Original Paper

Human Heredity

Hum Hered 2011;72:35–44
DOI: 10.1159/000330168

Received: February 22, 2011
Accepted after revision: June 17, 2011
Published online: August 17, 2011

A Geographic Cline of Skull and Brain Morphology among Individuals of European Ancestry

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Key Words
Biological anthropology · Cortex · Craniometry · Genetic drift · Imaging genomics · Neuroimaging · Population genetics

Abstract
Background: Human skull and brain morphology are strongly influenced by genetic factors, and skull size and shape vary worldwide. However, the relationship between specific brain morphology and genetically-determined ancestry is largely unknown. ***Methods:*** We used two independent data sets to characterize variation in skull and brain morphology among individuals of European ancestry. The first data set is a historical sample of 1,170 male skulls with 37 shape measurements drawn from 27 European populations. The second data set includes 626 North American individuals of European ancestry participating in the Alzheimer's Disease Neuroimaging Initiative (ADNI) with magnetic resonance imaging, height and weight, neurological diagnosis, and genome-wide single nucleotide polymorphism (SNP) data. ***Results:*** We found that both skull and brain morphological variation exhibit a population-genetic fingerprint among individuals of European ancestry. This fingerprint shows a Northwest to Southeast gradient, is independent of body size, and involves frontotemporal cortical regions. ***Conclusion:*** Our findings are consistent with prior evidence for gene flow in Europe due to historical population movements and indicate that genetic background should be considered in studies seeking to identify genes involved in human cortical development and neuropsychiatric disease.

Introduction

Human brain morphology is highly heritable [1] and is influenced by specific genetic factors [2, 3]. Magnetic resonance imaging (MRI) studies have shown that genetically mediated variations in brain morphology are associated with disease states, e.g. autism [4], schizophrenia [5], and Williams [6] and Down [7] syndromes.

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (www.loni.ucla.edu/ADNI). As such, the investigators within the ADNI contributed to the design and implementation of ADNI or provided data but did not participate in analysis or writing of this report (a complete listing of ADNI investigators is available at www.loni.ucla.edu/ADNI/Collaboration/ADNI_Authorship_list.pdf).

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However, the relationship between genetic background – the accumulation of genetic variants due to prehistoric and historic demographic processes, including migrations and bottlenecks – and brain morphology among individuals distributed globally and regionally has not been directly studied. Our goal is to examine the effect of genetic background in Europe on brain and skull morphology.

Human skull morphology varies greatly worldwide [8, 9] largely due to the accumulation of genetic and environmental differences within populations but also between geographically separated populations [10, 11]. Since the skull and brain are structurally and genetically linked during development [12, 13], it follows that the brain should also differ in size and shape among individuals. However, evaluation of the relationship between population level skull and brain morphological variation should consider at least three important questions: (1) the degree to which body size can explain variation in brain size among individuals from different populations; (2) the degree to which brain variation is associated with genetic (as opposed to environmental) differentiation among these populations, and (3) whether specific brain regions exhibit differences consistent with skull morphology differences between populations.

To address these questions, we studied data from individuals of European ancestry. We focused on this geographic region because populations in Europe are genetically differentiated in a continuous manner consistent with known historical migrations [14–16], and these populations vary in skull size and shape [8, 17, 18]. We leveraged two independent data sets in our analyses. The first data set is a historical sample of skulls drawn from 27 European populations [9, 19, 20] and is comprised of 1,170 individuals with 37 skull shape measurements (online suppl. tables S1 and S2; for all online suppl. material, see www.karger.com/doi/10.1159/000330168). The second data set includes 626 North American individuals of European ancestry participating in the Alzheimer's Disease Neuroimaging Initiative (ADNI) with MRI, height and weight, neurological diagnosis, and genome-wide single nucleotide polymorphism (SNP) data.

We found consistent evidence from these data sources that variation both in skull and specific brain morphology among individuals of European ancestry follows a Northwest (NW) to Southeast (SE) gradient, which is independent of body size or neurological diagnosis and involves predominantly the frontotemporal cortex. This finding is consistent with previous evidence of gene flow due to historical population movements in Europe [14–16] and indicates that genetic background should be considered in studies seeking to identify specific genes involved in human cortical development and neuropsychiatric disease.

Materials and Methods

Craniometric (Skull) Measures

Craniometric Data

1,170 male crania (skulls) from 27 European populations (including nearby Turkey and Syria; online suppl. table S1) with 37 measurements were included from a much larger worldwide data set [9, 19, 20]. Populations included skulls from all 4 geographic quadrants of Europe (centered on Austria): NW (n = 476), NE (n = 227), SW (n = 219), and SE (n = 248). Crania were obtained predominantly from modern European populations with some from medieval European populations. A logistic regression showed no association between the population latitude ($p = 0.22$) or longitude ($p = 0.26$) and whether skulls were of medieval or modern origin. Population sample sizes ranged from 11 to 93 individuals, and the average size (±SD) was 43.3 ± 27.6. All measurements were made by one person (Dr. Hanihara) [19, 20] using standardized landmarks and included a broad range of facial and neurocranial lengths (online suppl. table S2). 241 female crania from 12 populations (range 2–55, average 20.1 ± 20.7) with complete data for the same 37 measurements were treated as an additional replication and validation data set.

Craniometric and Geographic Distance Comparison

A multivariate ANOVA was used to test differentiation between populations based on 37 standardized measures. Mahalanobis squared distances (D^2) were calculated between population clusters based on 37 standardized (i.e. zero mean and unit variance) craniometric measures and the whole sample covariance matrix. A 4-dimensional (stress = 0.11) ordination of the craniometric distance matrix was generated by non-metric multidimensional scaling (MDS). Longitude and latitude coordinates have previously been estimated for each of the populations studied [21]. Great circle distances were calculated within the R statistical software package [22], and MDS was used to generate a 3-dimensional ordination. Pairwise craniometric distances were plotted versus geographic distances, and a moving average was computed to reveal trends. A permuted Mantel statistic was used to test the correlation between craniometric and geographic distance matrices.

Craniometric and geographic ordinations were aligned using Procrustes analyses, and the coordinates of each population estimated from craniometric distances were plotted. Residuals from the Procrustes analysis gave predicted geographic location errors for each population in kilometers. 100 bootstrap replications were performed by sampling different sets of individual crania from each population and then recalculating the Mahalanobis pairwise distances, ordinations, and Procrustes residuals. In addition, ordination labels were randomly permuted 100 times, and Procrustes residuals were computed. A one-tailed t test was used to test the hypothesis that the mean bootstrap prediction errors for each population were less than what was expected by chance.

Cranial Classification

Linear discriminant analysis was used to build a subpopulation classifier based on 37 standardized measurements of 538 male crania in 12 populations for which there were also female cranial measures available. For each female skull, the geographic distance was calculated between the classified population (based on cranial morphometry) and the correct population to which that individual belonged. A cumulative distribution of predicted distance errors in kilometers was plotted and compared to 10 similarly constructed distributions based on random assignment of individuals to populations. A one-sided Kolmogorov-Smirnov test was used to test the difference between the distributions.

Geographic Variation

Redundancy analysis was applied to the 37 standardized cranial measures with longitude and latitude as constraints. Permutation tests were used to test model and axis significance, and this identified a geographic axis that explained a maximal amount of craniometric variance. Population eigenvalues for this constrained axis were interpolated by kriging to create an isocline map of Europe. Individual cranial measures were tested for association with distance along the NW-SE axis that showed a trend in the isocline map. p values were adjusted by progressive Bonferroni correction [23] in the order of the loadings of the measures on this axis.

MRI Measures and Genotype Information

ADNI Subjects

Data used in the preparation of this article were obtained from the ADNI database (www.loni.ucla.edu/ADNI). 626 ADNI subjects were included in this study who were self-reported as white and non-Hispanic and included 181 controls, 305 subjects with mild cognitive impairment (MCI), and 140 subjects with Alzheimer's disease (AD), aged 75.3 $\pm$ 6.9 years.

Brain Imaging

MRI data was collected on 1.5-Tesla scanners at many study centers across the United States. The LONI website (www.loni.ucla.edu/ADNI/Research/Cores/index.shtml) describes specific protocols. Raw Digital Imaging and Communications in Medicine MR images were downloaded from the ADNI Data page of the public ADNI site at the LONI website (www.loni.ucla.edu/ADNI/Data/index.shtml) published in 2007. MRI scans were analyzed with software developed at the University of California at San Diego Multi-Modal Imaging Laboratory, based on the freely available FreeSurfer software package (freesurfer-software.org).

Ancestry Estimation

PLINK [24] was used to merge ADNI genotypes with 34 European reference populations from POPRES, HGDP, and HapMap (online suppl. table S3). 59,908 SNPs remained after filtering based on quality and linkage disequilibrium. The smartPCA software [25] was used to find the first two eigenvectors that explained the most variance in the reference genotypes. All individuals, including those from the ADNI data set, were projected along these eigenvectors and outliers were removed. Procrustes analysis was used to align the average principal component coordinates of each population to longitude and latitude coordinates of the reference populations. For each ADNI subject, NW-SE genetic ancestry was inferred by projecting the rotated principal components onto a 45° line oriented NW and SE. In order to quantify the geographic distribution of inferred ancestry, ADNI subjects were grouped into 1 of 4 quadrants in Europe based on their genetic ancestry, and the Austrian reference population was defined as the center. Individuals were located in all 4 geographic quadrants: NW (n = 447), NE (n = 46), SW (n = 29), and SE (n = 104).

Association Analyses

Estimated NW-SE European ancestry was tested for association with 12 brain region summary measures, cortical surface area in 66 regions of interest, and cortical surface area at vertices across the surface of the brain with univariate linear models, while controlling for known covariates including sex, age, height, weight, BMI, and neurological diagnosis.

Results

We initially tested the hypothesis that skull shape differs between 27 European populations using a multivariate analysis of variance (ANOVA) test and found overwhelming evidence for an association ($p < 1 \times 10^{-182}$). We then tested whether these population differences exhibit geospatial structure using a Mantel test and found that geographic distances between populations are strongly ($r_M = 0.51$, $p < 1 \times 10^{-5}$) positively correlated with craniometric distances between populations (fig. 1a).

This geospatial structuring of populations led us to construct a map of Europe based solely on cranial morphology. We used non-metric MDS to represent craniometric distances and aligned the resulting coordinates by Procrustes scaling and rotation to the longitude and latitude of each population (online suppl. table S1), while preserving the relative distances between the points. A plot of these craniometric coordinates along with the results of hierarchical clustering confirmed that this ordination captures the relative distances between populations well (fig. 1b), although clusters have somewhat indeterminate membership (online suppl. fig. S1). Populations defined purely on the basis of cranial morphology are remarkably close to the known origins of the skulls, and 15 out of 27 populations have predicted geographic locations significantly closer than is expected by chance (fig. 1c).

We further tested the hypothesis that variation in skull shape is geographically structured by testing how well individuals can be identified with populations based solely on craniometric measures. A linear classifier was built from a training data set of 538 male skulls and was applied to a test data set of 241 female skulls from 12 populations. 34% of female skulls are correctly classified, which is highly statistically significant ($p < 1 \times 10^{-6}$)

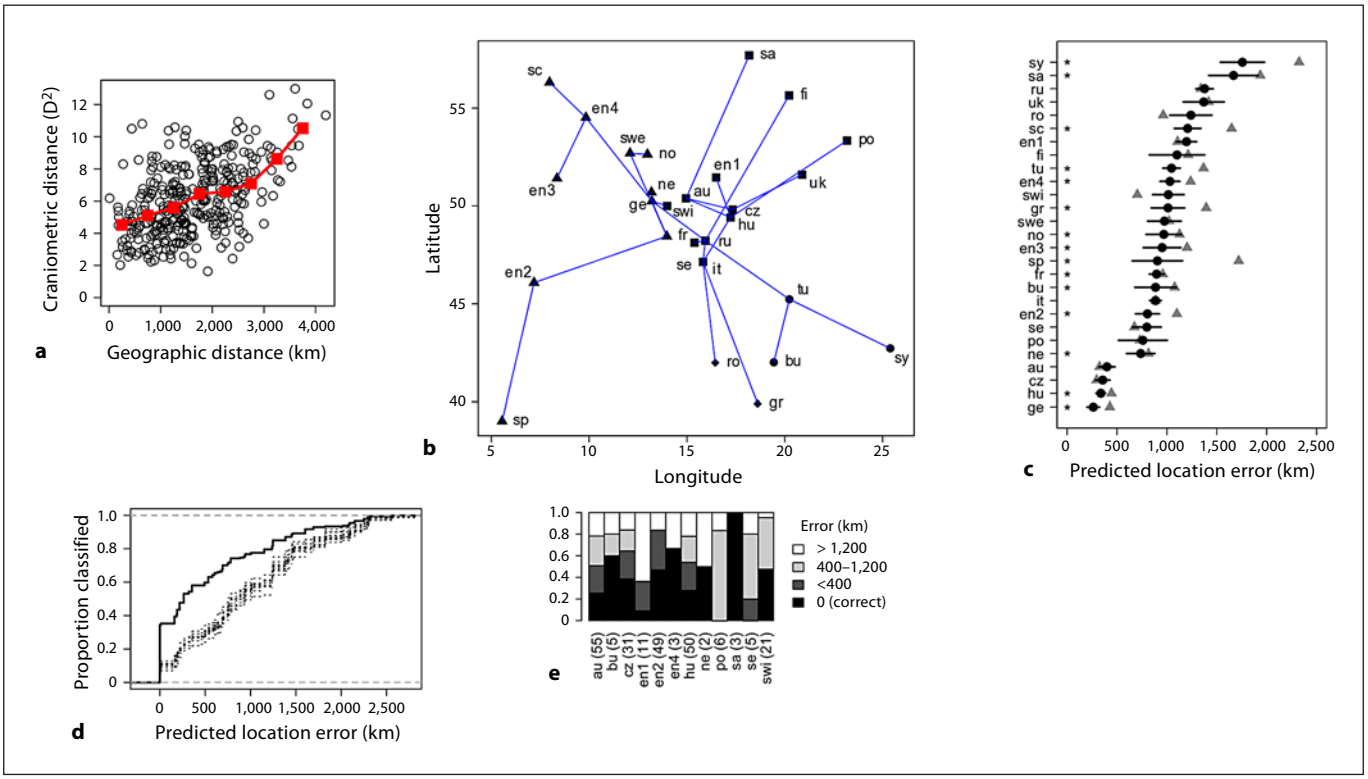

Fig. 1. Cranial morphology reflects geography across Europe. **a** Pairwise distances between 27 European populations. Craniometric distance is significantly correlated ($r_M = 0.51$, $p < 1 \times 10^{-5}$) with geographic distance. **b** Non-metric multi-dimensional scaling ordination of craniometric distances aligned to geographic coordinates of populations. Population symbols identify 4 clusters, and lines form a minimum spanning tree. **c** Distances between predicted locations based on craniometric ordination and population locations. Average ± SD plotted for 100 bootstrap replications (black) and random permutations (gray). * $p < 0.001$. **d** Individual female skulls were identified with correct or nearby populations based on cranial morphometry (solid) significantly better than chance (dotted). **e** Proportion of female skulls that were correctly classified (black) and misclassified with populations at different distances (gray shades). Sample sizes are listed after population name.

and similar to the cross-validated classification performance of the male skull training set. Misclassified crania are more likely than is expected by chance ($p = 6.4 \times 10^{-5}$) to be from geographically proximal populations (fig. 1d, e; online suppl. fig. S2), which is consistent with the spatial structure found at the population level in this study.

We then tested the hypothesis that skulls exhibit clinal variation along geographic axes within Europe. A directional Mantel correlogram shows a monotonic decrease in craniometric similarity with distance in two orthogonal directions, NW-SE and NE-SW (online suppl. fig. S3B), and this result motivated us to search for a geographic axis that can explain a significant fraction of the craniometric variation between populations. Redundancy analysis, a constrained version of principal components analysis (PCA), was used to find a projection that maximizes the variation of the 37 cranial measures under the condition that this projection is a linear combination of longitude and latitude. The first principal component is statistically significant ($p = 0.005$) and explains 12.8% of total craniometric variation, more than a third of the variation (30.5%) explained by the first component of an unconstrained PCA. Population eigenvalues for the first principal component were interpolated by ordinary kriging and plotted to create an isocline map of Europe (fig. 2a). Cross-validation indicated that predicted population eigenvalues were highly correlated with observed values ($r = 0.88$). Significantly, this map shows a clear gradient along a NW-SE axis that was not specified a priori and emerged from the redundancy analysis as the direction of maximum cranial variation. Therefore, a subset of cranial measures exhibits clinal variation along this geographic axis.

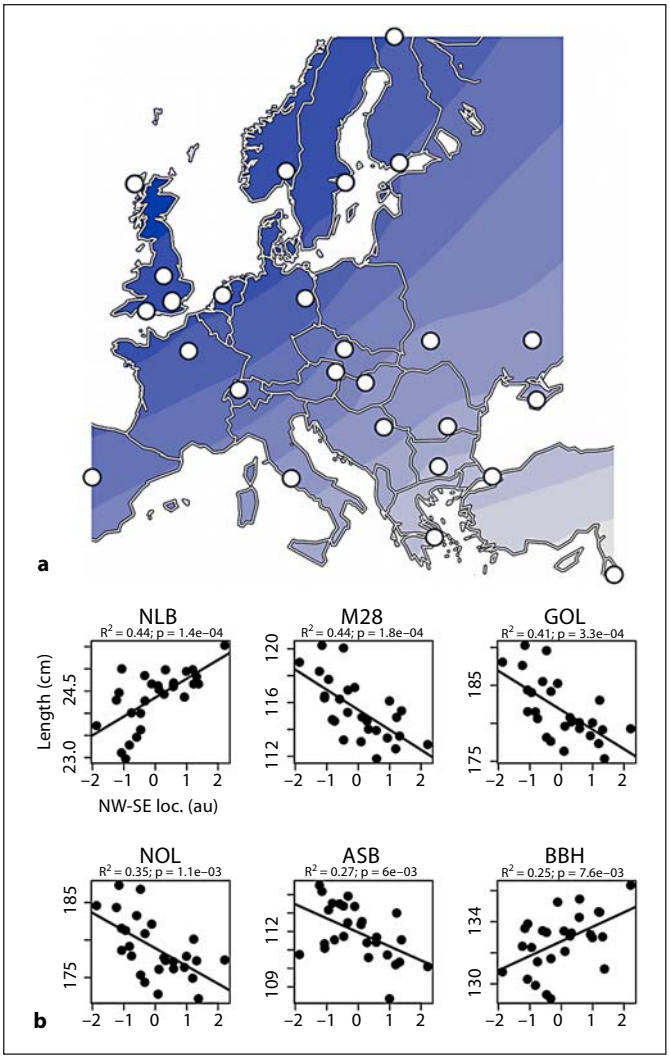

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Fig. 2. Cranial measures show significant variation along a NW-SE axis within Europe. **a** Isoclines of interpolated eigenvalues for first spatially constrained component of a redundancy analysis and geographic locations of populations. **b** Cranial measures plotted in order of their contribution to this map. Negative abscissas correspond to a more NW location. Proportion of variance explained (R^2) and nominal p values are indicated. NLB = Nasal breadth; M28 = sagittal occipital arc; GOL = glabello-occipital length; NOL = nasio-occipital length; ASB = biasterionic breadth; BBH = basion-bregma height.

Six measures are significantly associated with NW-SE location, and this NW-SE axis explains 25–44% of the variance of these individual measures (fig. 2b; online suppl. fig. S4). Of these measures, glabello-occipital length (maximum length) and nasio-occipital length, biasterionic breadth, and sagittal occipital arc are approximately 5% longer in NW populations, while basion-bregma height and nasal breadth are 5% shorter. We note that although there are clear trends in the population means of these measures along the NW-SE axis, the majority of variation in the measures is within populations.

To determine if brain morphometry exhibits similar geospatial population trends to the skull morphometry data, we estimated the ancestry of each individual in the ADNI sample using available genome-wide genotype data and confined attention to 626 individuals with a high probability of having European ancestry. In order to assign the European region of origin most likely to reflect the genetic background of each individual, genotypes from ADNI subjects were merged with publically available genotypes from 34 reference populations geographically distributed across Europe, and PCA was pursued. Procrustes analysis was used to align the average principal component coordinates of each population to longitude and latitude coordinates of the reference populations. A plot of the first two principal components separates ADNI subjects into two main clusters: one overlaps NW populations and one lies SE of Europe (fig. 3a) and overlaps individuals with self-reported Ashkenazi Jewish ancestry (online suppl. fig. S5). For each ADNI subject, NW-SE genetic ancestry was inferred by projecting the rotated principal components onto a 45° line oriented NW and SE in figure 3a. This PCA-based assignment of regional genetic origin to individuals has been validated in several groups of admixed European Americans with self-reported ancestry [26–29]. For example, Need et al. [26] found that a PCA plot of genetic distances placed individuals with grandparents of both NW and SE European and Ashkenazi Jewish origin in between these reference populations.

We found that ADNI individuals with a NW ancestry are on average 4 cm taller than ADNI individuals with a SE or Ashkenazi Jewish ancestry ($p = 7.3 \times 10^{-6}$), consistent with previously observed differences in height across Europe [30]. Our sample is evenly distributed along the NW-SE axis based on MCI ($p = 0.84$) and AD ($p = 0.13$) diagnosis. Our sample is slightly unevenly distributed based on age ($p = 0.014$) and sex ($p = 0.002$) as ADNI individuals with a NW ancestry are on average 2.5 years younger and include a greater number of female subjects.

We tested the correlation between degree of estimated NW-SE European ancestry and 12 summary measures of MRI-derived brain morphology, while controlling for height, weight, BMI, sex, age, and neurological diagnosis. Intracranial and brain volumes and total cortical surface area are significantly ($p < 0.0006$) correlated with degree of ancestry along the NW-SE axis (fig. 3b). These three

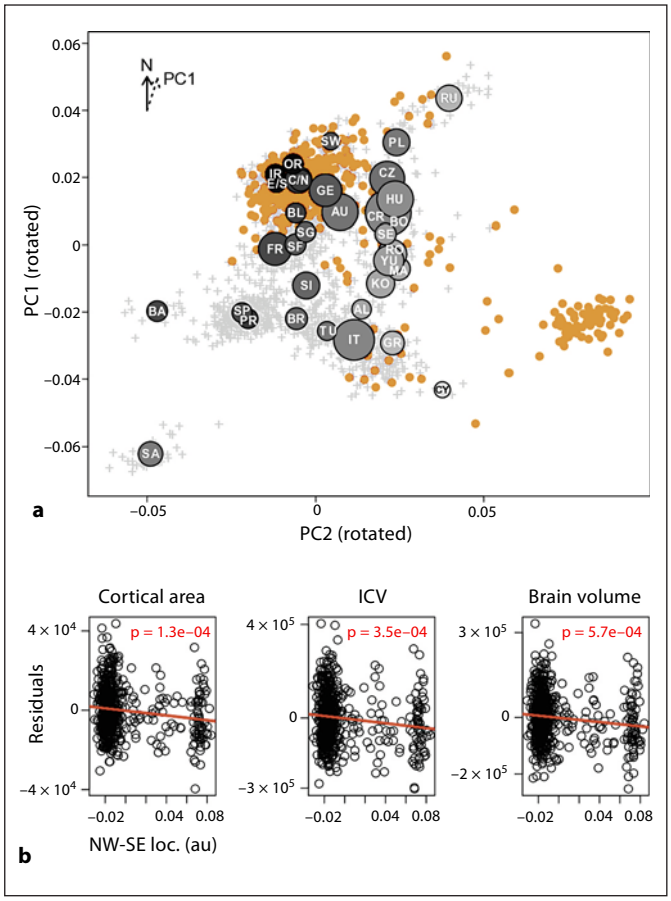

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Fig. 3. Structural brain measures follow a predicted trend in a group of individuals with European ancestry. **a** The first two principal components of genotypes of ADNI subjects (yellow/small points; color refers to online version only) and individuals from European reference populations (gray crosses) rotated 18° to align with a map of Europe. For each reference population (see online suppl. table S3 for labels), the average (SD) of principal components for all individuals in that population are indicated by disc position (diameter). Geographic origin of each population is indicated by disc shade of gray from NW (black) to SE (light gray) Europe. ADNI subjects are spread out primarily along a NW-SE axis and form two distinct clusters corresponding to NW European and Ashkenazi Jewish ancestry (see also online suppl. fig. S5). **b** Brain structural measures tested for association with estimated NW-SE ancestry, while controlling for height, weight, BMI, age, sex, and diagnosis. Negative abscissas correspond to a larger proportion of NW ancestry.

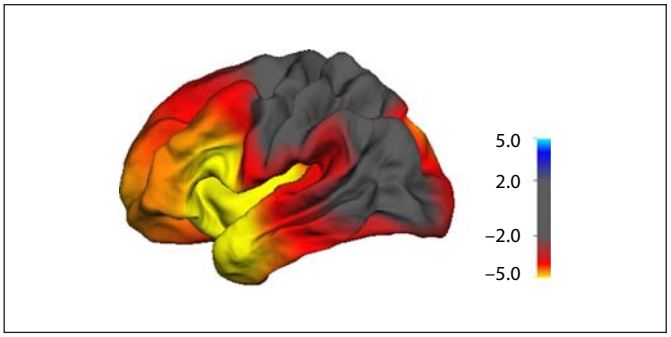

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Fig. 4. Frontotemporal cortical regions are most affected by NW European ancestry. Lateral view of the left hemisphere with color map that indicates nominal $-\log_{10}$ (p value) of association between estimated NW-SE ancestry and cortical surface area across the reconstructed cortical surface, while controlling for height, weight, BMI, age, sex, and diagnosis.

brain measures remain significantly associated ($p < 0.05$) with NW-SE ancestry among males (n = 368), females (n = 258), and subjects without a diagnosis of AD (n = 486), and cortical surface area shows a trend level association (p = 0.09) in the small sample of healthy controls (n = 181). Therefore, the observed association between degree of NW-SE ancestry and brain morphology is unlikely to be an artifact caused by differences in sex or neurological diagnosis.

Intracranial and brain volumes and cortical surface area progressively increase with the amount of inferred NW European ancestry (fig. 3b), and these measures are approximately 5% larger in the 10% of individuals with the most NW European ancestry compared to the 10% with the most SE European ancestry. This percentage increase matches the percentage increase in cranial length and breadth observed along the same NW-SE geographic axis in the skull data set (fig. 2b) and cannot be attributed to a correlation with body size since we controlled for height and weight. This correlation involves specific – not global – brain morphology because hippocampal, basal ganglia, ventricular, and cerebellar volumes and average cortical thickness are not associated with NW-SE ancestry.

Next, we performed both a region of interest analysis and vertex-based tests across the cortex to test whether the surface area of specific cortical regions showed more significant association with the degree of NW-SE ancestry. We found that cortical surface area predominantly in the frontal and temporal lobes from both hemispheres is significantly associated (online suppl. table S4) and is 4–9% larger among 10% of individuals with the most NW European ancestry compared to 10% with the most SE European ancestry. We found a similar frontotemporal pattern of association with the degree of NW-SE ancestry with a vertex-based analysis (fig. 4; online suppl. fig. S6).

Discussion

In this study, we leveraged brain imaging and genome-wide genotyping from 626 European Americans, as well as skull measurements obtained on an independent set of 1,170 individuals of European ancestry, to test the hypothesis that skull and brain morphology, like genetic background [14, 15], mirror geography within Europe. We found that skull and brain morphology vary continuously across Europe as evidenced by the weak clustering between and large variation within populations and are geospatially structured. In particular, we observed a significant NW-SE gradient in morphology that is independent of body size and involves predominantly frontotemporal cortical areas.

Sokal and Uytterschaut [17] and Sokal et al. [18] reported that craniometric variation is spatially structured across Europe but found no clear continental trend and further could not recapitulate a map of Europe. Moreover, these studies found that cranial variation was associated with geography and language family more than time period (Early and Late Middle Ages and a Recent Period). Therefore, European cranial diversity has retained some of its spatial patterning for at least 1,500 years. However, the data by Sokal and Uytterschaut [17] and Sokal et al. [18] were limited to population averages of only 10 different cranial measurements from populations with non-uniform geographic coverage of Europe. In contrast, our finding of significant skull and brain variation along a NW-SE cline is consistent with genetic and archaeological evidence for range expansions and gene flow due to prehistoric population movements along this geographic axis in Europe. In this 'demic diffusion' model [31], human populations gradually expanded into new geographic areas and carried with them novel genetic (and hence phenotypic) variation. If migrating humans had progressive admixture with individuals local to those regions or replaced local individuals and experienced a succession of bottlenecks [32], then a gradient of gene frequencies should have been formed. For example, the migration of anatomically modern humans out of Africa approximately 40,000 years ago, postglacial re-expansions from refugia in southern Europe, and the introduction of farming from SW Asia within the last 10,000 years could have generated a NW-SE gradient that is manifest in the genetic diversity across populations since these events involved movement of people primarily from southern to northern Europe [9, 16, 33–36].

However, historical population expansions and invasions – e.g. Greek, Jewish, Phoenician [37], and Viking [38] – have also contributed to patterns of genetic diversity in Europe [39] and may have masked genetic clines formed by earlier migrations. Furthermore, in contrast to demic diffusion, cultural diffusion could explain the spread of agricultural technology and cultural adaptations across Europe due to information – not gene – flow, and the pattern of Y chromosome diversity in modern Europeans reinforces this model [36]. Therefore, the existence of genetic and craniometric clines in modern European populations suggests at least two theories: (1) prehistoric population movements made such a dominant contribution to the structure of genetic variation in Europe that more recent gene flow has not masked it, and (2) local environmental factors and selection generated clinal variation or acted to restore clinal variation after gene flow occurred. One intriguing possibility for such an environmental factor is the cultural conditions associated with possessing agricultural technologies, e.g. sedentarism, altered diet including milk consumption [40], and new disease exposures [41]. As these technologies spread progressively from SE to NW Europe over several 1,000 years [33], natural selection may have acted either directly or indirectly to alter brain morphology, thus creating the clinal variation found in this study.

The genetic basis for this geographic trend in skull and brain variation is strengthened by the observation of the trend in North American individuals of European ancestry. The environment – e.g. nutrition and prenatal healthcare – can influence skull morphometry due to developmental plasticity [12, 13] and may be correlated with genetic variation across Europe. In contrast, environmental exposures may vary among European Americans, but this variation is less likely to be geographically structured based on individuals' European ancestry. Furthermore, Ashkenazi Jewish individuals are geographically dispersed in Europe and yet are genetically quite similar and genetically intermediate between SE European and Middle Eastern populations [26, 42–44]. This provides further support that the observed NW-SE clinal variation in brain morphology is driven by genetic more than environmental differentiation of these populations.

Brain volume and cortical surface area in humans increased dramatically after our evolutionary divergence from non-human primates, peaking some 35,000 years ago, and has since decreased in conjunction with body size [45]. Ruff et al. [45] state that some modern humans may be expressing a genetic potential for increased body size and cranial capacity that we inherited from our prehistoric ancestors, and these genes may also contribute to the craniometric clinal variation found in this study. Cra-

niometric studies have shown that random genetic drift can explain the morphological differences between Neanderthals (who had significantly larger brains) and modern humans [46] and can account for most differences between human populations worldwide [9, 11]. Larger brain size in modern humans is thought to be weakly correlated with increased cognitive performance [47], and therefore could have been subject to mild positive selection. Alternatively, larger skulls may have been selected for their increased globularity – i.e. a greater volume to surface area ratio – and thus reduced heat loss for a given brain size. Consequently, larger skulls may have provided a small selective advantage to humans in colder climates [48] and indirectly resulted in modestly increased brain volumes in northern European populations. However, whether or not the morphological diversity we have observed across Europe resulted from neutral genetic drift, natural selection, or a combination of both is an open question.

There are limitations to the data sets we analyzed, but we contend that they are sources of noise that would mask and not falsely generate the clinal geographic variation in skull and brain morphology that we have described. First, the collection of European skulls that we analyzed came from individuals living during a broad range of time periods within the past 500 years. Average skull morphology at any particular location in Europe has likely evolved over time as the result of environmental changes and genetic demographic shifts due to regional migrations. However, we found no relationship between the age (medieval or modern) and geographic location of the populations from which the skulls were obtained. Therefore, this sampling of skulls from different time periods had the potential to obscure continental variation in skull morphology. It is striking that, despite this source of noise, we find strong evidence for clinal skull variation along a NW-SE axis.

Second, both skull and brain morphological data sets include individuals with estimated genetic ancestry from all 4 geographic quadrants of Europe, but NW and SE populations were more highly represented than NE and SW populations. In particular, 62% of skull measurements and 88% of brain measurements come from individuals with NW or SE European ancestry. Therefore, we had greater statistical power to detect morphological variation along a NW-SE geographic axis. In addition, greater than 87% of craniometric variation must be attributed to processes other than NW-SE clinal variation.

A third limitation of this study is the use of subjects diagnosed with MCI and AD. Reductions in brain volume, cortical area, and cortical thickness have been consistently observed in AD and, to a lesser extent, in MCI. If subjects with a diagnosis of AD or MCI were more likely to have inferred genetic ancestry from SE Europe, then we might have falsely attributed differences between NW and SE European brain morphology to genetic background and not neurological disease status. However, neither AD nor MCI diagnosis were significantly associated with degree of inferred NW-SE ancestry. Moreover, brain morphology remains significantly associated with NW-SE ancestry if we exclude subjects with AD, and cortical surface area shows trend level association in the small sample of healthy controls.

In summary, we found consistent and highly significant clinal variation in both skull and brain morphology in Europe despite these sources of noise. We predict that this geographic trend and additional subtle trends will be even more apparent in a modern cohort of healthy, non-elderly adults sampled from across the European continent.

It is plausible that genes responsible for cortical expansion during human evolution retain a role in brain development and contribute to normal variation in brain morphology within and between modern human populations [3, 49]. Identifying these genes could contribute to our understanding of developmental abnormalities associated with neuropsychiatric diseases such as autism and schizophrenia. In this context, admixture mapping may prove to be a powerful strategy for identifying genomic regions responsible for overt brain morphology differences among individuals of European ancestry. Independent of the use of inferred ancestry for identifying genes, our results indicate that studies seeking to identify genes that influence brain morphology should consider genetic background, as it reflects historical mixing and then isolation of populations.

Acknowledgements

We thank T. Hanihara for access to his craniometric data set and O.A. Andreassen and C.N. Carlo for helpful comments on the manuscript. This work is the authors' sole responsibility and does not necessarily represent funding agencies' views. This work was supported in part by the following research grants: U19 AG023122-01, R01 MH078151-01A1, N01 MH22005, U01 DA024417-01, P50 MH081755-01, UL1 RR025774, RC2 DA029475, R01 AG031224, U54 NS056883, the Price Foundation, and Scripps Genomic Medicine. Data collection and sharing for part of this project was funded by the ADNI (National Institutes of Health Grant U01 AG024904). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineer-

ing, and through generous contributions from the following: Abbott, AstraZeneca AB, Bayer Schering Pharma AG, Bristol-Myers Squibb, Eisai Global Clinical Development, Elan Corporation, Genentech, GE Healthcare, GlaxoSmithKline, Innogenetics, Johnson and Johnson, Eli Lilly and Co., Medpace, Inc., Merck and Co., Inc., Novartis AG, Pfizer, Inc., F. Hoffman-La Roche, Schering-Plough, Synarc, Inc., as well as non-profit partners the Alzheimer's Association and Alzheimer's Drug Discovery Foundation, with participation from the U.S. Food and Drug Administration. Private sector contributions to ADNI are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuroimaging at the University of California, Los Angeles. This research was also supported by NIH grants P30 AG010129, K01 AG030514, and the Dana Foundation.

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