annotated with information from NCBI RefSeq using ANNOVAR [36]. We estimated the amount of novel variation expected to be observed from the first individual in a previously uncharacterized population utilizing the 1000 Genomes Project Phase 3 VCF files [37]. To do this, we carried out a leave-one-population-out procedure; i.e., we excluded one of the 26 populations at a time and for each individual in the excluded population, calculated the fraction of variants not seen in any of the individuals from the remaining 25 populations. The calculated fractions of novel variants were used to understand the expected novelty when sequencing an individual from a new population, given a sample of a particular size of previously sequenced individuals from different populations.

Structural variants (SVs) were called using Structural Variation Engine (SVE) and FusorSV [38]. SVE is an execution engine for an ensemble of SV calling algorithms containing BreakDancer [39], BreakSeq2 [40], cnMOPS [41], CNVnator [42], DELLY [43], GenomeSTRiP [44, 45], Hydra [46], and LUMPY [47]. The Docker image of SVE was used to run all the stages with default parameters. All but GenomeSTRiP completed without errors. The Docker image of FusorSV was then used to merge the results from the remaining seven SV callers, using the default fusion model. SVint [48] was used to subsequently annotate the structural variants. Scripts and documentation for parameters used to run all the pipelines described in this study were added to the Personal Genome Project website, participant ID hu3BDC4B.

Principal component analysis

Principal component analysis (PCA) was carried out using the `smartpca` program from EIGENSOFT (v6.0.1; https://github.com/DReichLab/EIG), on the Serbian genome combined with the SNV data (600,841 loci) from Lazaridis et al. [3]. Only the subset of European individuals from their curated fully public dataset was used, reducing the original set of 1,964

individuals to 260. A projection to the first two principal components was used to establish the correspondence between genetic and geographic distance in our results.

Neanderthal introgression

To test for Neanderthal introgression in the Serbian genome, we computed D-statistics [49, 50] using this genome and the dataset from Lazaridis et al. [9]. This dataset includes 294 ancient individuals (only one of which was used here) and a diverse set of 2,068 present-day humans, genotyped on the Affymetrix Human Origins array. Both the archaic and modern genotype data were provided in the PACKEDANCESTRYMAP format, and were combined using the `mergeit` program from EIGENSOFT (v6.1.2; https://github.com/DReichLab/EIG). The merged dataset, in total, contains 2,362 samples genotyped at 621,799 SNV loci. Upon request, we completed the consent form and obtained approval from David Reich's laboratory before using this dataset. Some individuals from the study of Lazaridis et al. [9] could not be included due to consent issues relating to data distribution.

We next genotyped the Serbian genome against these predefined SNVs using GATK HaplotypeCaller and following the GATK best practices recommendations [34, 35]. We converted the resulting VCF files to the EIGENSTRAT format using VCFtools (v0.1.12a, [51]), and integrated the Serbian genotype with the modern and ancient datasets. Finally, we ran qpDstat from AdmixTools (default setting, v701) to calculate D-statistics and to test for Neanderthal gene flow into the Serbian genome [50].

Burden of pathogenic variation

Variants of putative clinical significance were identified using genotype-phenotype databases as well as computational variant-effect prediction. Manually curated genotype-phenotype databases, such as the Human Gene Mutation Database (HGMD) [52], ClinVar [53] and PharmGKB [54], annotate variants with a known relationship to phenotype [52, 55]. Clinical Annotations from PharmGKB were compared against dbSNP v142 rsIDs [56] obtained using the annotate_variation.pl script in ANNOVAR and `avsnp142`. Variants identified by GATK were compared against HGMD and ClinVar to identify potentially disease-causing and disease-associated mutations.

All variants in protein-coding regions were extracted and inputted to the MutPred suite of tools [57–60]. The remaining variation observed in the proband was interrogated using CADD [61]. For disease and gene ontology associations, the hypergeometric test in WebGestalt was used with Benjamini-Hochberg correction for multiple hypothesis-testing [62]. The background set that was used for these analyses included all protein-coding genes from the human reference genome. For the significance of an ontology term to be confirmed, at least five genes were required to be associated with it.

Results

Effect of genotyping software

The choice of computational tools and their parameters in processing raw sequencing reads can significantly impact the resulting genome and the entirety of subsequent analysis [63, 64]. To understand the uncertainty of variant identification in our subject, we evaluated two different read mappers, BWA-MEM [29] and Bowtie2 [30], and two different variant callers, GATK [31] and Platypus [32].

The results from four different platforms are compared and contrasted in Fig 1. The SNV calling shows good concordance between both read mappers and variant callers, with a large

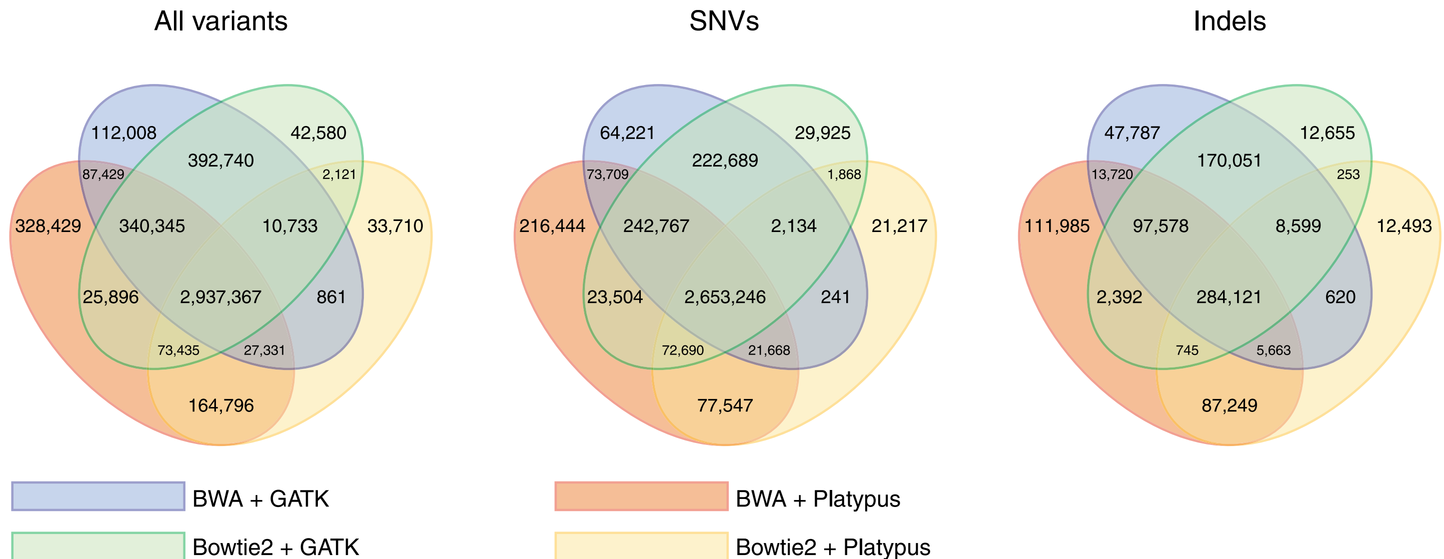

Fig 1. Venn diagrams showing the total numbers of identified variants using two read mappers (BWA [29], Bowtie2 [30]) and two variant callers (GATK [31], Platypus [32]).

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proportion of variants identified by either platform being identified by all platforms. Using the BWA-MEM mapper (which we refer to simply as "BWA" from now on), for example, 2,991,390/3,280,434 = 91.2% of SNVs identified by GATK were also identified by Platypus and 89.1% of SNVs identified by Platypus were also identified by GATK (Fig 1). Indel calling, on the other hand, is less reliable, with 401,082/627,519 = 63.9% variants identified by GATK also identified by Platypus and only 66.7% of variants identified by Platypus being also identified by GATK. The influence of read mappers was markedly lower; i.e., using the GATK variant caller, we found that 95.1% of SNVs and 89.3% of indels identified with BWA were also identified with Bowtie2, and 98.3% SNVs and of 97.6% of indels identified with Bowtie2 were also identified with BWA. Smaller percentages of overlap were observed for Platypus. Based on the results observed in this work (Table A in S1 File) and the extent of usage of these tools in resequencing human genomes, we selected BWA+ GATK as our main platform.

Identification of genetic variants

The genome of a Serbian individual was sequenced according to the protocols described in the Materials and Methods, with all 22 autosomes having similar coverage and the X and Y chromosome having approximately half this coverage. The genome sequencing and mapping achieved an average read depth of 34.7, with 98.3% of GRCh37 reference bases having coverage of 10-fold or more and 89.4% having coverage of 20-fold or more. The number of zero-depth positions were 7,649,443 (0.3%). The coverage distribution is shown in the Supporting Information (S1 Fig).

Using the BWA+ GATK pipeline, we identified a total of 3,908,814 variants (83.9% SNVs, 16.1% indels; Fig 1) in the Serbian genome, of which 2,195,638 (56.2%) were heterozygous with one non-reference allele, 23,095 (0.6%) were heterozygous with two non-reference alleles, and 1,690,081 (43.2%) were homozygous for a non-reference allele. The reported variants passed all quality filters of GATK (marked as "PASS") and were subsequently mapped to GRCh37 human reference genomic regions using ANNOVAR [36]. It is important to mention that ANNOVAR considers all heterozygous positions with both alternative alleles as two

Table 1. Summary of identified variants using BWA+ GATK. Variants not present in gnomAD [66] are listed as novel and variants identified by all four genotyping platforms are listed as confident.

Type of Variant	Variant	Novel	Confident variants	Confident novel
upstream	23094	320	16211	90
upstream; downstream	881	8	624	4
UTR5	5205	54	4055	22
UTR5; UTR3	16	1	12	0
exonic	20706	145	17114	115
exonic; splicing	33	1	22	0
splicing	151	0	107	0
intronic	1410507	20531	1078226	4336
UTR3	31066	409	24095	101
downstream	26685	398	19351	61
ncRNA_exonic	13064	129	9520	30
ncRNA_exonic; splicing	3	0	2	0
ncRNA_intronic	235936	3376	173168	832
ncRNA_splicing	65	1	51	0
ncRNA_UTR5	1	1	0	0
intergenic	2164496	34779	1597484	6848

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different variants. Mechanisms by which heterozygous alternative alleles can arise include sequencing errors and highly variable sites, some of which are tri-allelic because of rare mutational events [65]. Therefore, the resulting genome contains a total of 3,931,909 variants, of which 2,940,042 (74.8%) were identified by all four platforms and are considered to be confident identifications. Unsurprisingly, the majority of identified variants were found to reside in the more expansive and less evolutionarily constrained intergenic and intronic regions (Table 1).

To identify novel variation, we compared the identified variants against the Genome Aggregation Database (gnomAD) [66]. We found that 1.5% (60,153) all variants and 0.4% (12,439) of confident variants were not present in gnomAD. We shall refer to these variants as "novel" and "confident novel" variants, respectively. The breakdown of all variants and novel variants with respect to genomic location is shown in Tables 1 and 2. The percentage of novel variants varied across categories, comprising 0.9% (80) of nonsynonymous variants, 0.4% of synonymous variants, 0.7% (145) of exonic variants, 1.5% (20,531) of intronic variants, and 1.6%

Table 2. Summary of identified exonic variants using BWA+GATK. Variants not present in gnomAD [66] are listed as novel and variants identified by all four platforms are listed as confident.

Type of Variant	Variants	Novel	Confident variants	Confident novel
synonymous SNV	10381	42	8965	36
nonsynonymous SNV	9328	80	7559	69
nonframeshift deletion	137	2	62	0
nonframeshift insertion	117	3	58	0
frameshift deletion	103	6	45	4
frameshift insertion	74	3	37	1
stopgain	87	6	54	4
stoploss	11	0	9	0
unknown	501	4	347	1

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(34,779) of intergenic variants. We found that 45.0% (9,328/20,739) of the exonic variants were nonsynonymous, whereas 50.1% (10,381/20,739) were synonymous. Similar fractions were observed for the confident variants (44.1% vs. 52.3%). Of the 3,871,756 GATK variants that are also observed in the gnomAD database, 3,805,264 (95%) of these variants are annotated to have allele frequency greater than 1% in gnomAD and 3,676,638 (95%) with allele frequency greater than 5%. The proportion of novel variation in the Serbian individual is at the lower end of the distribution compared to 1000 Genomes Project participants (S6 Fig), consistent with a significantly larger size of gnomAD that currently integrates 15,708 whole-genomes and 125,748 exomes.

Using SVE and FusorSV, we identified 848 deletions and 3 duplications, which include the most confident calls generated by FusorSV after merging call-sets from seven different SV-callers using the default fusion model. The numbers of structural variants called by individual SV-callers are reported in (Table B in S1 File). The deletions in the Serbian genome have a length distribution (S7 Fig) similar to the deletions in the 27 deep-coverage samples of the 1000 Genomes Project reported by FusorSV [38]. The lengths of the three duplications are 313101, 362391 and 471821 bp. We used SVint to annotate the functional impact of the structural variants. The genes that overlap with the identified structural variants are listed in S1 File Tables C and D.

Genetic variation and geographic distance

The projection of the Serbian individual to the first and second principal components against European groups from [3] confirms that individuals from the same geographic region cluster together (Fig 2). We clearly distinguish clusters of major populations composed of individuals from the same region, approximately mirroring a map of Europe. The PCA plot demonstrates

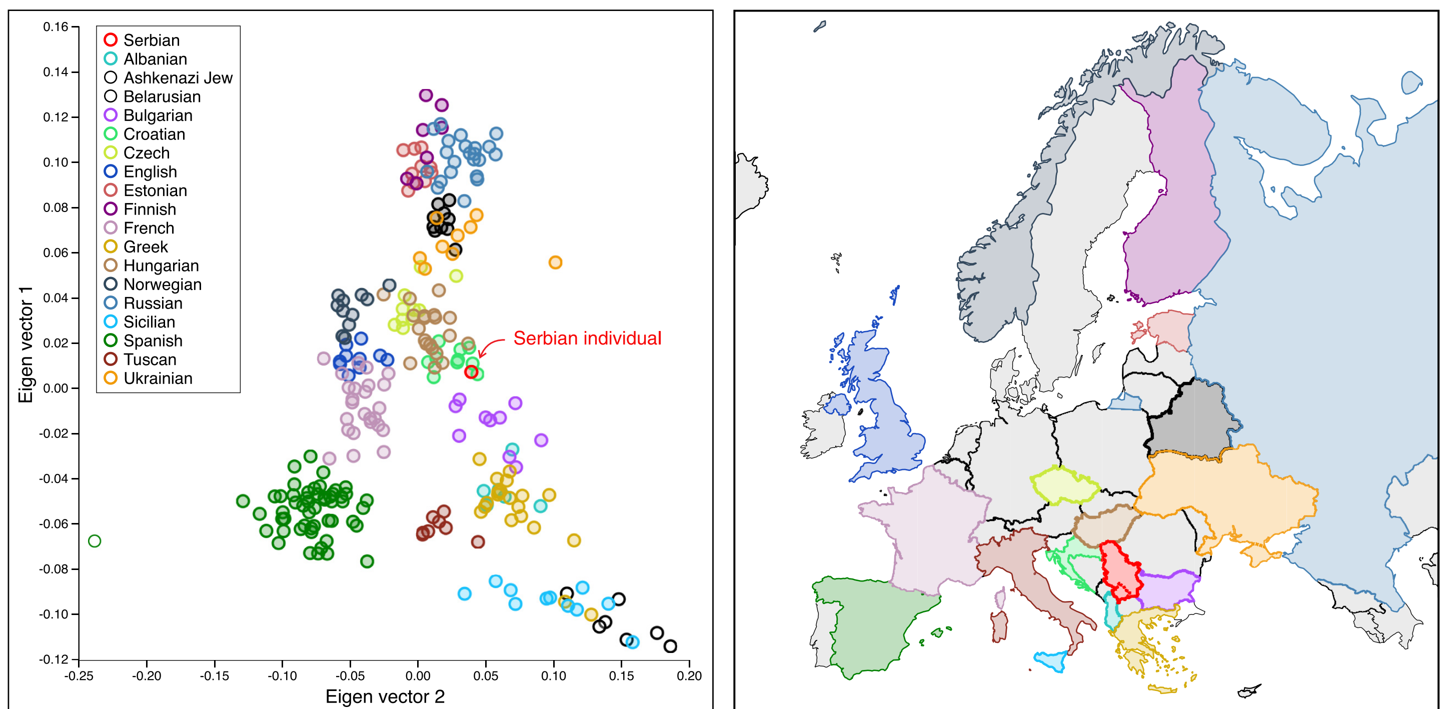

Fig 2. Principal component analysis (PCA) plot showing the proximity of the genome sequenced in this study to other European genomes. As observed in previous studies [2, 3], genomic distance correlates with geographic distance.

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that the genetic ancestry of the Serbian individual analyzed in the present study corresponds to its geographic distance from other populations. It is positioned in close proximity of the Croatian, Bulgarian, and Hungarian populations.

A somewhat surprising finding is the similarity of distances between the Serbian individual and other mostly Slavic populations (Russian, Belarus, Ukrainian) relative to distances to various Central, Western, and Southern European groups (Czech, French, English, Albanian, Greek). The average Euclidean distance and variance between the Serbian individual and each of the available populations in the two-dimensional space of major PCA components is as follows: Croatian (0.016826 ± 0.010526), Bulgarian (0.033603 ± 0.000225), Hungarian (0.037121 ± 0.000177), Czech (0.053687 ± 0.000033), Albanian (0.058875 ± 0.000117), Ukrainian (0.064328 ± 0.000062), Belarusian (0.069803 ± 0.000043), Greek (0.071108 ± 0.00062), Tuscan (0.0736441 ± 0.000028), French (0.083077 ± 0.000159), English (0.084570 ± 0.000142), Norwegian (0.092721 ± 0.00088), Russian (0.095968 ± 0.000079), Estonian (0.098421 ± 0.000046), Finnish (0.108523 ± 0.000154), Sicilian (0.120370 ± 0.000481), Spanish (0.134602 ± 0.000776), Ashkenazi (0.156692 ± 0.000538). The three closest individuals to the Serbian genome were of Croatian ancestry (0.0038, 0.0046, and 0.0108).

We note that combining the Serbian individual with the set of 260 European individuals from Lazaridis et al. [3] caused 50 formerly biallelic sites to become triallelic (no monoallelic sites became triallelic). The triallelic sites were removed from the analysis, leaving 600,791 sites in the analysis. The `smartpca` program was applied to the 261-by-600,791 genotype matrix.

Gene flow with Neanderthals

Comparisons between Neanderthals and modern humans have previously revealed evidence of gene flow from Neanderthals to Europeans [49, 50, 67, 68]. To test whether the Serbian genome shares an excess of alleles with the Neanderthal genome, we integrated the Serbian genotype with a published panel of ancient and modern humans (Materials and Methods). We calculated D-statistics as a formal test for gene flow based on a four-taxon phylogeny, $D(P_1, P_2, P_3, O)$, where P_i ($i \in \{1, 2, 3\}$) are populations and O is an outgroup. Given a scenario where gene flow is absent, the derived alleles of P_3 are expected, with equal likelihood, to match those of P_1 and P_2; i.e., $D = 0$. Alternatively, either P_1 or P_2 could share alleles with P_3 more often than not, in which case D deviates from zero.

We computed *D*(Yoruba, Serbian, Altai, Chimpanzee) for testing for gene flow between Neanderthals ("Altai") and the given Serbian genome. We expected a positive *D* value, given previous evidence that Neanderthals exchanged more alleles with Europeans than with Africans. The test returned a *D* value of 0.0241 ± 0.004476, which significantly deviated from zero (Z-score = 5.39; Table 3), suggesting gene flow between Neanderthal and the lineage leading to the Serbian genome. To validate this result, we also ran the test for other European populations (Table 3). D-statistics calculated for Croatian, French, Greek and Russian genomes were comparable to our result, all falling within the expected range of values reported in previous studies [49, 67, 68].

We further attempted to ensure that the calculated D-statistics were unbiased. To do this, we repeated the analysis by replacing Yoruba with Mbuti, as some of the Yoruba samples could have had some recent European admixture. The calculation for *D*(Mbuti, Serbian, Altai, Chimpanzee) yielded a *D* value of 0.0186 ± 0.004763 (Z-score = 3.99; Table 3), consistent with our results using the Yoruba samples. We next checked whether the Serbian individual has reference biases in genotyping that could have inflated the *D* value. We performed D-statistics tests in the form of *D*(other European population, Serbian, Mbuti, hg19ref) and chose Croatian, French, Greek and Russian as the "other European population". We obtained no test

Table 3. Testing gene flow with Neanderthals. The results show the D-statistic (*D*), its standard error (SE) and Z-score (*Z*) for the test using the set of populations P_1, P_2, and P_3, with Chimpanzee as an outgroup (*O*). The last two columns show ABBA vs. BABA counts over the four genomes (P_1, P_2, P_3, *O*).

P_1	P_2	P_3	*O*	*D*	SE	Z-score	ABBA	BABA
Yoruba	Serbian	Altai	Chimpanzee	0.0241	0.004476	5.393	18158	17302
Yoruba	Croatian	Altai	Chimpanzee	0.0233	0.003192	7.302	18268	17436
Yoruba	French	Altai	Chimpanzee	0.0266	0.003012	8.821	18284	17338
Yoruba	Greek	Altai	Chimpanzee	0.0270	0.003034	8.906	18266	17305
Yoruba	Russian	Altai	Chimpanzee	0.0288	0.003096	9.306	18328	17302
Mbuti	Serbian	Altai	Chimpanzee	0.0186	0.004763	3.909	18817	18129
Mbuti	Croatian	Altai	Chimpanzee	0.0178	0.003693	4.832	18891	18229
Mbuti	French	Altai	Chimpanzee	0.0210	0.003532	5.941	18902	18125
Mbuti	Greek	Altai	Chimpanzee	0.0214	0.003578	5.978	18897	18106
Mbuti	Russian	Altai	Chimpanzee	0.0232	0.003600	6.434	18932	18074

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results indicating the bias of Serbian genotypes toward the reference (Croatian: 0.0054 ± 0.004183; French: 0.0038 ± 0.004078; Greek: 0.0090 ± 0.004182; Russian: 0.0074 ± 0.004192).

Analysis of medically relevant variants

The sequenced genome contains 2,343 genetic variants that are present in HGMD by virtue of their having been previously associated with a risk of disease; the proportions of variants within each effect category are shown in Table 4. Several homozygous variants, manually annotated as disease-causing (DM) are observed in the genome, shown in Table 5. Of these,

Table 4. Amount of disease-causing and potentially disease-relevant variation in the Serbian genome. Identified variants were searched against HGMD and broken down into the phenotypic categories of HGMD. Variants were broken down into exonic and noncoding as well as homozygous and heterozygous.

	Exome		Noncoding	
	Hom	Het	Hom	Het
Disease-causing mutations (DM)	1	9	4	6
Likely disease-causing mutations (DM?)	29	51	8	31
Disease-associated polymorphisms with additional supporting functional evidence (DFP)	78	139	203	301
Disease-associated polymorphisms (DP)	233	356	189	322
Polymorphisms that affect gene/protein structure, function or expression but with no reported disease association (FP)	63	95	95	130

The number of homozygous and heterozygous variants that are associated with variants reported in HGMD. HGMD labels correspond to the strength and/or evidence for the relationship between variant and disease.

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Table 5. Disease-causing variants observed in the proband. The table summarizes the analysis of five homozygous variants form the sequenced genome that are listed by HGMD as disease-causing.

Gene	Variant	rsID	Phenotype
MIR137HG	NC_000001.10:g.98502934G>T	rs1625579	Schizophrenia increased risk
SLC12A3	NM_000339.2:c.1670-8C>T	NA	Gitelman syndrome without hypomagnesaemia
DUOXA2	NM_207581.3:c.554+6C>T	NA	Hypothyroidism
F13A1	NM_000129.3:c.-19+12C>A	rs2815822	Factor XIII deficiency
PNPLA2	NP_065109.1:p.P481L	rs1138693	Myopathy late-onset

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one is a youth-onset phenotype, Factor XIII deficiency, associated with homozygosity for the disease-causing allele (NM_000129.3:c.-19+12C>A) in the proband's genome. The disease phenotypes associated with these homozygous mutations typically become apparent in childhood, and therefore their occurrence in a healthy adult is indicative of variable penetrance. The other homozygous disease-causing variants result in phenotypes that have not yet been observed in either the individual or in their family history; perhaps reflecting either low expressivity or late-onset. Observed heterozygous disease-causing mutations are primarily childhood-onset without presentation in the individual, although they may represent recessive conditions; thus, their failure to manifest may not necessarily be indicative of poor reporting or curation quality. Next, we identified several variants with pathogenic annotation in the ClinVar database, an open-access alternative to HGMD [53]. These variants are either low-confidence or without known family history; more details are available in the Supporting Information (S1 File).

We also identified several variants of potential pharmacogenetic relevance using PharmGKB. Variants in PharmGKB are assigned Clinical Annotation Levels of Evidence from variants with preliminary evidence (Level 4) to high confidence variant-drug combinations with medically endorsed integration into health systems (Level A1). The genome contains a single variant with a high-confidence annotation (Level 1B): rs2228001, associated with toxicity and adverse drug reaction to cisplatin, a chemotherapeutic agent. A further 17 variants were annotated with moderate evidence to impact the dosage, efficacy, metabolism and/or toxicity of drugs for diverse phenotypes including chronic hepatitis C, organ transplantation rejection, glaucoma, depression, schizophrenia, asthma, epilepsy and HIV infections, as well as several chemotherapy drugs.

Pathogenicity prediction. In addition to known disease-associated variants, we identified missense variants predicted to be pathogenic by MutPred2 [57]. Of the 11,206 missense variants called by GATK, 9,329 passed all quality filters (annotated as 'PASS'). Of these, 9,305 variants were unambiguously mapped to the correct protein isoforms and hence were amenable for prediction by MutPred2. Based on a score threshold of 0.8 (estimated 5% false positive rate), 95 missense variants were predicted to be 'pathogenic.'

Of these, 14 variants were found in the homozygous state and 81 were found in the heterozygous state. Genes for these variants were enriched in GO terms related to peptidase activity S8 Fig). A similar analysis for disease associations revealed that the subject may be at risk for cardiovascular disorders (Table I in S1 File).

Next, we applied computational predictors on the remaining protein coding variation with the MutPred family of tools. First, we assessed the pathogenicity of 180 nonsense and frameshifting insertion and deletion variants with MutPred-LOF [58]. From this set, we identified a total of 7 variants with scores above the 0.5 score threshold (corresponding to a 5% false positive rate) (Table E in S1 File). Next, we assessed 279 non-frameshifting insertion and deletion variants with MutPred-Indel and identified 12 variants described in (Table F in S1 File. Finally, we assessed the pathogenicity of the 90 SNV splicing variants with MutPred Splice [59]. Of these, 28 of the variants scored at least 0.6 and were therefore classified as a "Splice Affecting Variant" by MutPred Splice. One of these variants is predicted to cause loss of natural 3' splice sites, two variants are predicted to interrupt cryptic 3' splice sites, and three variants are predicted to disrupt cryptic 5' splice sites, described in the Supporting Information (Table G in S1 File).

To ensure assessment of the complete variome of the proband, we utilized CADD v1.3 [61] to evaluate all noncoding variants. To do this, we utilized a scaled C-score cutoff of 20 to identify the 1% most damaging variants. In total, we found 16 UTR variants, 1,630 intronic variants, 3,911 intergenic variants, 80 regulatory variants, 839/533 upstream/downstream variants,

and 9 variants annotated as "noncoding_change." All of these were predicted to be deleterious. The noncoding variants with the highest C-scores are described in the Supporting Information (Table H in S1 File).

Discussion

This work describes the first whole-genome sequencing of a Serbian individual. Ancestry analysis positioned the Serbian individual in closest proximity to the Croatian population, consistent with its Southern Slavic ancestry [69]. Our analyses further support the hypothesis of gene flow between Neanderthal and pan-European ancestral populations, with the level of introgression into the Serbian genome being within the range observed in other European populations. Previous genetic studies involving Slavic populations employed mitochondrial, Y-chromosome and SNV-panel data to investigate the relationship between geographic, genetic and linguistic distances [69, 70]. Consistent with this work, our analyses expand the scope beyond Slavic populations and further contribute to the understanding of human genetic variation and its geographic distribution.

In contrast to studies using genotyping arrays [2, 3, 69, 70], the availability of whole-genome sequences presents the opportunity for a high-resolution individualized analysis. To this end, we found that the sequenced genome contains a significant number of previously unobserved variants, which emphasizes the importance of continued sequencing of a large number of individuals, especially from previously uncharacterized ethnic groups. Subsequent sequencing of other Serbian individuals could provide further insight into these novel variants; e.g., whether they are private to the population or to the individual. Such results would in turn contribute important information regarding variants that are currently considered to be rare, with implications for improved variant interpretation. Furthermore, new algorithms and reduced sequencing costs will have the potential to provide higher-quality analysis of structural variants. Our analysis also found a number of variants of clinical and pharmacogenomic significance that might extend beyond an individual's disease risks to facilitate possible future medical interventions although conclusions are limited without validation and knowledge of allele frequencies in the Serbian population [71, 72]. Such variants might contribute to better outcomes in studies of disease penetrance, mechanistic understanding of population risks, and database curation.

Recent advances in high-throughput sequencing and reduced costs of genotyping have greatly facilitated whole-genome data generation, and have become key to understanding both human phenotypes and early human history [2, 3]. However, modern technology and cost structure continue to pose challenges in determining and interpreting one's genome [73]. Variation in read mapping and variant calling contribute to the uncertainty of interpretation with different software packages, identifying different sets of variants. We found that inter-software discrepancies ranged from relatively small for SNVs to considerable for insertions and deletions, especially for structural variants. Therefore, variant and genome interpretation demand caution, since thousands of SNVs and tens of thousands of indels may simply constitute genotyping errors [74, 75].

It is worth mentioning that in addition to the technical aspects of genome sequencing, an important aspect of genome interpretation concerns psychosocial uncertainty due to phenotypic and privacy-associated risks [76]. The geographic distance analysis in this study has provided evidence that supports the individual's own sense of Serbian ancestry; however, the finding of multiple predicted youth-onset pathogenic mutations in a healthy individual provides cautionary lessons for predictive medicine.

Supporting information

S1 Fig. Histogram of read depths.
(EPS)

S2 Fig. Read depth across SNV and insertion/deletion variants across the four pipelines.
(EPS)

S3 Fig. Size of insertion/deletion variants across the four pipelines.
(EPS)

S4 Fig. Number of heterozygous and homozygous variants across the four pipelines.
(EPS)

S5 Fig. Size of insertion/deletion variants in the Serbian genome.
(EPS)

S6 Fig. Proportion of novel variants in thousand genomes project participants.
(EPS)

S7 Fig. Length distribution of deletions called by FusorSV.
(EPS)

S8 Fig. GO terms enriched in the set of 81 genes that harbored the 95 missense variants predicted to be pathogenic.
(EPS)

S1 File. Annotation descriptions and tables of variants scored as pathogenic.
(PDF)

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