Original Research Article

Fibroblast Growth Factor Receptor 1 (*FGFR1*) Variants and Craniofacial Variation in Amerindians and Related Populations

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Objectives: The polymorphic site rs4647905 of the *FGFR1* gene was previously associated with a decrease in cephalic index (CI). Here, we evaluate the relationships between genotypes and cephalometric measurements and indices in one Mexican Native and two mestizo Mexican populations using two haplotype-tag SNPs (rs4647905 and rs3213849) that represent >85% of the *FGFR1* variability, plus three other SNPs (rs2293971, rs2304000, and rs930828) situated nearby. In addition, we genotyped five South American natives, two European, one African, and one Siberian populations to evaluate their intra and intercontinental population diversity.

Methods: The five SNPs were tested and the craniofacial measurements and indices were collected using standardized procedures. Principal Component Analysis was used to verify individual/population comparisons. Associations were performed through the generalized linear model (GLM), coefficient of determination R^2 and linear regression tests.

Results: We found a tendency for a decrease in CI in individuals homozygous for allele rs4647905*C*, regardless of the population to which they belong, though the effect is more pronounced in mestizo. When the GLM analyses were performed using the absolute/linear cephalometric measurements, a statistically significant association was found between four SNPs and head length in the mestizo population.

Conclusions: *FGFR1* polymorphisms, especially rs4647905, can have an important role in the normal human skull variation, primarily due to their influence in head length, which would affect other cephalometric absolute/linear measures as well as indices like CI as a result of the pervasive nature of the morphological integration that characterizes the human skull. Am. J. Hum. Biol. 00:000–000, 2012. © 2012 Wiley Periodicals, Inc.

The understanding about how the genome contributes to determine the phenotype of an organism is considered as one of the most important goals of genetics today (Lee et al., 2008). This task is particularly difficult when complex traits are the subject of investigation since their expressions are controlled by genetic, environmental, mechanical, and epigenetic factors. Morphological traits characterizing the human head perfectly fit into this scenario. At the molecular level, Nei (2007) proposed the so-called "major gene effect hypothesis," according to which morphological evolution would occur by the action of a small number of mutations of large effect on structural or regulatory genes. Several recent studies have revealed that this suggestion is tenable (Chouard, 2010). However, a significant portion of the normal morphological variation can be attributed to several genes of small effect, as well as other non-genetic factors as noted above (Lawson et al., 2006, Roseman et al. 2010). Additionally, genes could have a significant redundant effect, since mutations in different genes could often lead to similar phenotypes (Kim et al., 1998), making the understanding of the genotype–phenotype map even more complicated. For instance, several genes are involved in the development of human craniofacial morphology (Kim et al., 1998; Szabo-

Rogers et al., 2010; Tapadia et al., 2005), but factually nothing is known about their roles in the normal variation found within and between human populations.

Human skull is comprised of two main regions: the neurocranium, formed by the cranial base plus the cranial vault (calvaria), which encapsulates the brain, and the face that surrounds the oral cavity. Unlike of the cranial base development, which is formed by the ossification of a pre-existing cartilaginous matrix (endochondral ossification), vault and face bones are formed by the proliferation and differentiation of multipotent mesenchymal cells into

osteoblasts in a process known as intramembranous ossification (Mooney et al., 2002; Sperber, 2001, 2002). Particularly important in the skull development is the closing of the sutures, the fibrous joints that connect the bones of the vault and face.

Sutures are formed during the embryonic stage at the sites linking the craniofacial membranous bones, which act as the main nuclei of bone expansion during the vault and facial complex pre and postnatal development (Opperman, 2000). Growth maintenance at the sutures osteogenic fronts requires a refined balance between proliferation and differentiation, to ensure appropriate changes in shape and growth during the skull development. Imbalance in these mechanisms result in premature closing of the sutures, leading to some different normal patterns (for instance, brachycephaly is characterized by the premature fusion of the coronal suture, while dolichocephaly is due to the premature fusion of the sagittal suture) or pathological conditions known as craniosynostoses (Morriss-Kay and Wilkie 2005; Levi et al. 2012).

Several investigations on animal models, as well as medical, evolutionary and *in vitro* research have revealed the role of *FGF/FGFR* genes in vertebrate development (Bobick et al., 2007; Eames and Schneider, 2008; Muenke et al, 1994; Rice et al., 2003; Tapadia et al., 2005; Wilkie, 1997). The importance of FGF/FGFR signaling in human craniofacial development has also been suggested after identification of mutations in *FGFR* genes in patients with craniosynostosis syndromes, some of them involving multiple sutures (Cooper, 1999). A large number of mutations have been related to other pathologies associated to closure/fusion suture patterns, leading to a wide range of abnormal craniofacial morphologies. Recently, Martinez-Abadías et al. (2011) studied the *FGFR2* gene using mouse models affected by a craniosynostosis (Apert syndrome), and proposed that cell communication and cell interactions that are influenced by FGF/FGFR signaling underlie basic developmental processes coordinating head morphogenesis and contribute to the coordinated growth and development of a functional and operational head.

The influence of *FGFR* mutations on normal craniofacial variation was studied only once in humans. Coussens and Dall (2005) sequenced the entire *FGFR1* gene in healthy individuals from four human groups: African-American, Asian, Caucasian, and Australian Aborigines. A total of 17 SNPs were identified, but only eight of them showed high variation between the four investigated populations. Nine common haplotypes could be inferred from these eight SNPs, three being the most common in all populations. Coussens and Dall (2005) also identified two haplotype tag SNPs (rs4647905 and rs3213849) responsible for >85% of the diversity in each population, concluding that these two SNPs could be very useful for population and association studies. They also showed that the rs4647905C allele is associated to a decrease in the cephalic index (CI; the ratio, in percentage, of the maximum breadth to the maximum length of a skull). Note that CI > 81 are normally associated to brachycephalous skulls, and CI < 75.9 with dolicocephalic forms (Coussens and Van Daal, 2005; and references therein).

Allele frequencies can significantly vary among human populations. For instance, a genetic variant present in Asians may be absent in their derived populations such as Native Americans or vice-versa (Acuña-Alonzo et al., 2010; Hünemeier et al., 2012a; Schroeder et al., 2009).

TABLE 1. Haplotype frequencies distribution among populations

| | | | Frequency per population (number of chromosomes) | | | | | | | | | | |
| | | | | | | Native American | | | | | | Mexican Mestizo | |
ID	Haplotype[a]	Frequency	African (52)	Eskimo (50)	European (78)	Kayapo (44)	Xavante (38)	Baniwa (40)	Kaingang (48)	Yanomama (44)	Totonaco (166)[b]	Tepango (24)[b]	Mexico city(82)[b]
h1	GCGCA	0.555556 (370)	0.673 (35)	0.660 (33)	0.538 (42)	0.659 (29)	0.816 (31)	0.675 (27)	0.646 (31)	0.568 (25)	0.470 (78)	0.458 (11)	0.341 (28)
h2	CCGCA	0.012012 (08)	0.077 (04)	0.040 (02)	0.026 (02)	0	0	0	0	0	0	0	0
h3	GCGTG	0.177177 (118)	0.173 (09)	0.180 (09)	0.154 (12)	0.023 (01)	0.105 (04)	0.200 (08)	0.083 (04)	0.182 (08)	0.181 (30)	0.042 (01)	0.390 (32)
h4	CGACA	0.171171 (114)	0.038 (02)	0.040 (02)	0.179 (14)	0.273 (12)	0	0.050 (02)	0.271 (13)	0.227 (10)	0.205 (34)	0.292 (07)	0.220 (18)
h5	GGGCA	0.003003 (02)	0.038 (02)	0	0	0	0	0	0	0	0	0	0
h6	GCGTA	0.027027 (18)	0	0.040 (02)	0	0	0.079 (03)	0	0	0	0.0542 (09)	0.167 (04)	0
h7	GCGCG	0.009009 (06)	0	0	0	0	0	0.050 (02)	0	0.0227 (01)	0.012 (02)	0.042 (01)	0
h8	GGACA	0.012012 (08)	0	0.040 (02)	0.051 (04)	0.045 (02)	0	0	0	0	0	0	0
h9	CCGTG	0.006006 (04)	0	0	0.051 (04)	0	0	0	0	0	0	0	0
h10	CGGCA	0.006006 (04)	0	0	0	0	0	0	0	0	0	0	0.049 (04)
h11	CGATA	0.010511 (07)	0	0	0	0	0	0	0	0	0.0422 (07)	0	0
h12	CGATG	0.003003 (02)	0	0	0	0	0	0	0	0	0.012 (02)	0	0
h13	CGGTG	0.003003 (02)	0	0	0	0	0	0.025 (01)	0	0	0.006 (01)	0	0
h14	GCACA	0.001502 (01)	0	0	0	0	0	0	0	0	0.006 (01)	0	0
h15	CCACA	0.003003 (02)	0	0	0	0	0	0	0	0	0.012 (02)	0	0

[a]Loci order: rs4647905 rs2304000 rs2293971 rs32138849 rs930828.
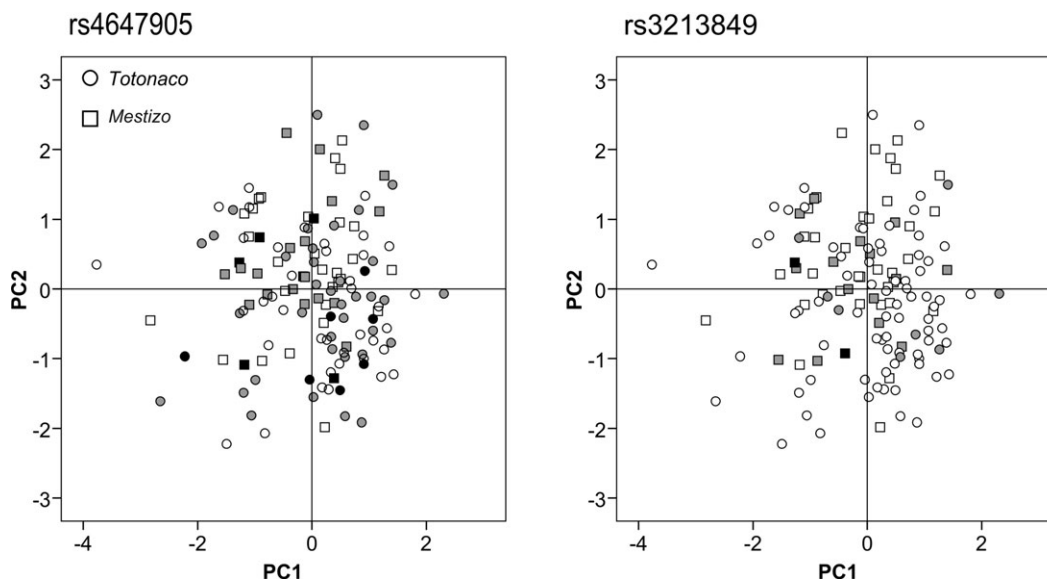[b]Populations with cephalometric and *FGFR1* data available.

Fig. 1. First two principal components depicting the variation in the sex and size standardized cephalometric variables (cumulative variances: PC1 = 46.75%; PC2 = 11.8%). PC1 is mainly explained by variation on head breadth, head length, head height, and bizygomatic breadth; PC2 is mainly explained by changes on facial length and nose breadth. Populations are identified by symbols and genotypes by colors: unfilled correspond to homozygotes from the ancestral allele, heterozygotes are in gray and filled black symbols correspond to the homozygotes from the derived allele.

Furthermore, linkage disequilibrium (LD; a condition in which the haplotype frequencies in a population deviate from the values they would have if the allele at each locus were combined at random) levels found in one population cannot be extrapolated to others.

Africans generally have lower LD levels (Lonjou et al., 2003), whereas Native Americans have high LD values. This happens for several reasons related to the evolutionary history of each population (Amorim et al., 2011). On the other hand, the gene pool of mestizo Latin Americans is characterized by different levels of contribution of their continental ancestral groups, Native American, European and African (Salzano and Bortolini, 2002), resulting in diverse LD patterns (Amorim et al., 2011). Furthermore it is important to emphasize that a SNP variant could be associated with a phenotype not because it is biologically functional, but because it is in LD with the causal allele. Based on these characteristics, LD patterns should be always considered in association studies.

The main objective of our work is to evaluate the contribution of the *FGFR1* polymorphisms to normal craniofacial morphology variation. More specifically we aim to test if the instigating Coussens and Dall's (2005) findings are observable on other human populations as well. We first studied the *FGFR1* alleles/haplotypes distributions in 136 Native American and Mexican mestizo individuals, to detect possible associations between *FGFR1* variants and craniofacial measurements or indices. Additionally, *FGFR1* was studied in 197 other native peoples from different continents (Africa, Asia, Europe, and America) to expand our knowledge about its distribution. The present investigation is related to a long-range project of our group which investigates the relationships between genetic variants and morphological traits (Hünemeier et al., 2009, 2010, 2012b; Paixão-Côrtes et al., 2011; Pereira et al., 2006).

SUBJECTS AND METHODS
Populations

Craniofacial and *FGFR1* data were obtained from 136 individuals from a Native American Totonaco population of Sierra Norte de Puebla ($n = 83$), as well as mestizo individuals from Mexico City ($n = 41$) and Tepango ($n = 12$). To evaluate the *FGFR1* haplotype distributions in other human populations, genetic data were also obtained from 107 Native Americans of five populations (Kayapo, Kaingang, Xavante, Yanomama, and Baniwa), 39 Europeans (Spaniards), 26 South Saharan Africans, and 25 Siberian Eskimo, based on samples already collected by our team and collaborators.

All Mexican subjects provided written informed consent and the study was approved by the Faculty of Medicine Research and Ethics Committee of the National Autonomous University of Mexico (Project number 008-2010). Local authorities gave their approval for the study, and a translator was used as needed. Additional ethical approval was also provided by the Brazilian National Ethics Commission (CONEP Resolution no. 123/98) for the Brazilian and Siberian Eskimo samples, as well as by the ethics committees of: (a) Hôpital Robert Debré, Paris, France (African samples); and (b) Universidad de Antioquia, Medellin, Colombia (European samples). For illiterate volunteers, individual and tribal informed oral consents were obtained according to the Helsinki Declaration. The ethics committees approved both, oral and written informed consent procedures as well as the use of these samples in population and evolutionary studies.

Laboratory tests

Two SNPs (rs4647905G/C and rs3213849C/T) which represent >85% of the *FGFR1* gene haplotype variability

TABLE 2. Association of FGFR1 polymorphism and cephalic indices in Mexican populations

Population		CI		TI		PI		JMI		JFI		CFI		NI		NFI		VCI		TCI	
		F	P value	F	P value	F	P value	F	P value	F	P value	F	P value	F	P value	F	P value	F	P value	F	P value
Mestizo	rs4647905	2.051	0.1392	0.763	0.4716	0.788	0.4603	0.338	0.7150	0.495	0.6126	0.464	0.6315	0.104	0.9015	0.566	0.5713	4.424	*0.0170*	0.315	0.7310
	rs2304000	2.118	0.1309	0.784	0.4621	1.382	0.2605	0.364	0.6969	0.551	0.5797	0.676	0.5133	0.172	0.8422	0.551	0.5799	4.670	***0.0138***	0.348	0.7079
	rs2293971	0.536	0.5886	1.586	0.2150	3.064	0.0556	0.557	0.5762	1.683	0.1962	0.673	0.5145	0.517	0.5992	0.780	0.4640	3.891	*0.0269*	1.746	0.1849
	rs3213849	3.157	0.0511	0.084	0.9197	0.565	0.5718	0.247	0.7824	0.510	0.6038	0.209	0.8120	0.000	0.9996	0.603	0.5511	0.309	0.7356	6.789	***0.0025***
	rs930828	3.157	0.0511	0.084	0.9197	0.565	0.5718	0.247	0.7824	0.510	0.6038	0.209	0.8120	0.000	0.9996	0.603	0.5511	0.309	0.7356	6.789	***0.0025***
Totonaco	rs4647905	0.209	0.8115	0.963	0.3860	0.979	0.3800	0.153	0.8581	0.397	0.6739	0.465	0.6299	1.149	0.3221	0.667	0.5160	0.640	0.5298	0.661	0.5191
	rs2304000	0.209	0.8115	0.963	0.3860	0.979	0.3800	0.153	0.8581	0.397	0.6739	0.465	0.6299	1.149	0.3221	0.667	0.5160	0.640	0.5298	0.661	0.5191
	rs2293971	0.209	0.8115	0.963	0.3860	0.979	0.3800	0.153	0.8581	0.397	0.6739	0.465	0.6299	1.149	0.3221	0.667	0.5160	0.640	0.5298	0.661	0.5191
	rs3213849	2.227	0.1395	0.001	0.9774	0.210	0.6478	0.033	0.8569	0.000	0.9848	0.004	0.9487	0.003	0.9534	0.298	0.5866	1.313	0.2552	0.239	0.6259
	rs930828	2.227	0.1395	0.001	0.9774	0.210	0.6478	0.033	0.8569	0.000	0.9848	0.004	0.9487	0.003	0.9534	0.298	0.5866	1.313	0.2552	0.239	0.6259
Overall	rs4647905	1.907	0.1525	0.450	0.6383	0.940	0.3933	0.322	0.7251	0.266	0.7668	0.122	0.8850	0.293	0.7463	0.959	0.3857	4.219	*0.0167*	1.170	0.3136
	rs2304000	1.917	0.1511	0.411	0.6638	1.073	0.3449	0.354	0.7026	0.314	0.7308	0.109	0.8969	0.454	0.6361	0.988	0.3749	4.214	*0.0168*	1.139	0.3231
	rs2293971	0.737	0.4804	0.656	0.5208	2.327	0.1015	0.456	0.6348	0.500	0.6077	0.052	0.9492	0.795	0.4538	1.004	0.3691	3.334	*0.0387*	1.750	0.1778
	rs3213849	3.328	***0.0389***	0.069	0.9329	0.670	0.5134	0.336	0.7154	0.349	0.7058	0.256	0.7748	1.251	0.2897	1.039	0.3566	0.384	0.6816	2.193	0.1156
	rs930828	3.328	***0.0389***	0.069	0.9329	0.6700	0.5134	0.336	0.7154	0.349	0.7058	0.256	0.7748	1.251	0.2897	1.039	0.3566	0.384	0.6816	2.193	0.1156

Generalized linear models (GLM) results are presented after cephalic measures were corrected for sex and size. Any significant ($P < 0.05$) results are marked in bold and italics. CI = cephalic index, TI = transverse frontoparietal index, PI = prosopic index, JMI = jugomandibular index, JFI = jugofrontal index, CFI = transverse cephalofacial index, NI = nasal index, NFI = nasofacial index, VCI = vertical cephalic index, TCI = transverse cephalic index.

found by Coussens and van Daals (2005), plus three other SNPs (rs2293971 G/A, rs2304000 G/C, and rs930828 A/G) situated nearby were investigated using TaqMan assays (which use probes designed to anneal within a DNA region amplified by a specific set of primers; Applied Biosystems). Haplotype phases, which indicate the allele combination of different loci in the same chromosome, were inferred with BEAGLE 3.3.2 (Browning and Browning, 2007) and linkage disequilibrium (LD) analysis was performed with the Haploview 4.1 software (Barrett et al., 2005).

Craniofacial measurements and indices

Nine craniofacial absolute/linear measures (head length, head breadth, head height, minimum frontal breadth, bizygomatic breadth, bigonial breadth, nose length, nose breadth, and facial length) were obtained using sliding and spreading calipers. To minimize errors, these anthropometric measurements were obtained according to international standards and by one researcher only (JAGV). Based on these absolute/linear measurements, ten standard craniofacial indices were calculated, as indicated by Comas (1983) (Supporting Information Table 1). All measurement values were standardized to account for sex and size differences following the procedures described in Ackerman et al. (2006) and Jungers, et al. (1995), respectively.

Data analysis

Mahalanobis squared distances were used to estimate morphological differences among samples coming from the Totonaco, Mexico City, and Tepango populations. In addition, cephalometric variation among individuals/populations was explored using principal component analysis (PCA) based on the above indicated nine absolute/linear measurements previously standardized to remove sex and size effects. PCA allows the reduction of the metric data to a smaller number of dimensions and the correlation of each original variable on the successive principal components can provide clues regarding regional differences between cranial (facial and vault) modules. Furthermore, standardized variables were employed to build cephalometric indices and to perform multivariate and association analysis. Association between common SNPs and craniofacial phenotypes was also tested using a generalized linear model (GLM), assuming an additive model, separately by population data and considering overall groups. The F statistics, which determines the likelihood ratio of the explained variance by the residual sum of squares was also calculated. The GLM analysis was performed independently on the set of nine craniofacial absolute/linear measurements and on the set of ten craniofacial indices generated from them (Supporting Information Table 1). These ten indices depict general aspects of head shape as well as some specific facial and vault structures. The proportion of variability due to SNP variation on the craniometric indices was tested by the coefficient of determination R^2. Finally, bar and regression graphics representing population mean indices values were obtained. All the statistical analysis was carried out using SPSS (v. 17.0) (www.spss.com).

RESULTS

Table 1 presents the *FGFR1* haplotype frequencies in African, European, Eskimo, South and Mesoamerican
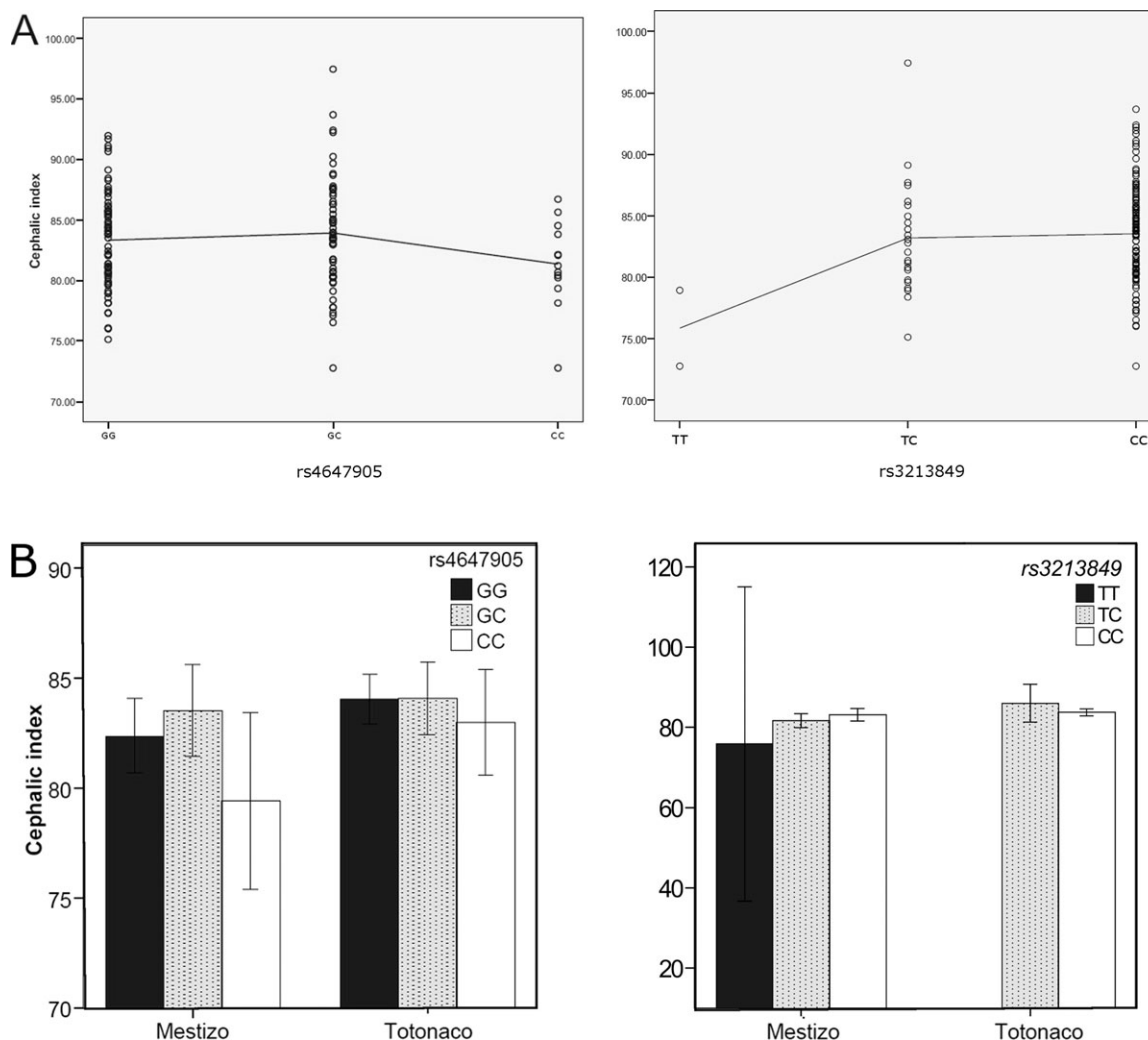
Fig. 2. A. Linear regression between rs4647905 and rs3213849 genotypes and CI considering Totonaco and mestizo together. B. Bar graphs showing the relationship between rs4647905 and rs3213849 genotypes and CI by population.

Native populations, as well as in two Mexican mestizo populations. Fifteen haplotypes were found, and four of them (h1, h3, h4, and h6) exhibited general prevalences of 3–55%, while the remaining eleven displayed frequencies ≤1.2%. The most frequent haplotype, h1 was present in all samples. South Amerindians had an average number of h1 (67%) practically identical to that of Africans and Eskimo. However, one Native Mexican population (Totonaco) showed a much lower frequency (47%), similar to that of Tepango (46%), while Mexicans from Mexico City and Europeans showed values of 34 and 54%, respectively. The second and third most frequent haplotypes (h3 and h4) also do not show very distinctive interethnic differences, but h6 is only found in Eskimos, Amerindians or the mestizos from Tepango. Haplotypes h11, h12, h14; h15 are observed only among the Totonaco; and h7 is detected only on two South Amerindian tribes (Xavante, Baniwa).

When Europeans, Native Americans, Eskimos, Africans, and mestizo populations were compared for LD patterns, the two previously LD blocks found by Coussens and van Daal (2005) were detected in Europeans and in all Mexican populations. South Amerindians, on the other hand, show just one LD block, while Africans do not present a clear pattern (Supporting Information Fig. 1), as expected due to the particular and distinct demographic and evolutionary history of these groups (Amorim et al., 2011). When only the Mexicans are considered, two similar LD blocks are observed in both, natives (Totonaco) and mestizos (Mexico City and Tepango), corroborating the suggestion that rs4647905 and rs3113849 are two haplotype-tag SNPs (Coussens and van Daals, 2005).

The squared Mahalanobis distances computed on the full space of variation evidenced a significant craniofacial difference ($P < 0.05$) between mestizo Mexicans (Mexico city and Tepango) and Totonaco, while no significant distance was obtained between the two mestizo groups. We therefore performed the following analyses considering just two population sets, mestizo (composed by Tepango plus Mexico City) and natives (Totonaco).

Figure 1 show that the first two principal component account for ~59% of the total variation on the sex and size standardized cephalometric measurements, and that only

TABLE 3. *Association of FGFR1 polymorphism and cephalic measures in Mexican populations*

Population		GOL		XCB		VTH		MFB		ZYB		GOB		NLH		NLB		NGH	
		F	P value	F	P value	F	P value	F	P value	F	P value	F	P value	F	P value	F	P value	F	P value
Mestizo	rs4647905	5.511	**0.0069**	0.017	0.9836	0.522	0.5968	1.162	0.3210	0.597	0.5543	0.561	0.5744	0.197	0.8218	0.361	0.6991	1.041	0.3607
	rs2304000	5.796	**0.0054**	0.010	0.9904	0.545	0.5834	1.185	0.3141	0.953	0.3923	0.822	0.4452	0.494	0.6133	0.346	0.7095	1.390	0.2585
	rs2293971	2.352	0.1056	0.154	0.8580	1.291	0.2840	1.463	0.2414	1.563	0.3128	1.563	0.2195	0.559	0.5754	0.605	0.5499	2.582	0.0857
	rs3213849	3.467	**0.0389**	1.016	0.3693	3.603	**0.0345**	0.187	0.8299	3.013	0.0581	1.439	0.2467	0.305	0.7385	0.292	0.7483	0.419	0.6601
	rs930828	3.467	**0.0389**	1.016	0.3693	3.603	**0.0345**	0.187	0.8299	3.013	0.0581	1.439	0.2467	0.305	0.7385	0.292	0.7483	0.419	0.6601
Totonaco	rs4647905	0.625	0.5378	0.108	0.8976	0.341	0.7123	1.043	0.3572	0.102	0.9032	0.082	0.9211	0.382	0.6836	0.785	0.4596	1.883	0.1588
	rs2304000	0.625	0.5378	0.108	0.8976	0.341	0.7123	1.043	0.3572	0.102	0.9032	0.082	0.9211	0.382	0.6836	0.785	0.4596	1.883	0.1588
	rs2293971	0.625	0.5378	0.108	0.8976	0.341	0.7123	1.043	0.3572	0.102	0.9032	0.082	0.9211	0.382	0.6836	0.785	0.4596	1.883	0.1588
	rs3213849	1.362	0.2467	0.903	0.3449	0.103	0.7496	0.770	0.3828	0.631	0.4292	0.187	0.6670	0.329	0.5676	0.373	0.5429	0.002	0.9640
	rs930828	1.362	0.2467	0.903	0.3449	0.103	0.7496	0.770	0.3828	0.631	0.4292	0.187	0.6670	0.329	0.5676	0.373	0.5429	0.002	0.9640
Overall	rs4647905	5.146	**0.0070**	0.043	0.9579	0.971	0.3813	0.473	0.6243	0.098	0.9063	0.415	0.6614	0.131	0.8773	0.289	0.7498	2.364	0.0980
	rs2304000	5.215	**0.0066**	0.049	0.9525	0.925	0.3990	0.417	0.6597	0.137	0.8719	0.581	0.5609	0.303	0.7393	0.326	0.7223	2.469	0.0886
	rs2293971	2.759	0.0670	0.180	0.8355	1.156	0.3180	0.445	0.6415	0.118	0.8891	0.942	0.3924	0.419	0.6589	0.589	0.5564	3.667	**0.0282**
	rs3213849	4.842	**0.0093**	0.774	0.4633	1.012	0.3661	0.169	0.8448	1.738	0.1799	1.257	0.2880	0.951	0.3891	0.481	0.6194	0.209	0.8118
	rs930828	4.842	**0.0093**	0.774	0.4633	1.012	0.3661	0.169	0.8448	1.738	0.1799	1.257	0.2880	0.951	0.3891	0.481	0.6194	0.209	0.8118

Generalized linear models (GLM) results are presented after cephalic measures were corrected for sex and size. Any significant ($P < 0.05$) results are marked in bold and italics. GOL = head length (g-op), XCB = head breadth (eu-eu), VTH = head height (v-t), MFB = minimum frontal breadth (ft-ft), ZYB = bizygomatic breadth (zy-zy), GOB = bigonial breadth (go-go), NLH = nose length (n-sn), NLB = nose breadth (al-al) and NGH = facial length (n-gn).

six of them (head breadth, head length, head height, bizygomatic breadth, facial length, and nose breadth) are correlated to these first two PCs. No clear pattern, however, was found between these traits and the populations or genotypes tested.

No association between rs4647905 and the cephalic index (CI) was found, in opposition to the results reported by Coussens and van Daal (2005), (Table 2). However, there is a tendency in both populations for a decrease in cephalic index in individuals homozygous for allele *C* (Fig. 2A, B). The only association found considering rs4647905 (as well as the two other polymorphisms in LD with it, rs2304000 and rs2293971) was with the vertical cephalic index (VCI) in the mestizo group (Table 2). Polymorphisms rs3213849 and rs930828, on the other hand, showed a statistically significant association with the transversal cephalic index (TCI) in the mestizo population. The above-described association between the rs4647905 and VCI is maintained when both groups are analyzed together, which is not true for the two others and TCI. Interestingly, when the two populations are considered together, we found a statistically significant association between rs3213849 and CI (lower CI values in the presence of the *T* allele; Table 2; Fig. 2A, B). Note that no association was found in the Totonaco native population.

When the GLM analyses were performed using the nine absolute/linear cephalometric measurements, a statistically significant association was found between four (rs4647905, rs2304000, rs3213849, and rs930828) of the five polymorphisms tested and head length in the mestizo population, as well as an association between two markers (rs3213849 and rs930828) and head height in the same population (Table 3). The association between head length and the four first above-mentioned polymorphisms is kept when the two groups (mestizo plus natives) are evaluated together. Again, no association was found in the Totonaco Native American population. Furthermore, the coefficient of determination (R^2 values) indicates a high contribution of rs4647905 (15%), rs2304000 (15%), rs3213849 (9%), and rs930828 (9%) SNPs on the determination of head length in mestizos.

DISCUSSION

Because *FGFs/FGFRs* have a crucial role in the development of the craniofacial structures, it is valid to suppose that some variants in these genes can be associated to the normal variation found in human populations. As already mentioned, Coussens and van Daal (2005) performed the first study searching for such putative associations, and reported that in European and Asian individuals the rs4647905*C* allele was associated with a decrease of the cephalic index. Although our results showed no significant association between CI and the rs4647905 SNP, we noted a reduction of the cephalic index in homozygote individuals carrying this allele. Moreover, it seems that there is a tendency for rs4647905*CC* subjects to present a transversely narrow and elongated antero-posterior head (dolicocephaly). This effect is seen in the mestizo group, while in the indigenous Totonaco this trend is less pronounced. These results are further corroborated by the association analyses, which shows that in the mestizo group the association between rs4647905 and length of the head was highly significant ($P = 0.007$). These results suggest that the effect of this SNP in the morphogenesis of the head is related
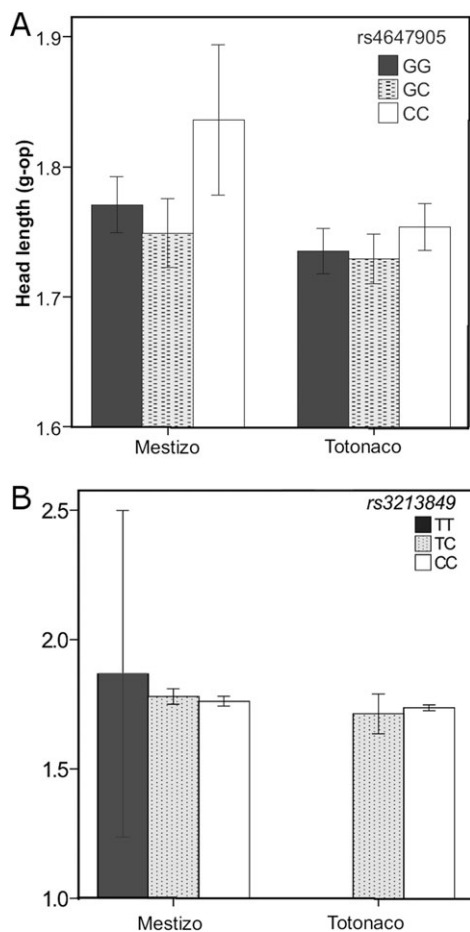
Fig. 3.   Bar graphs showing the relationship between rs4647905 (A) and rs3213849 (B) genotypes and length of the head by population.

mainly to the length of the cranial vault since individuals carrying the *CC* genotype have a head length dramatically increased in the antero-posterior way (Fig. 3). Moreover, we can see that the rs3213849 SNP also present an effect on the cephalic index, CI decreases with the presence of the rs3213849*T* allele, probably also related to its influence in the absolute length of the head (Figs. 2 and 3). Based on these results it is possible to suggest that this allelic combination promote a premature closure of the sagittal suture.

In conclusion, we observed that the common *FGFR1* polymorphisms studied here can have an important role in the normal human skull morphological variation, primarily due to their influence in head length, which would affect other cephalometric absolute/linear measures as well as indices like CI as a result of the pervasive nature of the skull's morphological integration (Martínez-Abadias et al., 2011). Our data revealed that some *FGFR1* variants, especially rs4647905, can have a major effect on normal human morphological variation corroborating Nei's (2007) proposition. However, only functional studies may reveal whether the SNPs studied here are causal or if they are in LD with unknown, true causal variants. The importance of considering population parameters in studies of this nature is clear, due to the differences found between the Mestizo and Native American populations, as

well as those studied by Coussens and van Daal (2005). Although some of these populations present the same two LD blocks and other similarities considering the *FGFR1* SNPs studied here, we cannot rule out that variants in other genes associated with craniofacial development may affect the results, since other differences in the genetic backgrounds of Mestizo and Native continental populations are expected.

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