Cancer predisposition in children: genetics, phenotypes & screening
Hopman, Saskia

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Face shape differs in phylogenetically related populations

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Abstract

3D analysis of facial morphology has delineated facial phenotypes in many medical conditions and detected fine grained differences between typical and atypical patients to inform genotype-phenotype studies. Next generation sequencing techniques have enabled extremely detailed genotype-phenotype correlative analysis. Such comparisons typically employ control groups matched for age, sex and ethnicity and the distinction between ethnic categories in genotype-phenotype studies has been widely debated. The phylogenetic tree based on genetic polymorphism studies divides the world population into nine subpopulations. Here we show statistically significant face shape differences between two European Caucasian populations of close phylogenetic and geographic proximity from the UK and the Netherlands. The average face shape differences between the Dutch and UK cohorts were visualized in dynamic morphs and signature heat maps, and quantified for their statistical significance using both conventional anthropometry and state of the art dense surface modeling techniques. Our results demonstrate significant differences between Dutch and UK face shape. Other studies have shown that genetic variants influence normal facial variation. Thus, face shape difference between populations could reflect underlying genetic difference. This should be taken into account in genotype-phenotype studies and we recommend that in those studies reference groups be established in the same population as the individuals who form the subject of the study.
**Introduction**

3D analysis of facial morphology using dense surface models (DSMs) has successfully delineated the facial phenotype of a variety of neurodevelopmental conditions and has attained high rates of discrimination between the face shape of affected and unaffected subgroups\(^1\),\(^2\). Using highly sensitive models of facial morphology, it has been possible to detect subtle differences in atypical patients and inform genotype-phenotype studies\(^3\),\(^4\). Advanced molecular genetics techniques have established increasingly detailed correlations between genotype and phenotype. It has been the subject of debate whether it is valid to distinguish ethnic or ancestral categories in such studies\(^5\)\(-\)\(^7\). Cavalli-Sforza et al. proposed a phylogenetic tree dividing the world population into nine subpopulations: New Guinean and Australian, Pacific Islander, Southeast Asian, Northeast Asian, Arctic Northeast Asian, Amerind, European, North African and West Asian, and African\(^8\)\(-\)\(^10\). This subdivision is based on genetic polymorphism studies in various populations grouped by continental sub-areas. \(F_{ST}\) statistics compute genetic distance between populations by measuring the portion of total genetic variation attributed to differences between them\(^6\). Smaller genetic distance, or \(F_{ST}\), is observed when populations live in closer proximity, but morphological differences are observed even in populations with the same phylogenetic origin or who live in relatively close geographic proximity\(^8\). Few investigators have addressed morphological differences between phylogenetically related populations\(^11\),\(^12\). Here, we aimed to determine morphological differences in the faces of two European Caucasian populations of close geographical proximity.

**Subjects and materials**

Permission to perform the study was obtained from Medical Ethics Review Committees of the Academic Medical Center Amsterdam and University College London. Both centers recruited scientific and medical professionals as well as unaffected parents. The scientific and medical recruited professionals were invited through internal ‘advertisement’ mailing. Unaffected members of families with children with a genetic condition were recruited at patient meetings and outpatient clinics. The family members had tested negative for the genetic condition of the child. All study subjects received written patient information and subsequently provided written consent.

The inclusion criteria were subjects who were from self-reported UK or Dutch descent up until the second degree of relatives. Study subjects who had undergone surgery or other treatments altering facial morphology were excluded. We captured 3D photogrammetric images of 400 Caucasian adults, 200 from the United Kingdom (UK) and 200 from the Netherlands (Dutch) (Table 1). Eight Dutch adults were excluded (5 females and 3 males) because of image quality or technical issues. The university scientific and hospital medical
professionals made up 40% of the study population (157/392) and the unaffected parents of children with a molecularly proven genetic syndrome contributed 60% (235/392).

Methods

A dense surface model (DSM) of all faces in the dataset was generated as the set of principal component analysis (PCA) modes covering 99% of shape variation from the overall mean face. DSM construction involved methods described in supplementary material. Animated morphs were generated from the face DSM. Mean Dutch male and female faces were normalized with respect to UK faces of the same sex. We investigated face shape discrimination at the individual face level using multi-folded cross validation. The shape differences identified in the animations and heat map comparisons were also investigated for significance in terms of linear and angular measures (defined in Supplementary Table S2 and derived from landmarks shown in Supplementary Figure S1).

Results

The animated morph between the mean Dutch female and mean UK female faces (Supplemental_movie_1.avi) showed the former to be broader and longer than the latter. Greater separation of outer canthi and nasal alae were noticeable, and the nose was shorter in the mean Dutch female face. The animated portrait morph between mean Dutch male face and mean UK male face (Supplemental_movie_2.avi) suggested the former to be broader at the exocanthi and temples. The profile view showed a more pronounced oral and supraorbital region in Dutch males compared to UK males.

To determine the significance of differences visible in the animations, we normalized the mean Dutch male and mean Dutch female faces with respect to UK faces of the same sex (Figure 1). The red/green/blue spectrum of the heat map corresponds to contraction/coincidence/expansion or to translation difference along lateral/vertical/anterior-posterior axes of the surface being compared. The greater width and length of the mean Dutch female face are highlighted respectively in the lateral (Figure 1B: opposing red-blue at the

<table>
<thead>
<tr>
<th></th>
<th>MALE (N=197)</th>
<th>FEMALE (N=195)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UK (N=100)</td>
<td>Dutch (N=97)</td>
</tr>
<tr>
<td>mean age (yrs)</td>
<td>39.7</td>
<td>38.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38.4</td>
</tr>
<tr>
<td></td>
<td>37/63</td>
<td>43/54</td>
</tr>
<tr>
<td></td>
<td>39/61</td>
<td>39/57</td>
</tr>
<tr>
<td>mean age (yrs)</td>
<td>39/40</td>
<td>33/42</td>
</tr>
<tr>
<td></td>
<td>33/42</td>
<td>31/38</td>
</tr>
</tbody>
</table>

Table 1: Demographics of 392 recruits. Prof = recruits from scientific and medical professionals; Family = recruits from unaffected family members attending clinics and support groups.
Face shape differs in phylogenetically related populations

Figure 1: Colour-coded heat-map comparisons. Heat map comparisons showing the shape differences (red-green-blue colour code spectrums) of the Dutch mean female face normalized against all UK female faces (A-D), of the Dutch mean male face normalized against all UK male faces (E-H), of the UK mean male face normalized against all UK female faces (I-L) and, of the Dutch mean male face normalized against all Dutch females (M-P). The first of each group of four columns is a heat map comparison of the raw mean faces, reflecting displacement normal to the face surface. Heat map comparisons parallel to three orthogonal axes are given in the second (x-axis), third (y-axis) and fourth (z-axis) columns. Colour-coded differences are depicted in standard deviations and correspond to the colour scales at ± the range indicated. In the Male -> Female comparisons, the degree and regional location of differences between the Dutch and UK are very similar, except that the former are slightly reduced in degree. In the Dutch -> UK comparisons, the differences for the females mean faces are greater and in different locations than those for the male mean faces.

left and right exocanthi at 0.7 S.D.) and vertical heat maps (Figure 1C: yellow on chin at 0.7 S.D.). The nasal shortness is shown in the vertical heat map (Figure 1B: blue nasal tip). Whereas in the surface normal comparison of the female Dutch and UK mean faces, there is widespread surface expansion reflecting greater face size (blue regions in Figure 1A) in the analogous male comparison significant regions of expansion are largely peri-oral (Figure 1E). The greater separation of outer canthi in the Dutch to UK male mean face comparison is highlighted by opposing red-blue hues in Figure 1F). The vertical heat mapped comparison (Figure 1G) does not reflect a shorter nose in the mean Dutch male face at the same significance level as in the female comparison.

Compared to the mean UK female face, the mean Dutch female face has as significant differences a greater face length, shorter nasal ridge length, greater nose width, greater nares anteversion; and, as highly significant differences, greater outer canthal separation, longer palpebral fissure width (Table 2). Relative to face length, the reduced nasal ridge and upper face lengths in the Dutch mean female face are both highly significantly different from the UK mean female face. Unlike females, the mean Dutch male face demonstrated an increased length that did not reach significance. In comparing faces of Dutch males to UK males, the anthropometric results confirm a significantly shorter nose relative to face
length; greater separation of the outer canthi; and both relatively and absolutely broader palpebral fissure width (*Table 2*).

Subsequently we investigated face shape discrimination at individual face level using multi-folded cross validation and the closest mean pattern matching algorithm. To check there were no internal biases in each ethnicity-gender subgroup we randomly partitioned them into an A and B subgroup and undertook 20-folded discrimination testing between the A and B subgroups. This was iterated five times and in each comparison dense surface model included PCA modes covering 99% of shape variance from the mean. The area under the corresponding ROC curves and standard error of the mean (SEM) were computed for the 100 comparisons:

<table>
<thead>
<tr>
<th></th>
<th>Dutch female</th>
<th>UK female</th>
<th>P</th>
<th>Dutch male</th>
<th>UK male</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>face length (mm)</td>
<td>113.7</td>
<td>111.9</td>
<td>&lt;0.05*</td>
<td>122.8</td>
<td>122.4</td>
<td>0.68</td>
</tr>
<tr>
<td>nasal ridge length (mm)</td>
<td>46.4</td>
<td>47.4</td>
<td>&lt;0.05*</td>
<td>49.6</td>
<td>50.5</td>
<td>0.10</td>
</tr>
<tr>
<td>relative nasal ridge length</td>
<td>0.41</td>
<td>0.42</td>
<td>&lt;0.0001**</td>
<td>0.40</td>
<td>0.41</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>upper face proportion</td>
<td>0.46</td>
<td>0.47</td>
<td>&lt;0.001**</td>
<td>0.45</td>
<td>0.46</td>
<td>0.06</td>
</tr>
<tr>
<td>nares anteversion</td>
<td>0.82</td>
<td>0.83</td>
<td>&lt;0.05*</td>
<td>0.83</td>
<td>0.84</td>
<td>0.09</td>
</tr>
<tr>
<td>nose width (mm)</td>
<td>33.4</td>
<td>32.7</td>
<td>&lt;0.05*</td>
<td>36.3</td>
<td>36.9</td>
<td>0.15</td>
</tr>
<tr>
<td>outer canthal separation (mm)</td>
<td>88.3</td>
<td>86.2</td>
<td>&lt;0.0005**</td>
<td>92.3</td>
<td>90.6</td>
<td>&lt;0.005**</td>
</tr>
<tr>
<td>palpebral fissure width (PBW) mm</td>
<td>28.3</td>
<td>27.3</td>
<td>&lt;0.001**</td>
<td>29.5</td>
<td>28.8</td>
<td>&lt;0.005**</td>
</tr>
<tr>
<td>Palpebral fissure length relative to face length (mm)</td>
<td>0.25</td>
<td>0.24</td>
<td>0.13</td>
<td>0.24</td>
<td>0.24</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

*Table 2:* Differences in facial measurements between Dutch and UK. Anthropometric calculations and t-tests were performed in Excel (Microsoft Office 2010). *= significant (p-value <0.05), **= highly significant (p-value <0.005, including <0.001 and <0.0001)

UK female A-B: 0.50 ± 0.003 UK male A-B: 0.52 ± 0.008
Dutch female A-B: 0.53 ± 0.007 Dutch male A-B: 0.48 ± 0.007

Each result is close to the expected chance rate of 0.5, supporting the hypothesis of lack of bias. As expected, discrimination between UK female and UK male faces and that between Dutch female and Dutch male faces was close to perfection (*Figure 2*). However, female UK-Dutch and male UK-Dutch comparisons produced much higher discrimination rates of 0.68 and 0.71 respectively than the expected chance rate of 0.5. This confirmed that the average differences found were reproducible at the individual face level.
Face shape differs in phylogenetically related populations

Discussion

Dutch people are significantly taller than the UK population\textsuperscript{13}. Therefore, it is to be expected that Dutch and UK faces differ dimensionally\textsuperscript{14, 15}. The differences identified here, however, include differences based on both shape and proportion, and some are contrary to the greater height of Dutch individuals. Dutch women have significantly longer and broader faces compared to UK women; their palpebral fissure and nasal widths are significantly greater, their nasal ridge length and upper face proportion are significantly reduced; and their nares are significantly more anteverted. In particular, the nasal differences from UK women show that the nose in Dutch women is more likely to be shorter and more retroussé. Dutch men did not have significantly longer faces despite their greater height. Their nasal ridge length relative to face length is significantly shorter; and, relative to face length, they have longer palpebral fissures than UK men. Dutch and UK females show significant difference for nearly every measure, whereas for Dutch and UK males few measures show significant difference. This could be explained by sexual dimorphism, which would mean that overall, Dutch and UK females differ more from each other than males do. Facial and cranial sexual dimorphism has been observed in many human populations\textsuperscript{16, 17}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Rates of discrimination between gender-ethnic subpopulations. In order to determine discrimination rates between the faces of particular sex-ethnicity subgroups multi-folded cross validation was undertaken using closest mean classification. Overall discrimination was calculated as the mean of the AUC estimates for the multiple cross validation results. AUC corresponds to the probability of correctly classifying a randomly selected pair of subjects, one from each classification subgroup. If there were no significant differences between the face shape of two subgroups being compared then the expected AUC would be 0.5 as indicated by the horizontal broken line. The vertical broken lines indicate the number of modes in the dense surface models used corresponding to 99\% of shape variation from the mean face.}
\end{figure}
It is unlikely that the differences in facial morphology we find between UK and Dutch populations were influenced by biased composition of the study group. For example, we recruited both medical/scientific professionals and family members covering a range of social backgrounds. The proportion of professionals to family members and the age ranges in both ethnic groups were comparable. Furthermore, we considered normalized mean difference of professionals from family members within ethnic groups. We also normalized the mean of the UK family members against UK medical/scientific professionals and detected no significant difference (Supplementary Figure 3A). We normalized Dutch family members against Dutch medical/scientific professionals producing minimal difference around the lips (especially the lower lips) and zygoma region (Supplementary Figure 3B). Neither of these comparisons shows any nasal bias which reconfirms the differences we find between our Dutch and UK subgroups as both realistic and generalizable.

In the present study, we describe morphological differences between mean Dutch and UK faces. However, it is unlikely that there is a typical UK or typical Dutch face considering that multiple waves of invasion and immigration in both countries have likely dispersed individual traits. Undoubtedly, there may be regions or subpopulations where less mixing has occurred for geographical or religious reasons, but we have not studied such relatively isolated populations.

Face shape differences are an important determinant of phenotype variation in humans. Craniofacial development is a complex process determined by genetic regulation and genetic variants influence facial morphology in the general population. Thus, face shape difference between populations reflects underlying genetic differences. Therefore, our findings indicate that different baselines for face shape norms for individual populations should be applied when considering craniofacial conditions. Genovese et al. have shown how differences between populations can help identify genomic “missing pieces” in the reference human genome. They described the location of these “missing pieces” using the patterns of variations in sequences that were a result of the admixture of human populations.

Our findings could have fundamental implications for genotype-phenotype correlation studies: a so-called “Caucasian” reference group, encompassing subjects from even close geographical proximity, may not be sufficiently reliable. The morphological differences in phylogenetically related individuals from close geographical proximity described here suggest that in genetic studies reference groups should be established in the same population as the individuals who form the subject of the study.

Supplementary data
Supplementary Methods
Supplementary Figure S1: Landmarks of the face
Supplementary Table S2: Landmark based linear and angular measures
Supplementary Figure S3: Normalized mean difference between medical volunteers and families within ethnic groups.
Supplementary_movie_1: Morph between mean Dutch and mean UK female faces
Supplementary_movie_2: Morph between mean Dutch and mean UK male faces

Acknowledgements
The authors would like to thank all participants of the study.

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Conflict of interest
The authors declare no conflict of interest.
Chapter 4

References


Supplementary methods

Image capture. Commercial stereo-photogrammetric cameras (Canfield Imaging Systems, New Jersey, USA and 3dMD, Atlanta, USA) were used for image capture. The devices used capture more than 25,000 3D points on the face surface. Each face image was manually annotated with 22 landmarks previously shown to be reliable and reproducible\(^1\), \(^2\). The landmarks were used to induce a dense correspondence across all faces of all surface points enabling average face surfaces to be calculated for any subgroup. A dense surface model (DSM) of all faces in the dataset was generated as the set of principal component analysis (PCA) modes covering 99% of shape variation from the mean face. DSM construction methods are described elsewhere\(^2\)-\(^5\).

Animated morphs (or rapidly interpolated image sequences) between the mean Dutch and mean UK female faces, and between the mean Dutch and mean UK male faces, were generated from a DSM using in-house developed and commercial software (VideoMach version 5.9.8, www.gromada.com).

Face signature and heat maps. We normalized the mean Dutch male and female faces with respect to UK faces of the same sex as follows. For each point on the mean male (respectively female) Dutch face, its displacement along the surface normal from the corresponding point on the mean male (respectively female) UK face was normalized with respect to analogous displacements at the corresponding point on faces of all UK males (respectively UK females). Heat maps of these differences using a red-green-blue colour scale, known as face signatures, were generated at min-0-max units of standard deviation for appropriate minima and maxima. Thus, for differences orthogonal to the face surface, the red/green/blue spectrum corresponded to contraction/coin cidence/expansion of the surface being compared. Analogous processes can be used to produce signatures for other reference subgroups (e.g. normalizing a male face against female faces) and for differences along lateral, vertical, and anterior-posterior axes.

Face shape discrimination testing. We investigated individual face shape discrimination using multi-folded cross validation. For example, the classification rate of female faces into Dutch and UK using a DSM representation of face shape was estimated from 20 random 90%-10% training-unseen test pairs of subject subsets (stratified with respect to Dutch/UK origin) for two pattern recognition algorithms. With closest mean discrimination testing, average faces were computed for each gender-ethnicity subset of a training set and each unseen test face was classified according to which average it was closest in terms of its DSM representation. For linear discriminant analysis, the goal was a linear combination of PC modes exhibiting the largest difference in the ethnic subgroup means relative to within-group variance. The validity of our discrimination was determined using receiver–operating characteristics (ROC) curve analysis. The discrimination success of a single test result was estimated as the area under the ROC curve (AUC) arising from a plot of pairs of false positive rate and true positive rate, when a classification parameter was varied through its full range. The overall discrimination was the mean AUC of multiple cross validation results. An intuitive
interpretation of AUC is the probability of correctly classifying a randomly selected pair of subjects, one from each classification subgroup, or, in the case described, individuals from each of the UK or Dutch female populations.
Supplementary data

Mid-line landmarks

n  nasion: the point in the midline of both the nasal root and the nasofrontal suture
prn pronasale: the most protruded point of the apex nasi identified in lateral view of the
  rest position of the head
sn  subnasale: the midpoint of the angle at the columella base where the lower border of
  the nasal septum and surface of the upper lip meet
lip1 labiale superius: the midpoint of the upper vermillion line
lip2 labiale inferius: the midpoint of the lower vermillion line
gn  gnathion: lowest median landmark on the lower border of the mandible

L-R paired landmarks

en  endocanthion: point at the inner commissure of the eye fissure
ex  exocanthion: point at the outer commissure of the eye fissure
al  alare: the most lateral point on each alar contour
ear  otobasion inferius: the most inferior point on the earlobe, located at the attachment
  (junction of the lobe to the face.
cp  christa philtrum: point where philtral ridge meets the vermillion border of the upper lip
ch  cheilion: the point located at each labial commissure
e1  palpebrale superius: the highest point in the mid portion of the free margin of each
  upper eyelid
e2  palpebrale inferius: the lowest point in the mid portion of the free margin of each lower
  eyelid
Supplementary Table S2 Landmark based linear and angular measures

<table>
<thead>
<tr>
<th>MEASURE</th>
<th>DEFINITION IN TERMS OF LANDMARKS</th>
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<tr>
<td>inner canthal separation</td>
<td>enL:enR</td>
</tr>
<tr>
<td>outer canthal separation</td>
<td>exL:exR</td>
</tr>
<tr>
<td>mean palpebral fissure width</td>
<td>0.5·(enL:exL+enR:exR)</td>
</tr>
<tr>
<td>upper face proportion</td>
<td>n:sn/n:gn</td>
</tr>
<tr>
<td>nasal ridge length</td>
<td>n:prn</td>
</tr>
<tr>
<td>face length</td>
<td>n:gn</td>
</tr>
<tr>
<td>relative nasal ridge length</td>
<td>n:prn/n:gn</td>
</tr>
<tr>
<td>nose width</td>
<td>alL:alR</td>
</tr>
<tr>
<td>nose tilt angle</td>
<td>(180/\pi)·acos((n:prn^2 + n:sn^2 - sn:prn^2)/(2·n:prn·n:sn))</td>
</tr>
<tr>
<td>nares anteversion</td>
<td>n:prn·cos(nose tilt angle)/n:sn</td>
</tr>
</tbody>
</table>

en_L = left inner canthus; en_R = right inner canthus; ex_L = left outer canthus; ex_R = right outer canthus; n = nasion; gn = gnathion; prn = pronasale; sn = subnasale; a:b denotes distance between landmarks a and b

Figure S3 Normalized mean difference between medical volunteers and families within ethnic groups

Figure S3 A: UK family members normalized against UK medical/scientific professionals. This comparison does not show any significant facial difference between the family members and the medical/scientific professionals within the UK subgroup of the research population.

B: Dutch family members normalized against Dutch medical/scientific professionals. This comparison shows minimal difference around the lips (especially the lower lips) and zygoma region. Neither of the family members-medical/scientific professionals comparisons show any nasal bias.
Animations (available online)
Supplementary_movie_1: Morph between mean Dutch and mean UK female faces
Supplementary_movie_2: Morph between mean Dutch and mean UK male faces
Supplementary references


