Patterns of genetic structure and adaptive positive selection in the Lithuanian population from high-density SNP data

A. Urnikyte1, A. Flores-Bello2, M. Mondal3, A. Molyte1, D. Comas2, F. Calafell2, E. Bosch2 & V. Kučinskas1

The analysis of geographically specific regions and the characterization of fine-scale patterns of genetic diversity may facilitate a much better understanding of the microevolutionary processes affecting local human populations. Here we generated genome-wide high-density SNP genotype data in 425 individuals from six geographical regions in Lithuania and combined our dataset with available ancient and modern data to explore genetic population structure, ancestry components and signatures of natural positive selection in the Lithuanian population. Our results show that Lithuanians are a homogenous population, genetically differentiated from neighbouring populations but within the general expected European context. Moreover, we not only confirm that Lithuanians preserve one of the highest proportions of western, Scandinavian and eastern hunter-gather ancestry components found in European populations but also that of an steppe Early to Middle Bronze Age pastoralists, which together configure the genetic distinctiveness of the Lithuanian population. Finally, among the top signatures of positive selection detected in Lithuanians, we identified several candidate genes related with diet (PNLIP, PPARD), pigmentation (SLC24A5, TYRP1 and PPARD) and the immune response (BRD2, HLA-DOA, IL26 and IL22).

Lithuania is a north-eastern European country where the most archaic Indo-European language is spoken1. The current Lithuanian population resulted from a complex amalgam between the former Baltic tribes each with potentially different contributions from Finno-Ugric and Slavic sources2. Moreover, since the Neolithic, the native population of what would become Lithuania has not been substituted by other peoples. Thus, the origin of the contemporary Lithuanian population may be traced back to the Neolithic settlers, with little admixture hereafter3.

The first settlement in the contemporary Lithuanian territory was founded in the late Palaeolithic in west Lithuania along the Baltic sea after the last glaciation around 11,000 years before present4,5. These people were highly related to hunter-gatherers (HG) from western Europe4. Notably, according to Lazaridis et al. (2014)6 modern eastern Baltic populations carry the largest proportion of western hunter-gatherer (WHG) ancestry of all Europeans. Moreover, recent results from Mittnik et al. (2018)7 point to continued gene flow between groups in the southeast Baltic region, who were more closely related to WHG, and the northern or eastern groups, more closely related to the eastern hunter-gatherers (EHG)6,7. Archaeological findings suggest that Finno-Ugrians had a minor contribution on the Lithuanian population3. However, Y-chromosome biallelic markers showed similarities between both Lithuanians and Latvians and the Finno-Ugric Estonians and Mari8. On the other hand, while mtDNA diversity also show this connection of Lithuanians to Finno-Ugric speakers, it also revealed similarities with Slavic (Indo-European) speaking populations2. Finally, analysis of molecular variance using the mtDNA HV1 region and Y STR haplotypes showed that Lithuania is a homogeneous population2.

1Department of Human and Medical Genetics, Biomedical Science Institute, Faculty of Medicine, Vilnius University, Santariskiu Street 2, LT-08661, Vilnius, Lithuania. 2Institut de Biologia Evolutiva (UPF-CSIC), Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Parc de Recerca Biomèdica de Barcelona, Doctor Aiguader 88, 08003 Barcelona, Catalonia, Spain. 3Institute of Genomics, University of Tartu, Riia 23b, Tartu, 51010 Tartu, Estonia. Correspondence and requests for materials should be addressed to E.B. (email: elena.bosch@upf.edu) or V.K. (email: vaidutis.kucinskas@mf.vu.lt)
The apparently long isolation of the Balts may have contributed to the preservation of an ancient social structure and of an archaic language. In addition, the establishment of the Baltic tribes led to the development of the current Lithuanian dialects, resulting in the actual regional linguistic differentiation. Six dialects can be currently distinguished in Lithuania: three groups from Aukstaitija (west, south and east) and three groups from Zemaitija (north, west and south).

The characterization of the adaptive history of local populations is of great interest because it provides knowledge about the genes that have been targeted by positive natural selection at a regional geographical scale. Besides their biological role in our past survival, these genes could be involved in rare, severe Mendelian diseases and contribute to current differences in resistance or susceptibility to disease. Indeed, several genetic variants that were adaptive in the past have been suggested to currently associate to particular common immune response and metabolic disease phenotypes due to recent environmental and dietary shifts. During the last years, the availability of high-throughput genotyping platforms and next generation sequencing techniques together with the increasing number of genotype-phenotype association studies and the development of new statistical and computational methods in the field of evolutionary genomics allow not only inferring putative selective events but also deciphering their potential phenotypic adaptive targets when analysing genomic patterns of variation from contrasting environmental conditions. To date many population genetics studies have been performed on large reference populations, but the need to analyse local patterns of population structure and adaptation remains. Without doubt, the analysis of geographically specific regions and the characterization of fine-scale patterns of genetic diversity may facilitate a much better understanding of the microevolutionary processes affecting local human populations.

To the best of our knowledge, there are no previous studies describing fine-scale genetic structure and recent positive selection in the Lithuanian population. Thus, in this study, we aimed to analyse local patterns of population structure and signatures of adaptive positive selection from genome-wide high-density SNP genotyping data we generated with the Illumina HumanOmmiExpress-12v1.1 and Infinium OmniExpress-24 arrays in 425 individuals from six regions in Lithuania. Furthermore, by combining our dataset with ancient data publicly available we aimed to explore the prehistorical ancestry components present in the Lithuania population. Notably, our results display Lithuanians as a homogenous population presenting one of the highest proportions of distinct pre-Neolithic HG (i.e. western, eastern and Scandinavian) and Early to Middle Bronze Age steppe pastoralist ancestry components found across contemporary Europeans. Moreover, among the top signatures of positive selection detected in Lithuanians we identified several candidate genes related to diet (PNLIP, PPARD), pigmentation (SLC24A5, TYRP1, PPARD) and the immune response (BRD2, HLA-DOA, IL26 and IL22).

Results
Genetic homogeneity across Lithuanian ethnolinguistic groups. We compiled and genotyped DNA samples from 425 Lithuanian individuals belonging to the six ethnolinguistic groups nowadays present in the Aukstaitija (western \(n=79\), southern \(n=67\), and eastern \(n=79\)) and the Zemaitija (northern \(n=79\), western \(n=43\), and southern \(n=78\)) regions of Lithuania (Fig. 1). Genetic inbreeding and similarity between all possible pairs of individuals in the Lithuanian population was investigated using the genome-wide SNP data generated through the kinship and the inbreeding coefficients, which were estimated at 0.00075 and 0.0022, respectively. Notably, four individuals had F values higher than expected for second cousin mating offspring (0.0156), and 19 pairs of individuals (including 1 duplicate) were inferred to present kinship coefficients higher than expected for 2nd degree relatives (0.0084). Moreover, a first exploratory principal component analysis (PCA) revealed 8 outliers (Fig. S1), which were subsequently removed from further analyses together with the aforementioned related and inbred individuals detected (Table S1, Fig. S2). Next, the genetic relationships of the six Lithuanian ethnolinguistic groups were explored by performing a second PCA using the 232,752 genome-wide...
pruned SNPs successfully genotyped in the remaining 399 Lithuanian samples. At that scale, the first two principal components (PC) explaining 0.81% of the variance showed that the six ethnolinguistic groups apparently formed a single cluster (Fig. 2A). However, when the average PC scores were computed for each ethnolinguistic group (Fig. 2B), a clear separation emerged. Both PC1 ($F = 30.108, P = 7 \times 10^{-26}$) and PC2 ($F = 2.586, P = 0.026$) were statistically significantly different among ethnolinguistic groups. If the main regions were compared to each other (Aukstaitija vs. Zemaitija), only PC1 scores were significantly different ($F = 83.443, P = 3 \times 10^{-18}$). Still, genetic differentiation assessed through calculation of pairwise $F_{ST}$ values revealed that the fraction of the genetic variance due to differences among ethnolinguistic groups ranged from 0.19% to 0.39% (Table 1). Thus, even if statistically significant, the genetic differentiation among the Lithuanian ethnolinguistic groups is quantitatively small.

**Population history of Lithuania.** To obtain a broader continental and historical context for the genetic diversity of the contemporary Lithuanian population, we next combined the autosomal data generated here with that of the Human Origins dataset from Lazaridis et al. (2016)[6] including a wide range of modern and ancient samples from west Eurasia and north Africa. Principal component analysis (PCA) of all modern samples displayed a tight clustering of the Lithuanian samples partly overlapping the north European group and in close proximity towards one of the extremes of the eastern and western European clusters (Fig. 3A). Interestingly, when adding ancient samples from different historical periods, Lithuanians positioned as the closest present-day European population to three different pre-Neolithic HG groups (the western, the Scandinavian and the eastern HG) and showed high proximity to the Steppe and Late Neolithic Bronze Age (LNBA) European samples (Fig. 3B).

**Table 1.** Weir and Cockerham $F_{ST}$ estimates between pairs of ethnolinguistic groups in Lithuania. NZ, North Zemaitija; SZ, South Zemaitija; WZ, West Zemaitija; EA, East Aukstaitija; SA, South Aukstaitija; WA, West Aukstaitija.

| Population | NZ | SZ | WZ | EA | WA | SA |
|------------|----|----|----|----|----|----|
| NZ         | 0  |    |    |    |    |    |
| SZ         | 0.001886 | 0  |    |    |    |    |
| WZ         | 0.003293 | 0.00333 | 0  |    |    |    |
| EA         | 0.002236 | 0.002485 | 0.003764 | 0  |    |    |
| WA         | 0.002096 | 0.00217 | 0.003641 | 0.001986 | 0  |
| SA         | 0.002738 | 0.002586 | 0.003884 | 0.002765 | 0.002201 | 0  |

**Figure 2.** Principal component analysis (PCA) of the six ethnolinguistic groups of the Lithuanian population. (A) Principal components 1 and 2 (after removing outliers) are shown. NZ, North Zemaitija; SZ, South Zemaitija; WZ, West Zemaitija; EA, East Aukstaitija; SA, South Aukstaitija; WA, West Aukstaitija. (B) Centroids of the PC scores by ethnolinguistic groups. Bars represent standard errors.
When comparing the three European populations to YRI (Fig. 5), among them, we detected a ~296 kb region in comparison and an additional one in the FIN-YRI comparison and thus indicate shared signatures of selection. Moreover, out of the nine signals identified when comparing LT to YRI, three were also detected in the CEU-YRI comparison mapping on chromosome 1, which comprises the \textit{COL6A5} and \textit{COL6A6} genes coding for the collagen type VI alpha 5 and alpha 6 chains, respectively. Interestingly, a non-synonymous variant in \textit{COL6A5} (rs12488457: 3838A > C, Thr1280Pro) with a CADD value of 23.2 was found among the top XP–EHH and \textit{FST} outliers along the region. Moreover, the \textit{COL6A5} SNP is reported as an eQTL for \textit{COL6A6} in testis \((P = 3.0 \times 10^{-17})\) and for \textit{COL6A6} in heart \((P = 4.1 \times 10^{-9})\) with the derived C allele implying lower expression in both cases according the Genotype-Tissue Expression (GTEx) portal (https://gtexportal.org/home/; accessed on 28/02/19). Notably, even if the signal was only significant in the LT-YRI comparison but the derived alleles at rs274750 and at rs274752, C and A respectively, are outliers (rs274750 and rs274752) with \textit{FST} rank score \(p\text{-value} < 0.01\). Thus, we detected a total of 42 candidate regions with signatures of recent selection in the Lithuanian population (Table 2). Interestingly, most recent signals were found when comparing Lithuanians to either the CEU (18 candidate regions) or the FIN populations (16 signals). Moreover, out of the nine signals identified when comparing LT to YRI, three were also detected in the CEU–YRI comparison and an additional one in the FIN–YRI comparison and thus indicate shared signatures of selection when comparing the three European populations to YRI (Fig. 5). Among them, we detected a ~296 kb region in chromosome 15, comprising the \textit{SLC24A5} gene, which codes for the collagen type VIII alpha 2 chain. Again, the signal was only significant in the LT–YRI comparison but the derived alleles at rs274750 and at rs274752, C and A respectively, are

**Genetic adaptation in the Lithuanian population.** Before analysing signatures of positive selection, we merged our original genotyping data in the Lithuanian population to an additional, but much denser, external SNP dataset, namely the 1000 Genomes Project Phase3 dataset. PCA and admixture analysis confirmed the expected clustering of Lithuanians within the European context as well as the consistent genetic differentiation and grouping of all six Lithuanian ethnolinguistic groups when compared to CEU, FIN, GBR and TSI (Supplementary Note 1). To detect putative recent selective events, we next calculated \textit{FST} and XP–EHH between all possible pairs of populations among LT, CEU, FIN and YRI, and focussed on those signals found in the Lithuanian population. For each population comparison, the genome-wide distribution of signals detected with \textit{FST} and XP–EHH is shown in Figs S5 and S6, respectively. In each comparison, we considered as top candidates for recent selection those genomic regions presenting at least 2 SNPs over the top 0.1% XP–EHH empirical values and a minimum of 1 SNP with an \textit{FST} rank score \(p\text{-value} < 0.01\). Thus, we detected a total of 42 candidate regions with signatures of recent selection in the Lithuanian population (Table 2). Interestingly, most recent signals were found when comparing Lithuanians to either the CEU (18 candidate regions) or the FIN populations (16 signals). Moreover, out of the nine signals identified when comparing LT to YRI, three were also detected in the CEU–YRI comparison and an additional one in the FIN–YRI comparison and thus indicate shared signatures of selection when comparing the three European populations to YRI (Fig. 5). Among them, we detected a ~225 kb region in chromosome 3, which comprises the \textit{COL6A5} and \textit{COL6A6} genes coding for the collagen type VI alpha 5 and alpha 6 chains, respectively. Interestingly, a non-synonymous variant in \textit{COL6A5} (rs12488457; 3838A > C, Thr1280Pro) with a CADD value of 23.2 was found among the top XP–EHH and \textit{FST} outliers along the region. Moreover, the SNP is reported as an eQTL for \textit{COL6A5} in testis \((P = 3.0 \times 10^{-17})\) and for \textit{COL6A6} in heart \((P = 4.1 \times 10^{-9})\) with the derived C allele implying lower expression in both cases according the Genotype-Tissue Expression (GTEx) portal (https://gtexportal.org/home/; accessed on 28/02/19). Notably, even if the signal was only significant in the LT–YRI comparison but the derived alleles at rs12488457, which is found at very low frequencies in YRI \((<0.013)\). Finally, an additional signal in the LT–YRI comparison mapping on chromosome 1 includes two extreme XP–EHH and \textit{FST} outliers (rs274750 and rs274752) at the 3’UTR of the \textit{COL8A2} gene, which codes for the collagen type VIII alpha 2 chain. Again, the signal was only significant in the LT–YRI comparison but the derived alleles at rs274750 and at rs274752, C and A respectively, are
nearly fixed in the three European populations and found at intermediate frequencies in YRI. Interestingly, polymorphisms in COL6A5 have been associated with body mass index19 and dermal phenotypes, such as eczema20, while mutations at COL8A2 have been linked to corneal endothelial dystrophies21.

As for the LT-CEU comparison, one of the strongest signals detected in Lithuanians was found at a ~184 kb region in chromosome 9 that comprises the TYRP1 gene, which codes for a melanosomal enzyme that participates in the melanin biosynthetic pathway. However, no obvious functional variant was identified among the top outlier XP-EHH and FST values in that region. In the same population comparison we also identified two signals probably related to immunity: one in chromosome 12 comprising the IL26 and IL22 interleukin genes, and the other in chromosome 6 containing the BRD2 and HLA genes.

A total of 35 candidate regions for older signatures of selection were identified in the Lithuania population when using the Tajima’s D statistic (Table 3, Fig. S7). Notably, 21 of these candidate regions were also detected in CEU (8), in FIN (6) or in both CEU and FIN (7) pointing to a high proportion of common old targets of selection among Europeans (Fig. 5). That is the case of the SLC24A5 region related to light pigmentation which, besides being identified as target of recent selection in the LT-YRI and CEU-YRI comparisons, emerged also as an outlier for old signatures in all three European populations. Interestingly, at least two of the candidate regions detected in Lithuanians for old signatures of selection may be related to dietary and environmental pressures: one in chromosome 6 containing the PPARD gene and another in chromosome 10 comprising the PNLIP and PNLIPRP3 genes.

Whereas PNLIP codes a pancreatic lipase required for the efficient digestion of dietary fats22, PPARD stimulates beta-oxidation not only in muscle and adipose tissue but also in the liver and other tissues and has been suggested to be involved in metabolic adaptation to environmental changes23. However, in none of these two cases any obvious functional variant specifically linked to the detected signatures was identified among the SNPs genotyped.

Figure 4. Geographic distribution of outgroup f3-statistics. Outgroup f3 values for the top ancient ancestries detected in the Lithuanian population were plotted across contemporary populations in western Eurasia: (A) f3(Mbuti; X, WHG). (B) f3(Mbuti; X, EHG). (C) f3(Mbuti; X, SHG). (D) f3(Mbuti; X, Europe_EN). (E) f3(Mbuti; X, Steppe_EMBA), being X a modern population.
Discussion

Geographically specific microevolutionary processes can be inferred when exploring local patterns of population structure and adaptation within the global and historical genetic context established from large general population and ancient publicly available reference panels. In this study we aimed to do that by analysing genome-wide high-density SNP genotyping new data generated in six ethnolinguistic groups from Lithuania.

At regional level, we found small genetic distances and a rather homogeneous genetic landscape across the six ethnolinguistic groups present in Lithuania. Previous analyses showed only statistically significant differences in the allele frequencies of the P1 and LWb blood groups when comparing south Aukstaitija with the remaining Lithuanian ethno-linguistic regions as well as on the Alu TPA25 allele distribution between north Zemaitija and south Aukstaitija. Similarly, according to Kasperaviciūtė et al. (2004), when considering Y-chromosome

| Genome coordinates | Genes | Population (SNPs)* |
|---------------------|-------|---------------------|
| chr:3:7197923–7197923 | MCL4, PDS | LT–FIN (3) |
| chr:1:116000000–116000000 | CTN | LT–FIN (5) |
| chr:1:116000000–116000000 | CTN | LT–FIN (5) |

Table 2. Candidate regions under recent positive selection in the Lithuanian population as detected by \( F_{ST} \) and XP–EHH. *Number of significant SNPs that were located at the extreme 0.1% of the empirical distribution for the XP–EHH and at least one SNP in the region had \( F_{ST} \) value < 0.01.
Fig. 5. Shared signals of adaptation between Lithuanians and the CEU and FIN populations from the 1000 Genomes Project Phase3 dataset. Venn diagrams showing the overlap of candidate regions for recent and older events of positive selection as detected by the combined FST and XP-EHH approach and the Tajima’s D statistic, respectively.

and mtDNA uniparental markers, Lithuania is a genetically homogeneous population. However, the sample size (180–196 Lithuanian samples) used in that study could be too small to identify minor differences between regions of Lithuania. Here, by increasing sample size to 399 individuals and covering the whole genome, we had statistical power to detect weak signals of genetic structure. However, even if we detected statistically significant genetic differentiation among the six Lithuanian ethnolinguistic groups, genetic distances among these groups were found to be rather small.

Archaeological findings locate the first settlement in the contemporary Lithuanian territory in the late Palaeolithic along the Baltic sea in west Lithuania (Zemaitija region), after the last glaciation around 11,000 years before present, probably favoured by the environment and the presence of marine food resources. In contrast, the middle Lithuanian land was unsuitable to live permanently due to the extreme cold climate before 11,000 years ago. People in the different regions of Lithuania seem to have lived in relative isolation for a long time because of the inaccessible nature of the terrain, bounded on the landward side by vast forests and swamps. Thus, if genetic differences between Lithuanians from different geographical regions existed, these disappeared in the contemporary population. Alternatively, the genetic composition of the Baltic peoples from which contemporary Lithuanians originated may have been relatively homogeneous.

When combining the new data we generated with external datasets, we confirmed that Lithuanians locate within the expected European context, even though they also present particular genetic distinctiveness when compared to neighbouring populations. In addition, the inclusion of ancient individuals from different periods across western Eurasia in the analysis allowed us to distinguish the genetic signature of three main prehistoric sources shaping the distinctiveness of present-day Lithuanians: pre-Neolithic HG groups, the Early to Middle Bronze Age Steppe pastoralists and Late Neolithic Bronze Age (LNBA) Europeans. Moreover, up to three HG populations can be inferred to contribute to the main genetic component identified the Lithuanians being the contribution of the WHG and the Scandinavian HG greater than that of the EHG. On the contrary, earlier European Neolithic movements from Levant/Anatolia known to contribute to genetically differentiated populations in Europe such as Sardinians or Basques are not especially predominant in Lithuania.

Partial genetic isolation of the Lithuanians is a possible explanation for the structure results observed. Until the late Middle Ages, the eastern Baltic region was one of the most isolated corners of Europe. Moreover, after the fall of the Roman Empire in the 5th century, the eastern Baltic region was spared by the subsequent population movements of the Migration Period, which allowed the most archaic of all the living speaking Indo-European languages to survive. Thus, Lithuanians could retain their cultural identity.

After establishing the genetic homogeneity and distinctiveness of Lithuanians within the European context, we next investigated whether specific signals of positive selection could be identified using different statistical approaches for recent and old classical selective sweeps. The two strongest signals for recent selection in our analysis comprise two candidate genes coding for different collagen chains (COL6A5 and COL6A6) as well as the TYRPI gene involved in pigmentation and were detected in the LT-YRI and LT-CEU comparisons, respectively. Interestingly, an additional collagen gene (COL8A2) and a well-known target of positive selection related skin pigmentation (SLC24A5) also emerged among the top signals of recent selection detected in the LT-YRI comparison comprising only genes related to the immune response (IL26, IL22 and BRD2, HLA-DOA) and two candidate regions for old signatures in LT with genes probably related to diet (PNLIP, PNLIPRP3 and PPARD) may probably result from specific pathogen driven and local dietary selection pressures in the Lithuania population. Notably, a significant number of the candidate regions for positive selection detected in the Lithuania population were also identified in FIN and/or CEU in our analyses and thus point to common selection signals across Europeans. Some of these signals had already been described in previous genome-wide scans of positive selection in Europeans and include the aforementioned SLC24A5 gene as well as other candidates not always completely characterized such as LRRC36 and PPARD.

Other forms of adaptation resulting from soft sweeps such as polygenic selection or selection from standing variation have not been explored here and may provide additional insights to the past selective pressures of Lithuanians. Moreover, in most candidate regions the putative adaptive variants targeted by selection remain
elusive since our genotyping data does not cover all the genetic variants present in each genomic region. However, even when the real adaptive variants are genotyped, their predicted functional relevance and subsequent assignment as putative adaptive is not always clear because different in silico prediction methods often provide non-congruent results. Despite all these limitations, we have identified several plausible candidate regions for selection in the Lithuanian population as well as some potential adaptive alleles, which after their subsequent experimental validation in follow-up studies may allow inferring new cases of local adaptation in the future.

Material and Methods

samples. The initial data set consisted of 425 samples from individuals who reported a minimum of three generations of Lithuanian nationality. The average age of the participants was 53 years. Lithuania is divided in two main regions (Aukstaitija and Zemaitija), which can be further subdivided ethnolinguistically into six dialectal groups. We sampled three groups from Aukstaitija (western ($n=79$), southern ($n=67$), and eastern ($n=79$)) and three groups from Zemaitija (northern ($n=79$), western ($n=43$), and southern ($n=78$)) (Fig. 1). All the study participants provided written informed consent in accordance with the Declaration of Helsinki.

Genomic DNA was obtained from whole blood using either a standard phenol-chloroform method of extraction or the automated DNA extraction platform TECAN Freedom EVO (TECAN Group Ltd., Männedorf, Switzerland). A NanoDropR ND-1000 spectrophotometer (NanoDrop Technologies Inc., USA) was used to assess DNA concentration and quality. This work is part of the LITGEN project, which was approved by the Vilnius Regional Research Ethics Committee 235 No. 158200-05-329-79, date: 3 May 2011.

Table 3. Candidate regions under positive selection in the Lithuanian population as detected by the Tajima's D statistic.

| Genome coordinates | Windows | P-value | Genes | Shared signal |
|---------------------|---------|---------|-------|---------------|
| chr1:35818960–35948959 | 4       | 0.0008  | KIAA0319L;ZMYM4 |
| chr1:49988960–50728959 | 6       | 0.0003  | AGBL4;ELAVL4 |
| chr1:18878960–188968959 | 9       | 0.0003  | LINCO1035;BRIP3 |
| chr2:1728675–21888674 | 7       | 0.0004  | TDRD15;LINCO1822 |
| chr2:179468675–179468674 | 9       | 0.0003  | TTN |
| chr3:50334312–51893411 | 11      | 0.0006  | C1orf18;CAGNA2D2;CISH;CYB561D2;DCAF1;DOCK3;GRM2;HEMK1;HTAL2,QCF2;QCF6;MANF;MAPKAPK2;NPRL2;RAD54L2;RASSF1;RM0158;TEX264;TMEM115;TNC2;ZMYND10 |
| chr3:128763412–128903411 | 5       | 0.0006  | CNBP;GP9;K5Y15Y1;RAB43;RAB45 |
| chr3:143543412–143683411 | 5       | 0.0001  | SLC9A9 |
| chr4:471930684–172360683 | 4       | 0.0002  | LINCO4331;MIR6082 |
| chr4:176190684–176400683 | 11      | 0.0005  | ADAM29;GPM6A |
| chr5:50531164–50691163 | 6       | 0.0003  | ISL1 |
| chr5:126311164–126441163 | 4       | 0.0005  | C1orf63 |
| chr6:35265879–35395878 | 4       | 0.0005  | DEF6;PPARD |
| chr6:97855879–98005878 | 6       | 0.0007  | MIR548H3 |
| chr7:30280729–30470728 | 8       | 0.0006  | NOD1;ZMNF2 |
| chr7:151370729–151870728 | 5       | 0.0002  | GALNT11;KMT2C |
| chr8:48621077–48801076 | 9       | 0.0007  | CEPBD;PRKDC;SPIDR |
| chr8:93731077–93901076 | 8       | 0.0002  | TRQK |
| chr9:38474202–384714201 | 5       | 0.0001  | ANKRD18A |
| chr9:12543202–125574201 | 5       | 0.0006  | OR1K1;OR1L3;OR1L4;OR1L6;OR5C1 |
| chr10:66065709–66215708 | 6       | 0.0002  | REEP3;ANX1AP3 |
| chr10:118195709–118325708 | 4       | 0.0008  | PNLIP;PNLIPPR3 |
| chr11:71554229–71734228 | 9       | 0.0004  | DEF8I138;RIL18B;NUMA1;RNF121 |
| chr12:1296234–1426234 | 4       | 0.0009  | ERC1 |
| chr12:15736235–15866234 | 6       | 0.0003  | EPS8;PTPRO |
| chr13:34108565–34278564 | 8       | 0.0005  | STARD13 |
| chr14:64046743–64236742 | 10      | 0.0005  | SGPP1;WDR89 |
| chr15:48357093–48470792 | 3       | 0.0005  | MYEP2;SLC24A5 |
| chr15:69670993–69740992 | 3       | 0.0002  | KIF23;PAQR3 |
| chr16:67321264–67501263 | 7       | 0.0003  | ATP6V0D1;HSD11B2;KCTD19;LRC36;PLEKHG4;TPPP3;ZDHHC1 |
| chr17:29252345–29392344 | 5       | 0.0002  | ADAP2;LOC107984974;RNF135 |
| chr18:303859383–30359382 | 8       | 0.0011  | CCDC178 |
| chr19:50580913–50709012 | 3       | 0.0005  | IZUMO2 |
| chr20:58399095–58399094 | 5       | 0.0006  | CDH126;FAM217B;PHACTR3;PPP1R3D;SYCP2 |
| chr21:44859932–44899931 | 3       | 0.0005  | HSF2BP |
Genotyping. Genotyping was performed at the Department of Human and Medical Genetics, Biomedical Science Institute, Faculty of Medicine, Vilnius University, Lithuania with the Illumina HumanOmniExpress-12v1.1 (296 samples) and the Infinium OmniExpress-24 (129 samples) arrays (Illumina, San Diego, CA, USA), which include an overlap of 707,138 genome-wide SNPs. Genotyping data quality control was performed according to the standard manufacturer recommendations. Individuals and SNPs with >10% missing data and SNPs with minor allele frequency (MAF) <0.01 were excluded from the analysis. SNPs with deviations from Hardy-Weinberg equilibrium (P < 10^-4) were eliminated from the study. After quality control 1 individual was excluded with more than 10% missing genotypes (MIND > 0.1) and 532,836 autosomal SNPs remained out of 589,752.

European context and ancient data. To obtain a broader geographical and historical context for the genetic diversity of the Lithuanian population, we merged our genotyped autosomal Lithuanian data with that of the Human Origins dataset from Lazaridis et al. (2016)48. Next, the whole Lithuanian dataset was randomly sampled to keep a sample size of 50 individuals and avoid any potential overrepresentation in subsequent analyses. After this procedure, we ended up with a total of 1,033 modern and 284 ancient samples, respectively, and 52,477 SNPs after LD pruning.

Principal component analysis, admixture and outgroup f3-statistics. Principal component analysis (PCA) for the six Lithuanian ethnolinguistic groups was carried out with independent pruned SNPs using SmartPCA from EIGENSOFT (v7.2.1)32. SNPs in linkage disequilibrium were removed with the indep-pairwise option of PLINK (v1.07) using a window size of 50 SNPs, a step size of 5, and a r^2 threshold of 0.545. Genetic relationship and consanguinity were inferred through the kinship and the inbreeding coefficients, which were estimated with KING v2.136 and PLINK v1.0737, respectively. Moreover, negative F values were converted to zero as they probably represent sampling errors44. Outlier samples on the PCA plot, as well as individuals with inbreeding coefficients higher than that expected for second cousin mating offspring (F values ≥ 0.0156) and 2nd degree relatives (kinship coefficient > 0.0084) were removed for all subsequent analyses (Table S1).

To visualize the closest ancient groups to our modern Lithuanians, we performed a PCA on the merged Lithuanian – Lazaridis dataset with the SmartPCA program (v. 1.3050) from EIGENSOFT package (v7.2.1)32. The analysis was first carried out on the present-day Eurasian samples, and subsequently we projected the ancient individuals in the analysis. PCA results were then plotted in R (v3.2.3).

Model-based individual ancestries were estimated with ADMIXTURE (v. 1.3.0)35. We used the unsupervised method implemented in the program including both ancient and present-day samples with K (number of distinct ancestral clusters) ranging from 2 to 12. The results of 10 iterations were combined and plotted by using PONG (v1.4.7)46.

Outgroup f3-statistics were computed using the qp3Pop program from ADMIXTOOLS package (v4.1)45. We considered Mbuti as outgroup in the analysis and calculated the shared drift between each putative ancient group and all the modern groups in the dataset in the form f3 (Mbuti; Ancient, Modern). We have focused the analyses on the three main European ancestral components: pre-Neolithic HG (western, eastern and Scandinavian), European Neolithic farmer and Bronze Age steppe components. These groups are represented in the analyses as in the reference WHG, EHG, SHG, Europe_EN, Steppe_EMBA, respectively.

Detection of signals of positive selection. To characterize signals of positive selection specific of the Lithuanian population we merged our original SNP genotyping data to that downloaded from the 1000 Genomes Project Phase3 dataset generating a pooled dataset of 264,950 autosomal SNPs distributed genome-wide in a total of 2,928 individuals. In particular, we included 20 populations from the 4 main geographical regions as described in the 1000 Genomes Project Phase3 dataset: Africa including the Yoruba in Ibadan, Nigeria (YRI), Luhya in Webuye, Kenya (LWK), Gambian in Western Divisions in the Gambia (GWD), Mende in Sierra Leone (MSL), and Esan in Nigeria (ESN) populations; Europe including Utah residents with ancestry from northern and western Europe (CEU), Toscani in Italy (TSI), Finnish in Finland (FIN), British in England and Scotland (GBR), and Lithuanians (LT); East Asia including Han Chinese in Beijing, China (CHB), Japanese in Tokyo, Japan (JPT), Southern Han Chinese, China (CHS), Chinese Dai in Xishuangbanna, China (CDX), and Kinh in Ho Chi Minh City, Vietnam (KHV); South Asia including Gujarati Indians in Houston, Texas (GIH), Punjabi from Lahore, Pakistan (PIL), Bengali from Bangladesh (BEB), Sri Lankan Tamil from the UK (STU) and Indian Telugu from the UK (ITU). Genotyping data was subsequently phased with SHAPEIT247.

Signatures of recent or ongoing positive selection were next investigated using the locus fixation index (F_L)38 and the XP-EHH statistic49 which were computed between all possible pairs of populations among LT, CEU, FIN, and YRI. F_L values were calculated with vcftools v0.1.1340. Negative F_L values were converted to zero. XP-EHH was run using selscan v1.2.0a40. For each comparison a XP-EHH score per SNP was obtained and XP-EHH scores >2 were considered as indicative of positive selection. We selected as candidates for positive selection any genomic region with two or more SNPs located at the 0.1% top extreme of the XP-EHH genome-wide empirical distribution and with at least one SNP presenting an F_L p-value < 0.01.

Older signals of selection were investigated through the Tajima’s D statistic, a widely used neutrality test that uses the site frequency spectrum43 and which have been suggested to capture selective sweeps occurring up to ~250,000 years ago43. The statistic was calculated through the PopGenome package implemented in R considering 100 kb sliding windows across all autosomal regions with a step size of 10 kb. Windows containing missing variants were ignored. As was done for F_L extreme negative Tajima’s D values were identified considering the rank of the score in the genomic distribution. In particular, windows were sorted in ascending order based on the Tajima’s D values and we considered for further analysis those with empirical p-values less than 0.01.

Finally, gene and variant annotation in candidate regions for selection was performed with ANNOVAR50 using GRC37 (hg19), RefSeqGene, dbSNP147 and CADD version 1.347.
Ethics approval and consent to participate. This research was approved by the Vilnius Regional Research Ethics Committee No. 158200-05-329-79, date: 2011-05-03. All the study participants provided written informed consent.

Data Availability
All the genotyping data generated in this study have been deposited at https://figshare.com/articles/Patterns_of_genetic_structure_and_adaptive_positive_selection_in_the_Lithuanian_population_from_high-density_SNP_data/7964159.

References
1. Klimas, A. Some unique futures of Lithuanian. Lituanus 30 (1984).
2. Kaspersaviciute`, D., Kucinskas, V. & Stoneking, M. Y chromosome and mitochondrial DNA variation in Lithuanians. Ann Hum Genet 68, 438–452, https://doi.org/10.1111/j.1529-8817.2003.00119.x (2004).
3. Groth, B., Bainville, B.,LAS, P. & Savontaus, M. L. Y-chromosomal diversity suggests that Baltic males share common Finno-Ugric-speaking forefathers. Hum Hered 53, 68–78, https://doi.org/10.1159/000057985 (2002).
4. Zinkevicius, Z. The history of the Lithuanian language. 333 (1998).
5. Blekhman, R. et al. Natural selection on genes that underlie human disease susceptibility. Curr Biol 18, 883–889, https://doi.org/10.1016/j.cub.2008.04.073 (2008).
6. Veljovic, I., Zuzovic, A., Pantic, M. & Gaggiotti, O. ‘Changes in selective pressures associated with human population expansion may explain metabolic and immune related pathways enriched for signatures of positive selection. BMC Genomics 17, 504, https://doi.org/10.1186/s12864-016-2783-2 (2016).
7. Laitinen, V., Lahermo, P., Sistonen, P. & Savontaus, M. Y chromosome and mitochondrial DNA variation in Lithuanians. Ann Hum Biol 38, 513–524, https://doi.org/10.1080/03014460.2011.578870 (2011).
8. Patterson, N., Price, A. L. & Reich, D. Population structure and eigenanalysis. PLoS Genet 1, e1000088, https://doi.org/10.1371/journal.pgen.1000088 (2005).
9. Lachman, M. The gene frequencies in European populations. Hum Hered 55, 313–317, https://doi.org/10.1159/000057985 (2002).
10. Behr, A. A., Liu, K. Z., Liu-Fang, G., Nakka, P. & Ramachandran, S. pangen: fast analysis and visualization of latent clusters in population genetic data. Bioinformatics 32, 2817–2823, https://doi.org/10.1093/bioinformatics/btw327 (2016).
11. Voight, B. F., Kudaravalli, S., Wen, X. & Pritchard, J. K. A map of recent positive selection in the human genome. PLoS Genet 5, e1003162, https://doi.org/10.1371/journal.pgen.1003162 (2009).
12. Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81, 559–575, https://doi.org/10.1016/j.ajhg.2007.08.010 (2007).
13. Excoffier, L. & Lischer, H. E. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10, 564–567, https://doi.org/10.1111/j.1471-8286.2010.02847.x (2010).
14. Alexander, D. H., Novembre, J. & Lange, K. Fast model-based estimation of ancestry in unrelated individuals. Genome Res 19, 1655–1664, https://doi.org/10.1101/gr.094012.109 (2009).
15. Behr, A. A., Liu, K. Z., Liu-Fang, G., Nakka, P. & Ramachandran, S. pangen: fast analysis and visualization of latent clusters in population genetic data. Bioinformatics 32, 2817–2823, https://doi.org/10.1093/bioinformatics/btw327 (2016).
16. Delaneau, O. & Zagury, J. F. Haplotype inference. Methods Mol Biol 888, 177–196, https://doi.org/10.1007/978-1-61779-870-2_11 (2012).
17. Weir, B. S. & Cockerham, C. C. Estimating F-statistics for the analysis of population structure. Evolution 38, 1358–1370, https://doi.org/10.1111/j.1558-5646.1984.tb05657.x (1984).
39. Sabeti, P. C. et al. Genome-wide detection and characterization of positive selection in human populations. *Nature* **449**, 913–918, https://doi.org/10.1038/nature06250 (2007).

40. Danecek, P. et al. The variant call format and VCFtools. *Bioinformatics* **27**, 2156–2158, https://doi.org/10.1093/bioinformatics/btr330 (2011).

41. Szpiech, Z. A. & Hernandez, R. D. selscan: an efficient multithreaded program to perform EHH-based scans for positive selection. *Mol Biol Evol* **31**, 2824–2827, https://doi.org/10.1093/molbev/msu211 (2014).

42. Tajima, F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**, 585–595 (1989).

43. Sabeti, P. C. et al. Positive natural selection in the human lineage. *Science* **312**, 1614–1620, https://doi.org/10.1126/science.1124309 (2006).

44. Pfeifer, B., Wittelsbürger, U., Ramos-Onsins, S. E. & Lercher, M. J. PopGenome: an efficient Swiss army knife for population genomic analyses in R. *Mol Biol Evol* **31**, 1929–1936, https://doi.org/10.1093/molbev/msu136 (2014).

45. Wang, K., Li, M. & Hakonarson, H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* **38**, e164, https://doi.org/10.1093/nar/gkq603 (2010).

46. Pruitt, K. D., Tatusova, T. & Maglott, D. R. NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res* **35**, D61–65, https://doi.org/10.1093/nar/gkq482 (2007).

47. Kircher, M. et al. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet* **46**, 310–315, https://doi.org/10.1038/ng.2892 (2014).

Acknowledgements

This study was supported by LITGEN project, which was approved by the Vilnius Regional Research Ethics Committee No. 158200-05-329-79, date: 2011-05-03, and by Direcció General de Recerca, Generalitat de Catalunya (2017SGR702), Agencia Estatal de Investigación (AEI) and Fondo Europeo de Desarrollo Regional (FEDER) with project grants BFU2016-77961-P and CGL2016-75389-P, and the Unidad de Excelencia María de Maeztu”, funded by the MINECO (ref: MDM-2014-0370). EB is the recipient of an ICREA Academia Award. We also thank all investigators (L. Ambrožitytė, I. Uktverytė, I. Domarkienė, R. Meškienė) who contributed to the sample genotyping.

Author Contributions

A.U., A.M. and V.K. contributed to sample preparation, genotyping and data manipulation. D.C., F.C., E.B. and V.K.; study conception, study design, and interpretation of results. M.M. participated in the design of the selection analyses. A.U. and A.F.B.; performed all the analyses and contributed to the interpretation of the results. A.U. and E.B.; writing of the original manuscript. All authors reviewed the manuscript.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-45746-3.

Competing Interests: The authors declare no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2019