Hum Genet
DOI 10.1007/s00439-015-1618-6

ORIGINAL INVESTIGATION

EDARV370A associated facial characteristics in Uyghur population revealing further pleiotropic effects

Qianqian Peng[1,2] · Jinxi Li[1,2] · Jingze Tan[2] · Yajun Yang[2,3] · Manfei Zhang[1,2] · Sijie Wu[1,2] · Yu Liu[1,2] · Juan Zhang[2,3] · Pengfei Qin[1] · Yaqun Guan[4] · Yi Jiao[4] · Zhaoxia Zhang[4] · Pardis C. Sabeti[5,6] · Kun Tang[1] · Shuhua Xu[1,2,7] · Li Jin[1,2,3] · Sijia Wang[1,2]

Received: 23 October 2015 / Accepted: 14 November 2015

Abstract An adaptive variant of human Ectodysplasin receptor, *EDARV370A*, had undergone strong positive selection in East Asia. In mice and humans, *EDARV370A* was found to affect ectodermal-derived characteristics, including hair thickness, hair shape, active sweat gland density and teeth formation. Facial characteristics are also largely ectodermal derived. In this study, taking advantage of an admixed population of East Asian and European ancestry—the Uyghur, we aim to test whether *EDARV370A* is affecting facial characteristics and to investigate its pleiotropic nature and genetic model. In a sample of 1027 Uyghurs, we discover that *EDARV370A* is significantly associated with several facial characteristics, in particular shape of earlobe ($P = 3.64 \times 10^{-6}$) and type of chin ($P = 9.23 \times 10^{-5}$), with successful replication in other East Asian populations. Additionally, in this Uyghur population, we replicate previous association findings of incisors shoveling ($P = 1.02 \times 10^{-7}$), double incisors shoveling ($P = 1.86 \times 10^{-12}$) and hair straightness ($P = 3.99 \times 10^{-16}$), providing strong evidence supporting an additive model for the *EDARV370A* associations. Partial least square path model confirms *EDARV370A* systematically affect these weakly related ectodermal-derived characteristics, suggesting the pleiotropic effect of *EDARV370A* mainly plays roles in early embryo development. This study extends our knowledge about the pleiotropic nature of *EDARV370A* and provides potential clues to its adaptation fitness in human evolution.

Keywords *EDARV370A* · Facial characteristics · Pleiotropic effect · Uyghur · Association study

Qianqian Peng, Jinxi Li, Jingze Tan and Yajun Yang contributed equally to this study.

Li Jin and Sijia Wang co-directed this study.

Electronic supplementary material The online version of this article (doi:10.1007/s00439-015-1618-6) contains supplementary material, which is available to authorized users.

✉ Li Jin
lijin@fudan.edu.cn

✉ Sijia Wang
wangsijia@picb.ac.cn

1 Chinese Academy of Sciences Key Laboratory of Computational Biology, Chinese Academy of Sciences-Max Planck Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yue Yang Road, 200031 Shanghai, China

2 State Key Laboratory of Genetic Engineering and Ministry of Education Key Laboratory of Contemporary Anthropology, Collaborative Innovation Center for Genetics and Development, School of Life Sciences, Fudan University, 200433 Shanghai, China

3 Fudan-Taizhou Institute of Health Sciences, 1 Yaocheng Road, 225300 Taizhou, Jiangsu, China

4 Department of Biochemistry, Preclinical Medicine College, Xinjiang Medical University, 830011 Urumqi, Xinjiang, China

5 The Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA

6 Department of Organismic and Evolutionary Biology, Center for Systems Biology, Cambridge, MA 02138, USA

7 School of Life Science and Technology, Shanghai Tec University, 200031 Shanghai, China

Published online: 24 November 2015

Introduction

Human evolution studies identified that an adaptive variant of ectodysplasin receptor, *EDARV370A* (*rs3827760*), had undergone strong positive selection in East Asia (Grossman et al. 2010, 2013; Sabeti et al. 2007). The derived *370A* allele has extremely high prevalence in East Asians and Native American populations, but it is almost absent in Europeans and Africans (Bryk et al. 2008; Grossman et al. 2010, 2013; Sabeti et al. 2007). This gain-of-function allele alters a highly conserved amino acid in the death domain of *EDAR*, and it influences the binding efficiency of ectodysplasin to *EDAR* thereupon affects the function of downstream pathways (Cluzeau et al. 2012). It is well established that mutations on *EDAR*, along with other genes in *EDA* pathway, are causing hypohidrotic ectodermal dysplasia (HED), a rare genodermatosis characterized by sparse hair (hypotrichosis), abnormal or missing teeth (hypodontia) and inability to sweat (anhidrosis) (Cluzeau et al. 2011). This led the researchers to hypothesize that the *EDARV370A* allele might be selected for functionally affecting certain ectodermal-related phenotypes. Indeed *EDARV370A* was found to be significantly associated with hair thickness (Fujimoto et al. 2008a, b), hair straightness (Tan et al. 2013), incisor shoveling (Kimura et al. 2009; Park et al. 2012; Tan et al. 2014) and active sweat gland density (Kamberov et al. 2013). Transgenic mice studies further confirmed that *EDARV370A* is the responsible causal functional variant (Kamberov et al. 2013). The strong selection and the pleiotropic effect of *EDARV370A* on ectodermal-derived phenotypes make it a very special case in human evolution.

Intriguingly, HED is known to affect facial appearance in the patients (e.g. prominent forehead, thick lips) (Cluzeau et al. 2011). Facial characteristics are largely shaped by skull bones, which are also ectodermal derived. Therefore, it is conceivable but remains to be tested whether *EDARV370A* could also affect facial morphology to some extent. In this study, we aim to explore the relation between *EDARV370A* and facial morphology by conducting an association study in the Uyghur population.

All of the previous association studies on *EDARV370A* were carried out in East Asian populations (Fujimoto et al. 2008a, b; Kamberov et al. 2013; Kimura et al. 2009; Park et al. 2012; Tan et al. 2013, 2014). One conspicuous limitation is that the ancestral allele, *370V*, is at a very low frequency in most East Asian populations (Sabeti et al. 2007). It is, therefore, hard to distinguish the genetic model of *EDARV370A* on its affected phenotypes. The low diversity of *EDARV370A* also leads to low power of the association studies in East Asian populations, making it hard to explore a large spectrum of potentially affected ectodermal-related phenotypes (with lesser effects than the reported ones). In order to overcome the above limitations, we choose to conduct a study in the Uyghur population, a typical admixed population with genetic contribution from both East Asian and European ancestry populations—close to a 50:50 proportion. Population genetic studies showed that the admixture event in Uyghurs was likely to happen more than 100 generations ago. Therefore, the whole Uyghur population is thoroughly admixed, and the ancestry proportion of most Uyghur individuals are close to a 50:50 ratio (Xu et al. 2008, 2009; Xu and Jin 2008). Such an admixture property makes Uyghur an extremely ideal population to study the phenotypic effects of *EDARV370A*, as (1) both the *370A* and *370V* alleles have a decent frequency in the population; and (2) the phenotypic diversity is also considerably high in the population.

By collecting the *EDARV370A* genotype and a number of ectodermal-derived phenotypes in 1027 Uyghur individuals, we aim to detect the potential facial characteristics which may be influenced by *EDARV370A*, and further expand our understanding of the pleiotropic nature of *EDARV370A*.

Materials and methods

Samples

This study recruited 1027 Uyghur samples, who were undergraduate students from Xinjiang Medicine University, including 393 males and 634 females. The age of the samples ranged from 17 to 25 years. The study only recruited individuals whose self-reported Uyghur origins traced back to all four grandparents. The research was conducted with the official approval from the Ethics Committee of Fudan University, Shanghai, China. All the participants had provided written consents.

For replication purpose, genotype and phenotype data from two other populations (323 samples in Mongolian and 973 samples in Han Chinese) were also included in this study. More details of those samples can be found in a previous study (Tan et al. 2013).

Genotyping

A qualified physician collected peripheral blood samples from each of the participants. DNA samples were extracted from the blood using the GENEray™ DNA extraction kit. The *EDARV370A* SNP (*rs3827760*) was genotyped using the SNaPshot Multiplex System. 24 Ancestral Informative Markers (AIMs) were also genotyped to calculate the ancestral contribution for each of the Uyghur samples. The detailed selection method of the AIMs is described in the "Statistical analysis" section. The 24 AIMs were genotyped using the LDR Multiplex System. Genotype calling was performed by GeneMapper v2.0.

Phenotyping

After signing a content form, each of the participants first completed a questionnaire including the information of age, gender, ethnic background, etc. We then collected phenotypic information of the participant by observing or measuring the following ectodermal related phenotypes (Table 1).

Facial characteristics We assessed 16 facial characteristics according to the human body assessment methods (Wu et al. 2010). The assessed characteristics include upper eyelid fold (right and left), epicanthic fold (right and left), type of forehead, type of cheekbone, type of chin, nasal root height, nasion depression, nasal bone, nasal-tip direction, nasal-tip shape, nasal base direction, nostril form, upper lip thickness and shape of earlobe. The rating scales and the frequencies of the facial characteristics are shown in Table 1.

Incisors shoveling and double shoveling Dental plaster models were applied to assess incisors shoveling and double incisors shoveling of the subjects. The rating criteria of incisors shoveling and double incisors shoveling follow the Arizona State University Dental Anthropology System, which includes seven degrees (0–2 indicates no incisors shoveling or double incisors shoveling, while 3–6 indicates incisors shoveling or double incisors shoveling) (Scott and Turner 1997; Turner et al. 1991).

Hair straightness The degree of hair straightness was rated on a three-point scale: straight, wavy, or curly, following the method used in previous studies (Medland et al. 2009; Tan et al. 2013). However, curly hair is scarce in the Uyghur sample, so we merged curly and wavy into one scale—"non-straight" in data analysis.

Table 1 List of assessed phenotypes by observation

Trait	*N*	Rating scales (frequency in %)			
Upper eyelid fold left	1005	No	Close to eyelash	1–2 mm	>2 mm
		1	1.69	15.32	81.99
Upper eyelid fold right	1005	No	Close to eyelash	1–2 mm	>2 mm
		1.09	1.89	16.42	80.60
Epicanthic fold left	1006	No	Part	Yes	
		90.36	7.65	1.99	
Epicanthic fold right	1006	No	Part	Yes	
		90.95	7.16	1.89	
Type of forehead	718	Convex	Normal	Oblique	
		12.12	45.54	42.34	
Type of cheekbone	1001	Convex	Normal	Closed	
		13.69	63.14	23.18	
Type of chin	1011	Protrude	Normal	Retrude	
		66.37	25.52	8.11	
Nasal root height	1013	Low	Normal	High	
		4.94	49.75	45.31	
Nasion depression	997	No	Slight	Notable	
		2.51	56.47	41.02	
Nasal bone	993	Spill	Normal	Convex	
		13.8	21.95	64.25	
Nasal-tip direction	1012	Upward	Ahead	Ptosis	
		45.36	49.11	5.53	
Nasal-tip shape	719	Sharp	Normal	Blunt	
		52.85	39.78	7.37	
Nasal-base direction	1012	Upward	Ahead	Ptosis	
		50.89	45.06	4.05	
Nostril form	1001	Narrow		Broad	
		51.75		48.25	
Upper lip thickness	718	Slim	Normal	Thick	
		18.11	79.25	2.65	
Shape of earlobe	1012	Circular	Square	Triangular	
		52.47	31.42	16.11	
Incisor shoveling	1007	No		Yes	
		49.95		50.05	
Double incisor shoveling	1010	No		Yes	
		91.68		8.32	
Hair straightness	988	No		Yes	
		47.06		52.94	

Statistical analysis

Ancestry analysis To ensure that the association analysis is not confounded by the ancestry of the samples, we inferred the ancestral proportion of each study sample and corrected the potential effect. First, we selected 24 Ancestral Informative Markers (AIMs) based on International HapMap Project Phase III (CHB as the East Asian ancestry and CEU as the European ancestry), under two criteria: (1) SNPs highly differentiate between East Asia and Europe (*Fst* >0.8); and (2) inter-marker distance is great enough (>0.01 cM) to avoid any substantial linkage disequilibrium. We then use STRUCTURE program to estimate the ancestral proportion for each Uyghur sample based on the 24 AIMs, using the CHB and CEU from HapMap Project Phase III as ancestral populations (Falush et al. 2003, 2007; Hubisz et al. 2009; Pritchard et al. 2000). An admixture-model with $K = 2$ was assumed and the program run with 5000 iterations and 10,000 burn-ins to convergence.

Association analysis General linear model was applied to estimate the association between *EDARV370A* and collected phenotypes. Gender and the ancestral proportion were taken as covariates in our study. We found that age was not a significant factor in any models which might be due to that all

Table 2 The association between *EDARV370A* and 16 facial characteristics

Facial characteristic	Coefficient	Standard error	*P* value	R^{2a}
Upper eyelid fold left	0.01	0.02	0.77	0.00
Upper eyelid fold right	0.02	0.02	0.40	0.00
Epicanthic fold left	**0.04**	**0.02**	**0.02**	**0.03**
Epicanthic fold right	0.03	0.02	0.09	0.04
Type of forehead	**0.07**	**0.03**	**0.02**	**0.34**
Type of cheekbone	0.01	0.03	0.76	0.01
Type of chin	*0.11*	*0.03*	9.23×10^{-5}	*0.06*
Nasal root height	0.01	0.02	0.58	0.27
Nasion depression	−0.02	0.02	0.45	0.16
Nasal bone	−0.04	0.03	0.24	0.02
Nasal-tip direction	−0.02	0.03	0.47	0.14
Nasal-tip shape	0.01	0.03	0.68	0.03
Nasal-base direction	−0.05	0.03	0.08	0.07
Nostril Form	−0.01	0.02	0.55	0.02
Upper lip thickness	**0.05**	**0.02**	**0.02**	**0.01**
Shape of earlobe	*0.16*	*0.03*	3.64×10^{-6}	*0.03*

Bold indicates nominal significant association results (*P* value <0.05)

Italicized indicates significant association results after multiple testing (*P* value $<3.13 \times 10^{-3}$)

[a] R^2 is calculated as the ratio of the sum of squares for the model to the corrected total sum of squares

the samples were from a young group (17–25 years old), so it was not considered as a covariate in the present models. Frequency bar charts along with results of Fisher exact test (for qualitative traits), or average histograms (for quantitative traits) were also applied to demonstrate the simple association between *EDARV370A* and significantly associated phenotypes obtained through general linear model.

Partial least square path model analysis Partial least square path model was applied to present the pleiotropic effect of *EDARV370A* on detected ectodermal-derived phenotypes (Tenenhaus et al. 2005). A full path model was constructed among *EDARV370A* and the phenotypes while controlling for gender and ancestral proportion. The bootstrap confidence interval test (bootstrap resampling times = 1000, significance level = 0.05) was applied to test the significance of each path coefficient.

Results

For *EDARV370A*, the frequencies of three genotypes in the Uyghur population are 0.38 (*370V/370V*), 0.47 (*370V/370A*) and 0.15 (*370A/370A*). The minor allele (*370A*) frequency is 0.39, showing a much higher diversity than other East Asian populations. All the genotyped SNPs including *EDARV370A* and 24 ancestral informative markers (AIMs) have passed Hardy–Weinberg equilibrium test (Table S1).

In a general linear model, we find significant associations between *EDARV370A* and a number of facial characteristics, including type of chin, shape of earlobe, epicanthic fold, type of forehead and upper lip thickness (Table 2). After Bonferroni correction, the associations between *EDARV370A* and type of chin and shape of earlobe are still significant. Additionally, we perform ancestral proportion correction for these associations and obtain similar results (Table S2).

Significant association between *370A* allele and triangular shape of earlobe

In the Uyghur population, individuals carrying more *370A* alleles are less likely to have circular shape of earlobe and more likely to have triangular shape of earlobe ($P = 3.91 \times 10^{-4}$, Fisher exact test; Fig. 1b). In a general linear model corrected for gender, *EDARV370A* is significantly associated with shape of earlobe and fitted well in an additive model ($P = 3.64 \times 10^{-6}$; Table 2). We confirm this association in two other East Asian populations ($P = 4.97 \times 10^{-5}$ in Han Chinese, $P = 9.60 \times 10^{-3}$ in Mongolian, Fisher Exact test; Fig. 1c, d). Furthermore, we have collected the frequency data of the *EDARV370A* allele as well as the shape of earlobe in 19 East Asian populations (Table S3). A linear regression analysis shows that the frequency of triangular earlobe is positively correlated with the frequency of *370A* at the population level ($R^2 = 0.27$, $P = 0.02$; Fig. 1e).

Significant association between *370A* allele and retruded chin

In the Uyghur population, we also find that individuals carrying *370A* alleles are more likely to have retruded chin and less likely to have protruded chin ($P = 4.75 \times 10^{-5}$, Fisher Exact test; Fig. 2b). In a general linear model corrected for gender, the association between type of chin and *EDARV370A* remains significant ($P = 9.23 \times 10^{-5}$, Table 2). Compared with individuals carrying *370A* homozygotes, the individuals carrying *370V* alleles are more inclined to have protruded chins. We further confirm the association between *EDARV370A* and type of chin in a Han Chinese population ($P = 0.03$; Fig. 2c).

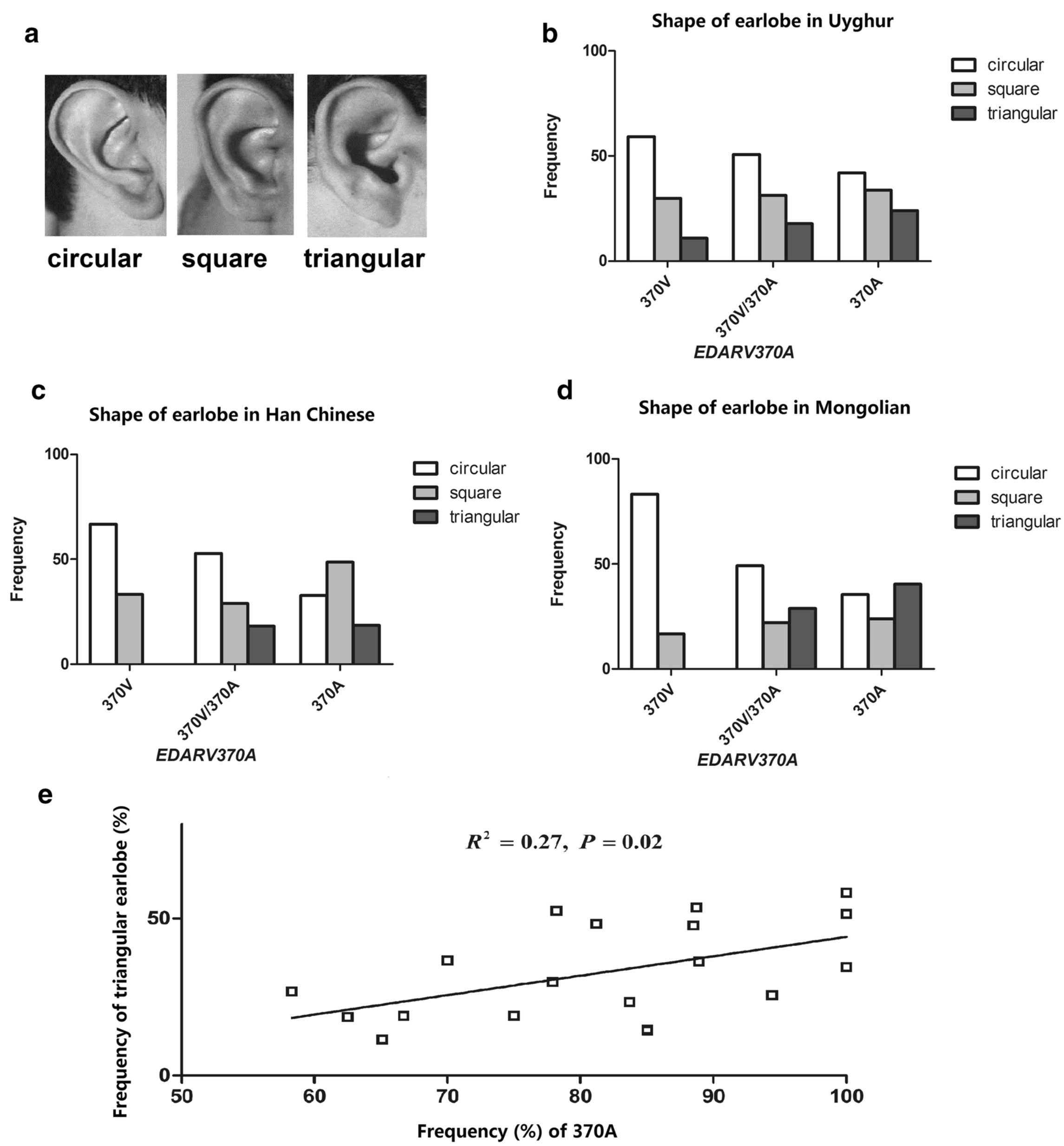

Fig. 1 *EDARV370A* is significantly associated with the shape of earlobe. **a** Different shapes of earlobe (*circular, square* and *triangular*); **b** the association between *EDARV370A* and shape of earlobe in the Uyghur population ($P = 3.91 \times 10^{-4}$); **c** The association between *EDARV370A* and shape of earlobe in a Han population ($P = 4.97 \times 10^{-5}$); **d** The association between *EDARV370A* and shape of earlobe in a Mongolian population (9.60×10^{-3}); **e** The linear regression on frequencies of triangular earlobe and *370A* frequencies in 19 Asian populations

Significant associations of *370A* allele on hair straightness and incisor shoveling best fitting in an additive genetic model

Previous studies found that *EDARV370A* is significantly associated with hair straightness and incisors shoveling in East Asian populations (Kimura et al. 2009; Tan et al. 2013, 2014). Our data on the Uyghur population confirm the trend that individuals with the *370A* allele tend to have straight hair ($P = 4.07 \times 10^{-16}$, Fisher Exact test; Fig. 3b), shovel-shaped incisors ($P = 1.76 \times 10^{-6}$, Fisher exact test; Fig. 3c) and double shovel-shaped incisors ($P = 5.15 \times 10^{-12}$, Fisher exact test; Fig. 3d). In a general linear model corrected for gender, the associations remain significant between *370A* and straight hair ($P = 3.99 \times 10^{-16}$), incisors shoveling ($P = 1.20 \times 10^{-7}$) and double incisors shoveling ($P = 1.86 \times 10^{-12}$). Moreover, our results clearly show that the associations fit best in an additive model (Table S4).

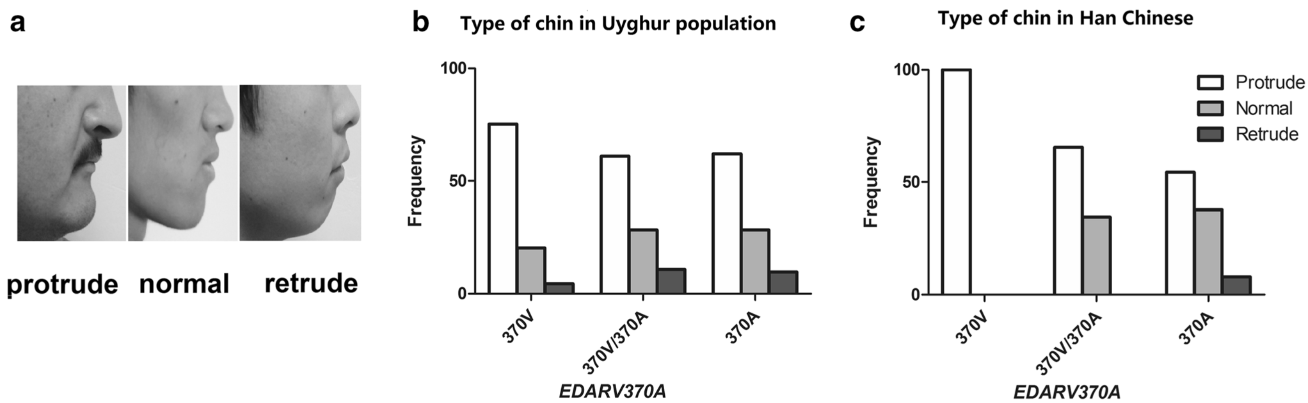

Fig. 2 *EDARV370A* is significantly associated with the type of chin. **a** Different types of chin (*protrude*, *normal* and *retrude*); **b** The association between *EDARV370A* and type of chin in the Uyghur population ($P = 4.75 \times 10^{-5}$); **c** The association between *EDARV370A* and type of chin in a Han population (general linear model: $P = 0.03$)

Systematical associations of *EDARV370A* and weakly related ectodermal-derived characteristics

Pairwise correlation analyses show that the eight ectodermal-derived characteristics detected in our study are weakly correlated with each other (Fig. 4a). Furthermore, we apply a full partial least square path model to systematically learn the relation among *EDARV370A* and eight ectodermal-derived characteristics. The result shows that *EDARV370A* is significantly associated with hair straightness (path coefficient = −0.27), incisors shoveling (path coefficient = 0.12), double incisors shoveling (path coefficient = 0.20), shape of earlobe (path coefficient = 0.10), type of chin (path coefficient = 0.11), upper lip thickness (path coefficient = 0.07) and type of forehead (path coefficient = 0.08) in the path model (Fig. 4b; Table S5). Each path coefficient represents the effect of *EDARV370A* on each studied characteristic. The path model also indicates that there are weak relations among eight ectodermal-derived characteristics, where only 4 out of 28 path coefficients among these characteristics are significant (Table S5). Those non-significant path coefficients are not presented in Fig. 4b, while more information about the path model is included in supplement Table S5. These results confirm that *EDARV370A* systematically influences a series of weakly related ectodermal-derived characteristics.

Discussion

EDARV370A and facial morphology

Bones are mostly mesodermal derived, with the exception of skull bones, which are mostly ectodermal derived. Since *EDARV370A* is already known to play a crucial role in the ectodermal-related phenotypes, we hypothesize that facial characteristics—largely affected by skull bones—could be associated with *EDARV370A* as well. In this Uyghur study, we did find at least two facial characteristics, the type of chin and the shape of earlobe, are significantly associated with *EDARV370A*. Both associations have been independently replicated in other East Asian populations and, therefore, should not be artifacts. For the type of chin, *370A* carriers tend to have retruded chins, while *370V* carriers are more likely to have protruded chins. The worldwide distribution does indicate that protruded chin is prominent in Europe, matching well with the distribution of *EDARV370A*. The type of chin might be a combined result from the shape of mandible and teeth (Aiello and Dean 1990; Fuerle 2008; Schwartz and Tattersall 2000).

The shape of earlobe is also largely affected by skull bone. The external ear derives from tissue in the area of the contiguous edges of the first pharyngel pouch where the mandibular and hyoid arches join (Hunter and Yotsuyanagi 2005; Moldenhauer 1877). Specifically, the earlobe is believed to derive from one or two hillocks in this region (Park 1999). In our results, we find that for the shape of earlobe, *370A* carriers tend to have more triangle earlobes. Kaustubh and colleagues also independently found this association in Latin American populations (Adhikari et al. 2015). Looking at the worldwide distribution of shape of earlobe, it may not be a coincidence that triangular earlobes mostly present in East Asia, where *370A* is predominant; while circular earlobes mostly present in Africa and Europe, where *370V* prevails (Bhowmik 1971; Nakamura et al. 1995; Overfield and Call 1983). It is also worth noting that the shape of earlobe is long thought to be a polygenetic trait with more than one gene

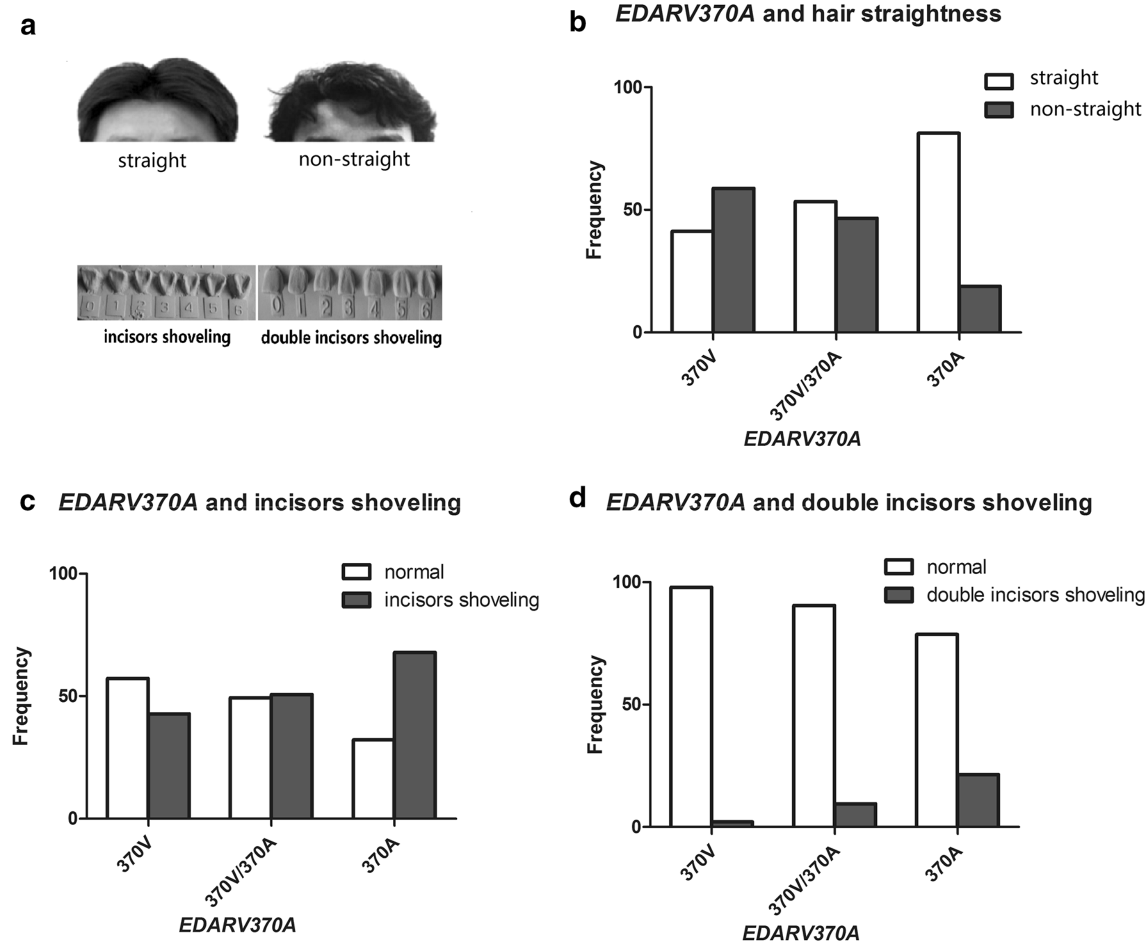

Fig. 3 *EDARV370A* is associated with hair straightness, incisors shoveling and double incisors shoveling, fitting well in an additive genetic model. **a** The rating scales of hair straightness, incisors shoveling and double incisors shoveling. For incisors shoveling and double shoveling, 0, 1 defined as normal while 2–6 defined as incisors shoveling or double shoveling. **b** The association between *EDARV370A* and hair straightness ($P = 4.07 \times 10^{-16}$). **c** The association between *EDARV370A* and incisors shoveling ($P = 1.76 \times 10^{-6}$). **d** The association between *EDARV370A* and double shoveling ($P = 5.15 \times 10^{-12}$)

involved. Indeed our results suggest that *EDARV370A* only explains a small portion of the variance of the phenotype. Future studies at the genome-wide level shall be able to discover other genes also responsible for the shape of earlobe phenotype.

Besides the type of chin and the shape of earlobe, there are also several facial characteristics (epicanthic fold, type of forehead and upper lip thickness) showing nominal association with *EDARV370A*. The association between *EDARV370A* and type of forehead and upper lip thickness are also confirmed in partial least square path model analysis. Interestingly, prominent forehead and thick lips are two most conspicuous facial characteristics in the HED patients (Cluzeau et al. 2011). Although we do not find significant associations of these two characteristics in our replicate populations, it would be interesting to confirm the results in further studies with increased samples size.

Pleiotropic effect of *EDARV370A*

EDARV370A arose in East Asia more than 30,000 years ago and since then had undergone strong positive selection in East Asia (Kamberov et al. 2013). The estimated selective coefficient of *EDARV370A* was found to be extremely high in various studies (Bryk et al. 2008; Fujimoto et al. 2008a; Kamberov et al. 2013; Sabeti et al. 2007). This could be due to the pleiotropic nature of *EDARV370A*, as many related traits could be the subjects under selection. It is even possible that at different time of human evolution, different traits could be the subjects under selection, collectively driving the *370A* allele into high frequency in East Asia. Unfortunately, also because of its pleiotropic nature, the selective pressure on this unique variant remains a mystery.

While it is difficult to determine the adaptive fitness of *EDARV370A* during its strong positive selection in East a

Correlation among eight epidermal-derived phenotypes								
P Value	Hair Straightness	Epicanthic Fold	Type of Forehead	Type of Chin	Upper Lip Thickness	Shape of Earlobe	Incisors Shoveling	Double Incisors Shoveling
Hair Straightness		-0.01[a]	-0.07	-0.03	-0.05	-0.04	-0.03	0.02
Epicanthic Fold	0.77[b]		-0.07	0.07	0.14	-0.02	-0.05	0.16
Type of Forehead	0.06	0.07		-0.17	0.02	0.08	0.08	0.02
Type of Chin	0.48	0.07	<0.01		0.03	-0.02	-0.12	0.00
Upper Lip Thickness	0.22	<0.01	0.61	0.36		0.00	0.04	0.08
Shape of Earlobe	0.28	0.56	0.03	0.68	0.96		-0.02	-0.02
Incisors Shoveling	0.39	0.21	0.03	<0.01	0.30	0.62		0.11
Double Incisors Shoveling	0.68	<0.01	0.62	0.95	0.04	0.63	<0.01	

b

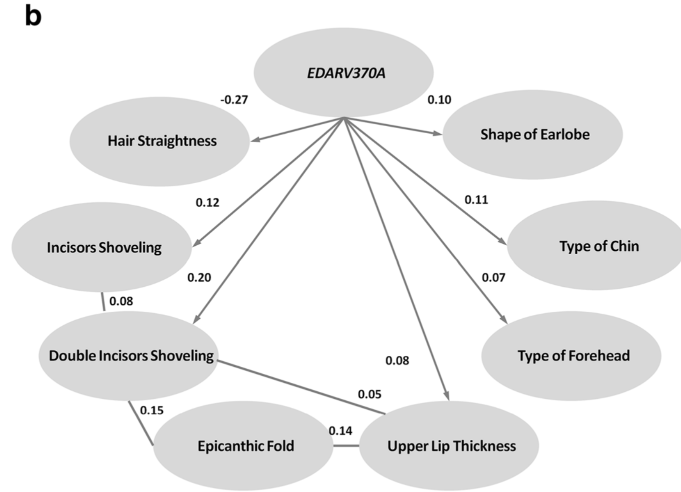

Fig. 4 The correlation among eight ectodermal-derived phenotypes and the partial least square path model among them and *EDARV370A*. **a** The correlation among eight ectodermal-derived phenotypes which are associated with *EDARV370A*. [a]*Upper panel* are the correlation coefficients among eight phenotypes; [b]*lower panel* shows the *P* values of significance test for the corresponding correlation coefficients. There is weak correlation among the eight ectodermal-derived phenotypes. **b** The partial least square path model between *EDARV370A* and eight ectodermal-derived phenotypes. Only path coefficients that are significant by bootstrap confidence interval testing (bootstrap resampling times = 1000, significance level = 0.05) are presented in this panel

Asia, exploring more potential traits which are influenced by *EDARV370A* may provide clue to its selective pressure. In previous studies, the *EDARV370A* allele was reported to be associated with hair straightness, hair thickness, sweat gland density, incisor shoveling and other dental morphologies (Fujimoto et al. 2008a, b; Kamberov et al. 2013; Kimura et al. 2009; Mou et al. 2008; Park et al. 2012; Tan et al. 2013, 2014). In this study, we further discover that *EDARV370A* is associated with a number of facial characteristics, particularly the type of chin and the shape of earlobe. This study indicates that *EDARV370A* may play a role in the development of facial morphology. With the new associated phenotypes reported in this paper, the pleiotropic nature of *EDARV370A* is further extending.

By applying a partial least square path model, we confirm that *EDARV370A* is systematically affecting a number of ectodermal-derived characteristics. However, these characteristics are weakly correlated. This might because that *EDARV370A* mainly plays roles in early embryo development, and subsequently various genes and environmental factors affected the development of these characteristics during late stage of development.

Use of the admixed Uyghur population in association studies

The Uyghur population used in this study is a typical admixed population between East Asia and Europe. Estimations of the admixture time range from 800 to more than 2000 years ago (Xu et al. 2008, 2009; Xu and Jin 2008). Therefore, the Uyghur admixture happened much earlier than the admixture events of African Americans or the Mestizos. The admixture happened quite thoroughly in the population at the individual level, making most of the Uyghur individuals ~ 50:50 admixture of their East Asian and European ancestries (Xu et al. 2008, 2009; Xu and Jin 2008). This unique feature helps to reduce substructure within the Uyghur population and makes it an ideal population for association studies. In this study, since *EDARV370A* has an extremely contrasting frequency between East Asia and Europe, one could raise the concern that the association between *EDARV370A* and a related phenotype may result from a real association between the ancestry proportion and the phenotype. We cautiously add the ancestry proportion as a co-variant in all of the association analysis to exclude this possibility. The association results before and after corrections remain largely the same, making it clear that ancestry proportion is not a confounding factor in the association results reported in this study. Our unpublished results using genome-wide SNP markers in a subset of the samples also confirm that substructure is not much a concern for the well-admixed Uyghur population. Contrarily, because of the admixture feature, the Uyghur population turns out to be an excellent subject to study the phenotypic effect of *EDARV370A*. Its high genetic diversity (minor allele frequency *0.39*) and phenotypic diversity bring at least ten times more power than a study of same size in Han Chinese (simulation results not shown).

Interestingly, by studying the Uyghur population, one can infer that *EDARV370A* is most likely not to be under any selection at all for its history of 800–2000 years. The *370A* allele, selected in most of the East Asian populations, is actually the minor allele in the Uyghurs, with a frequency of 39 %. Had it been under selection in the Uyghurs, one would expect the allele to also raise to a high frequency, significantly higher than 50 %, the genome-wide average contribution from East Asia. The absence of selection in Uyghurs could shed light on the intriguing case of *EDARV370A*. It also raises an interesting question as to whether *EDARV370A* is no longer under positive selection in East Asia in the recent human history, or if *EDARV370A* is just not under positive selection in the environment that the Uyghurs live in.

Acknowledgments This work was supported by the National Natural Science Foundation of China (31322030, 91331108 to S.W., 30890034, 31271338 to L. J., 91331204, 31171218 to S. X., 31401061 to Q. P., 31071102 to J. T., 31071096 to Y. Y.), National Science and Technology Infrastructure Program (2015FY111700 to L. J.), the National Basic Research Program (2012CB944600 to L. J.), Ministry of Science and Technology (2011BAI09B00 to L. J.), Ministry of Education (311016 to L. J.), National High-Tech Research and Development Program (2012AA021802 to Y.Y.), Chinese Academy of Sciences (XDB13040100 to S.X., 2014KIP214 to Q. P.), the National Program for Top-notch Young Innovative Talents of The "*Wanren Jihua*" Project to S. X., National Thousand Young Talents Award to S. W., Max Planck-CAS Paul Gerson Unna Independent Research Group Leadership Award to S. W., the State Key Laboratory of Genetic Engineering, Fudan University to S. W., and key lab of computational biology of Chinese Academy of Sciences to J. T.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Adhikari K, Reales G, Smith AJ, Konka E, Palmen J, Quinto-Sanchez M, Acuna-Alonzo V, Jaramillo C, Arias W, Fuentes M, Pizarro M, Barquera Lozano R, Macin Perez G, Gomez-Valdes J, Villamil-Ramirez H, Hunemeier T, Ramallo V, Silva de Cerqueira CC, Hurtado M, Villegas V, Granja V, Gallo C, Poletti G, Schuler-Faccini L, Salzano FM, Bortolini MC, Canizales-Quinteros S, Rothhammer F, Bedoya G, Calderon R, Rosique J, Cheeseman M, Bhutta MF, Humphries SE, Gonzalez-Jose R, Headon D, Balding D, Ruiz-Linares A (2015) A genome-wide association study identifies multiple loci for variation in human ear morphology. Nat Commun 6:7500. doi:10.1038/ncomms8500

Aiello L, Dean C (1990) An introduction to human evolutionary anatomy. Academic Press, London

Bhowmik D (1971) Earlobe attachment in Uttar Pradesh. Man India 51:157–161

Bryk J, Hardouin E, Pugach I, Hughes D, Strotmann R, Stoneking M, Myles S (2008) Positive selection in East Asians for an EDAR allele that enhances NF-kappaB activation. PLoS One 3:e2209. doi:10.1371/journal.pone.0002209

Cluzeau C, Hadj-Rabia S, Jambou M, Mansour S, Guigue P, Masmoudi S, Bal E, Chassaing N, Vincent MC, Viot G, Clauss F, Maniere MC, Toupenay S, Le Merrer M, Lyonnet S, Cormier-Daire V, Amiel J, Faivre L, de Prost Y, Munnich A, Bonnefont JP, Bodemer C, Smahi A (2011) Only four genes (EDA1, EDAR, EDARADD, and WNT10A) account for 90% of hypohidrotic/anhidrotic ectodermal dysplasia cases. Hum Mutat 32:70–72. doi:10.1002/humu.21384

Cluzeau C, Hadj-Rabia S, Bal E, Clauss F, Munnich A, Bodemer C, Headon D, Smahi A (2012) The EDAR370A allele attenuates the severity of hypohidrotic ectodermal dysplasia caused by EDA gene mutation. Br J Dermatol 166:678–681. doi:10.1111/j.1365-2133.2011.10620.x

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol Ecol Notes 7:574–578. doi:10.1111/j.1471-8286.2007.01758.x

Fuerle RD (2008) Erectus walks amongst us. Spooner Press, New York

Fujimoto A, Kimura R, Ohashi J, Omi K, Yuliwulandari R, Batubara L, Mustofa MS, Samakkarn U, Settheetham-Ishida W, Ishida T, Morishita Y, Furusawa T, Nakazawa M, Ohtsuka R, Tokunaga K (2008a) A scan for genetic determinants of human hair morphology: EDAR is associated with Asian hair thickness. Hum Mol Genet 17:835–843. doi:10.1093/hmg/ddm355

Fujimoto A, Ohashi J, Nishida N, Miyagawa T, Morishita Y, Tsunoda T, Kimura R, Tokunaga K (2008b) A replication study confirmed the EDAR gene to be a major contributor to population differentiation regarding head hair thickness in Asia. Hum Genet 124:179–185. doi:10.1007/s00439-008-0537-1

Grossman SR, Shlyakhter I, Karlsson EK, Byrne EH, Morales S, Frieden G, Hostetter E, Angelino E, Garber M, Zuk O, Lander ES, Schaffner SF, Sabeti PC (2010) A composite of multiple signals distinguishes causal variants in regions of positive selection. Science 327:883–886. doi:10.1126/science.1183863

Grossman SR, Andersen KG, Shlyakhter I, Tabrizi S, Winnicki S, Yen A, Park DJ, Griesemer D, Karlsson EK, Wong SH, Cabili M, Adegbola RA, Bamezai RN, Hill AV, Vannberg FO, Rinn JL, Lander ES, Schaffner SF, Sabeti PC (2013) Identifying recent adaptations in large-scale genomic data. Cell 152:703–713. doi:10.1016/j.cell.2013.01.035

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour 9:1322–1332. doi:10.1111/j.1755-0998.2009.02591.x

Hunter AG, Yotsuyanagi T (2005) The external ear: more attention to detail may aid syndrome diagnosis and contribute answers to embryological questions. Am J Med Genet A 135:237–250. doi:10.1002/ajmg.a.30723

Kamberov YG, Wang S, Tan J, Gerbault P, Wark A, Tan L, Yang Y, Li S, Tang K, Chen H, Powell A, Itan Y, Fuller D, Lohmueller J, Mao J, Schachar A, Paymer M, Hostetter E, Byrne E, Burnett M, McMahon AP, Thomas MG, Lieberman DE, Jin L, Tabin CJ, Morgan BA, Sabeti PC (2013) Modeling recent human evolution in mice by expression of a selected EDAR variant. Cell 152:691–702. doi:10.1016/j.cell.2013.01.016

Kimura R, Yamaguchi T, Takeda M, Kondo O, Toma T, Haneji K, Hanihara T, Matsukusa H, Kawamura S, Maki K, Osawa M, Ishida H, Oota H (2009) A common variation in EDAR is a genetic determinant of shovel-shaped incisors. Am J Hum Genet 85:528–535. doi:10.1016/j.ajhg.2009.09.006

Medland SE, Nyholt DR, Painter JN, McEvoy BP, McRae AF, Zhu G, Gordon SD, Ferreira MA, Wright MJ, Henders AK, Campbell MJ, Duffy DL, Hansell NK, Macgregor S, Slutske WS, Heath AC, Montgomery GW, Martin NG (2009) Common variants in the trichohyalin gene are associated with straight hair in Europeans. Am J Hum Genet 85:750–755. doi:10.1016/j.ajhg.2009.10.009

Moldenhauer W (1877) Die Entwickelung des mittleren und des äusseren Ohres. Morph Jahrb 3:106–151 **(cited in Streeter, 1922)**

Mou C, Thomason HA, Willan PM, Clowes C, Harris WE, Drew CF, Dixon J, Dixon MJ, Headon DJ (2008) Enhanced ectodysplasin-A receptor (EDAR) signaling alters multiple fiber characteristics to produce the East Asian hair form. Hum Mutat 29:1405–1411. doi:10.1002/humu.20795

Nakamura M, Ikeda T, Shioya N (1995) Side-to-side earlobe variations with respect to surface area and shape: a quantitative study. Aesthetic Plast Surg 19:561–565

Overfield T, Call EB (1983) Earlobe type, race, and age: effects on earlobe creasing. J Am Geriatr Soc 31:479–481

Park C (1999) Lower auricular malformations: their representation, correction, and embryologic correlation. Plast Reconstr Surg 104:29–40

Park JH, Yamaguchi T, Watanabe C, Kawaguchi A, Haneji K, Takeda M, Kim YI, Tomoyasu Y, Watanabe M, Oota H, Hanihara T, Ishida H, Maki K, Park SB, Kimura R (2012) Effects of an Asian-specific nonsynonymous EDAR variant on multiple dental traits. J Hum Genet 57:508–514. doi:10.1038/jhg.2012.60

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Sabeti PC, Varilly P, Fry B, Lohmueller J, Hostetter E, Cotsapas C, Xie X, Byrne EH, McCarroll SA, Gaudet R, Schaffner SF, Lander ES, Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, Belmont JW, Boudreau A, Hardenbol P, Leal SM, Pasternak S, Wheeler DA, Willis TD, Yu F, Yang H, Zeng C, Gao Y, Hu H, Hu W, Li C, Lin W, Liu S, Pan H, Tang X, Wang J, Wang W, Yu J, Zhang B, Zhang Q, Zhao H, Zhou J, Gabriel SB, Barry R, Blumenstiel B, Camargo A, Defelice M, Faggart M, Goyette M, Gupta S, Moore J, Nguyen H, Onofrio RC, Parkin M, Roy J, Stahl E, Winchester E, Ziaugra L, Altshuler D, Shen Y, Yao Z, Huang W, Chu X, He Y, Jin L, Liu Y, Sun W, Wang H, Wang Y, Xiong X, Xu L, Waye MM, Tsui SK, Xue H, Wong JT, Galver LM, Fan JB, Gunderson K, Murray SS, Oliphant AR, Chee MS, Montpetit A, Chagnon F, Ferretti V, Leboeuf M, Olivier JF, Phillips MS, Roumy S, Sallee C, Verner A, Hudson TJ, Kwok PY, Cai D, Koboldt DC, Miller RD, Pawlikowska L, Taillon-Miller P, Xiao M, Tsui LC et al (2007) Genome-wide detection and characterization of positive selection in human populations. Nature 449:913–918. doi:10.1038/nature06250

Schwartz JH, Tattersall I (2000) The human chin revisited: what is it and who has it? J Hum Evol 38:367–409. doi:10.1006/jhev.1999.0339

Scott GR, Turner CGI (1997) The anthropology of modern human teeth. Cambridge University Press, Cambridge

Tan J, Yang Y, Tang K, Sabeti PC, Jin L, Wang S (2013) The adaptive variant EDARV370A is associated with straight hair in East Asians. Hum Genet 132:1187–1191. doi:10.1007/s00439-013-1324-1

Tan J, Peng Q, Li J, Guan Y, Zhang L, Jiao Y, Yang Y, Wang S, Jin L (2014) Characteristics of dental morphology in the Xinjiang Uyghur and correlation with the EDARV370A variant. Sci China Life Sci 57:1–9

Tenenhaus M, Vinzi VE, Chatelin Y-M, Lauro C (2005) PLS path modeling. Comput Stat Data Anal 48:159–205. doi:10.1016/j.csda.2004.03.005

Turner CG, Nichol CR, Scott GR (1991) Scoring procedures for key morphological traits of the permanent dentition: the Arizona State University dental anthropology system. In: Kelley M, Larsen C (eds) Advances in dental anthropology. Wiley-Liss, New York, pp 13–32

Wu X, Xi HZC (2010) Human body measurements (Chinese Version). Science Press, Beijing

Xu S, Jin L (2008) A genome-wide analysis of admixture in Uyghurs and a high-density admixture map for disease-gene discovery. Am J Hum Genet 83:322–336. doi:10.1016/j.ajhg.2008.08.001

Xu S, Huang W, Qian J, Jin L (2008) Analysis of genomic admixture in Uyghur and its implication in mapping strategy. Am J Hum Genet 82:883–894. doi:10.1016/j.ajhg.2008.01.017

Xu S, Jin W, Jin L (2009) Haplotype-sharing analysis showing Uyghurs are unlikely genetic donors. Mol Biol Evol 26:2197–2206. doi:10.1093/molbev/msp130