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ARTICLE

Association between the *FGFR1* rs13317 single nucleotide polymorphism and orbitale-nasion depth based on cephalometric images

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Abstract

The fibroblast growth factor receptor 1 (*FGFR1*) gene plays an important role in craniofacial morphogenesis. In our previous study, an association between *FGFR1* single nucleotide polymorphisms (SNPs) and craniofacial morphology was demonstrated in Japanese and Korean subjects. The present study aimed to evaluate the relationship between a common *FGFR1* SNP (rs13317) with craniofacial morphology, increasing the number of measurements and examining Egyptian subjects ($n = 191$) in addition to the Japanese ($n = 211$) and Korean ($n = 226$) subjects. Genotyping for rs13317 was performed using the TaqMan assay, and its associations with 81 craniofacial measurements derived from lateral and posteroanterior cephalograms were analyzed by multiple regression analysis controlling sex and facial size. The results from each of the populations were then statistically combined. In the Egyptian subjects, rs13317 was significantly associated with the nasion-orbitale depth ($P = 0.00040$), and a suggestive association was also observed in the Japanese ($P = 0.037$) and Korean subjects ($P = 0.045$). The combined analysis revealed that only the nasion-orbitale depth showed a significant association ($P = 0.000062$) and that several measurements showed a suggestive association. Our results strongly indicate that rs13317 is associated with a smaller depth between the nasion and orbitale, representing a relative protrusion of the cheekbones and retrusion of the nasal root. A similar characteristic is also observed in individuals with Pfeiffer syndrome, which is caused by a dysfunctional *FGFR1* mutation.

Introduction

Individual human facial features are distinctive and unique, and are key to identification and social interactions. Previous studies on the development of the human face have identified strong links between genetics and the patterning of facial traits, although environmental factors also play an important role [1, 2]. The heritability of human facial morphology has long intrigued researchers, indicating its multifactorial nature.

Growth of the cranial vault occurs by intramembranous ossification at the fibrous joints between the bones, known as cranial sutures. The developmental biology of this process is not yet fully understood. However, genetically determined disorders of premature cranial suture fusion, termed craniosynostosis, provide a means of identifying some of the factors involved [3]. Pfeiffer syndrome results from dysfunctional mutation in the fibroblast growth factor receptor 2 (*FGFR2*) gene or the immunoglobulin-like

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domain II–III linker region of *FGFR1* [4]. *FGFR1*, which is located on chromosome 8p11.1, contains 19 exons spanning 55 kb, and encodes at least nine isoforms of FGFR1 [5]. FGFR1 is involved in FGR/FGFR signaling, and functions in multiple biological processes including mesoderm induction and patterning, cell growth and migration, organogenesis, and bone growth [6]. During embryonic development, its signaling causes immature cells to become bone cells. *FGFR1* mutations alter the protein function and cause prolonged signaling, which can promote the premature fusion of cranial and/or craniofacial sutures [7]. Early fusion that occurs before growth of the underlying structures has been completed prevents the skull from growing normally, and affects the shape of the head and midface [4, 8, 9].

In many genetic disorders, a clear association is observed between genetic alterations and facial anomalies. It is also likely that genetic polymorphisms in genes involved in genetic disorders may also contribute to normal facial variation [10]. It is thus important to study the link between pathological and normal variations. Relatively few studies have described the association between *FGFR1* single nucleotide polymorphisms (SNPs) and normal phenotypic variation of the craniofacial complex [7, 11–14]. However, several have suggested the possibility of a relationship between *FGFR1* variants and the incidence of non-syndromic cleft lip with or without cleft palate (NSCL/P). Cephalometric studies revealed unique facial characteristics, particularly in the mid-facial region, in individuals affected by NSCL/P and even unaffected relatives [15–17]. This could be considered evidence for the role of *FGFR1* SNPs on the patterning of human craniofacial morphogenesis. Our previous study of lateral and posteroanterior cephalograms obtained from Japanese and Korean subjects showed that *FGFR1* SNPs were associated with a shape component representing midface protrusion/recession and the wideness of cheekbones and orbits [18]. Therefore, in this study, we examined the association between the *FGFR1* rs13317 SNP and craniofacial measurements by adding healthy Egyptian subjects and increasing the number of measurements to confirm the effect of *FGFR1* polymorphisms on normal variation in craniofacial morphology.

Table 1 Landmarks of cephalograms

Cephalogram	Landmark	Abbreviation
PA	Roof of orbit (R, L)	RO
	Orbitale (R, L)	Or
	Latero orbitale (R, L)	LO
	Zygion (R, L)	Zy
	Zygomatic arch (R, L)	ZA
	Condylion (R, L)	Cd
	Koronion (R, L)	Ko
	Mastoidare (R, L)	Ma
	Gonion (R, L)	Go
	Antegonion (R, L)	Ag
	Nasal cavity (R, L)	NC
	Neck of crista galli	Cg
	Anterior nasal spine	ANS
	Menton	Me
LAT	Glabella	G
	Nasion	N
	Rhinion	Rh
	Orbitale	Or
	Key ridge	KR
	Pterygomaxillary fissure	Ptm
	Anterior nasal spine	ANS
	Posterior nasal spine	PNS
	Sella	S
	Condylion	Cd
	Porion	Po
	Articulare	Ar
	Gonion	Go
	basion	Ba
	Point A	A
	Point B	B
	Incision superius	Is
	Incision inferius	Ii
	Apex superius	As
	Apex inferius	Ai
	Gnathion	Gn
	Pogonion	Pog
	Retrognathion	RGn
	Menton	Me
	Occlusal plane	
	Frankfurt plane	

PA posteroanterior, *LAT* lateral, *R* right, *L* left

Materials and methods

Subjects

This study was approved by the Ethics Committee and other related committees of the Suez Canal University Faculty of Dentistry, Showa University Dental Hospital, and the University of the Ryukus. Egyptian participants were patients at the clinic of the Orthodontic Department, Faculty of Dentistry, Suez Canal University (Ismailia, Egypt) or volunteers made up of students and workers at the Faculty of Dentistry; all the participants provided written informed consent. Craniofacial data and saliva specimens were obtained from 191 Egyptian subjects (92 males and 99 females). The age range was 18–55 years (mean age, 22.4 years ± standard deviation (SD) 4.3 for both males and females). Inclusion

Table 2 Summary of the distance measurements obtained from posteroanterior cephalograms

Category	Measurement	Egyptians ($n = 191$)				Japanese ($n = 211$)				Koreans ($n = 226$)			
		Male ($n = 92$)		Female ($n = 99$)		Male ($n = 42$)		Female ($n = 169$)		Male ($n = 132$)		Female ($n = 94$)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Height, PA	RO (R)–Or (R) (*y*)	42.08	5.24	41.39	5.37	42.03	19.33	41.64	18.46	42.08	6.93	40.83	4.89
	RO (L)–Or (L) (*y*)	41.58	5.31	41.53	5.64	42.32	19.45	41.98	18.60	41.99	6.85	40.79	4.78
2D distance, PA	LO (R)–LO (L)	95.83	4.34	92.80	5.31	99.92	45.77	97.28	42.93	102.36	15.73	98.70	10.73
	Or (R)–Or (L)	70.00	4.12	66.66	3.60	76.33	35.13	73.26	32.57	78.96	13.35	75.75	10.95
	Zy (R)–Zy (L)	136.09	34.34	125.88	13.93	138.02	63.26	132.38	58.41	147.76	25.93	139.15	15.16
	ZA (R)–ZA (L)	139.81	35.14	129.14	14.08	145.33	66.63	139.34	61.48	154.34	35.11	145.59	15.67
	Cd (R)–Cd (L)	110.54	12.58	103.64	4.26	114.17	52.43	111.48	49.26	124.36	19.87	117.73	13.36
	Ko (R)–Ko (L)	99.40	11.62	93.35	4.77	109.94	50.58	107.61	47.64	114.51	18.37	108.49	12.51
	Ma (R)–Ma (L)	119.22	21.98	111.62	31.12	123.82	56.69	118.81	52.47	130.40	20.23	123.68	13.87
	Go (R)–Go (L)	103.12	5.74	94.75	5.68	111.13	51.03	105.29	46.59	118.26	18.96	110.28	12.71
	Ag (R)–Ag (L)	90.89	5.35	85.42	5.05	97.18	44.61	93.21	41.18	101.12	15.96	96.80	10.89
	Cg–ANS	41.48	3.68	38.27	3.50	44.16	20.43	41.53	18.52	44.35	7.40	41.27	5.58
	NC (R)–NC (L)	31.83	3.02	29.89	2.79	36.76	17.03	36.09	16.10	37.67	7.78	35.53	4.54
	ANS–Me	71.53	11.94	67.34	5.21	74.07	34.85	68.76	30.58	71.63	11.89	67.97	8.48

criteria were: unrelated individuals, no congenital disorders such as cleft palate or general physical disease, and no previous orthodontic or orthopedic treatment. Korean (132 males and 94 females) and Japanese (42 males and 169 females) subjects were healthy volunteers recruited from Pusan and patients from Tokyo who underwent orthodontic treatment at an orthodontic clinic in Showa Dental Hospital, respectively. The Japanese and Korean subjects were the same as those used in the previous study and details were described elsewhere [18].

Genotyping

Saliva was used as the source of DNA from all subjects. The Oragene DNA Kit (DNA Genotek, Kanata, ON, Canada) was used for saliva collection, storage, and DNA purification according to the manufacturer's recommendations. The *FGFR1* SNP rs13317 (A/T) was genotyped using the TaqMan genotyping assay (Life Technologies, Carlsbad, CA, USA), and the frequency of the derived allele (T) in the subjects was determined.

Craniofacial measurements

Craniofacial morphology was analyzed by plotting facial landmarks on posteroanterior and lateral cephalograms using the ImageJ software (version 1.48; National Institute of Health, Bethesda, MD, USA) (Table 1). On the lateral cephalogram, points on the overlapping bilateral structures were identified by establishing a middle point between the two structures. The Frankfurt plane was defined by Or–Po, and the occlusal plane was also defined by the midpoint of Ii and Is and the occlusal point of first molars. We defined *x*-axis (horizontal) and *y*-axis (vertical) in the posteroanterior cephalogram and *z*-axis (horizontal) and *y*-axis (vertical) in the lateral cephalogram. Two-dimensional Euclidean distances and angles between landmarks were calculated based on their coordinates (Tables 2–4). Only *y* coordinates were used in the calculation of the height values (vertical distances; e.g., RO (R)–Or (R) (*y*)), whereas only *z* coordinates were used for the depth values (horizontal distances in the lateral cephalograms; e.g., N–Or (*z*)). In total, 81 measurements were obtained. Additionally, the geometric mean (GM) of facial height, depth, and breadth, $GM=[(N-Pg)\times(N-Cd)\times(Zy-Zy)]^{1/3}$, was calculated to evaluate the craniofacial size of the individual and to rule out size effects on the measurements. To investigate intra-operator error, 10 lateral and posteroanterior cephalograms were chosen randomly and re-traced in separate sessions with a 2-week interval under identical conditions. Measurement error was estimated according to Dahlberg's formula ($S2 = \sum d2/2n$) [19, 20].

Statistical analysis

Multiple regression analysis was performed to test the association between the rs13317 SNP and obtained measurements. The number of the derived (T) allele was used as the explanatory variable, and sex and GM were covariates. We preliminary tested the effect of age, but age showed no significant association with the morphological variables. The commercial software Statistical Package for the Social

Table 3 Summary of the distance measurements obtained from lateral cephalograms

Category	Measurement	Egyptians ($n = 191$)				Japanese ($n = 211$)				Koreans ($n = 226$)			
		Male ($n = 92$)		Female ($n = 99$)		Male ($n = 42$)		Female ($n = 169$)		Male ($n = 132$)		Female ($n = 94$)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Height, LAT	Is–Ii (*y*)	2.75	1.87	2.71	1.96	3.04	2.44	3.19	2.25	3.65	2.20	3.71	1.83
Depth, LAT	N–G (*z*)	5.02	1.99	1.72	1.50	5.91	3.49	3.38	2.30	5.78	1.90	3.53	1.90
	N–Rh (*z*)	15.33	2.79	15.84	3.10	12.45	6.49	11.52	5.74	12.87	2.87	11.98	3.23
	N–Or (*z*)	16.65	5.02	12.28	4.02	14.01	7.14	11.56	5.73	12.73	3.21	10.84	3.32
	Is–Ii (*z*)	4.32	2.00	4.75	1.83	4.38	3.12	4.26	2.83	3.67	1.82	3.79	1.62
2D distance, LAT	N–Rh	25.61	3.44	26.17	3.64	29.44	13.70	28.76	12.84	31.42	3.41	29.90	5.19
	N–ANS	58.25	3.46	55.20	3.34	60.09	27.57	57.28	25.06	62.79	3.28	58.98	9.00
	N–Gn	129.61	7.71	121.92	6.09	136.13	62.51	128.55	56.21	138.21	6.59	130.01	23.73
	N–S	75.99	3.55	71.37	3.22	72.02	33.09	68.98	30.12	76.48	3.45	72.64	10.93
	N–Cd	93.76	4.84	87.38	4.44	88.62	40.68	85.35	37.31	95.10	4.55	88.74	13.46
	N–Po	105.75	5.04	98.10	4.79	101.18	46.40	96.97	42.35	106.41	4.24	100.28	15.06
	N–Ba	115.96	5.21	109.12	4.35	111.91	51.38	107.18	46.82	119.33	11.43	111.88	20.13
	KR–PNS	25.40	4.19	22.75	3.94	24.62	11.71	23.26	10.55	26.98	3.98	25.41	4.99
	ANS–Gn	72.22	6.49	68.10	5.41	76.91	35.60	72.78	32.03	76.23	5.52	72.20	13.62
	ANS–Ptm	60.56	4.13	57.35	3.68	57.74	26.59	54.41	23.87	59.03	9.38	55.79	8.52
	ANS–Cd	96.76	5.83	91.48	4.66	94.84	43.61	90.28	39.53	97.48	4.60	92.05	13.83
	ANS–Po	107.11	5.92	101.02	5.23	105.63	48.56	100.41	43.91	108.73	5.07	102.94	15.43
	ANS–Ba	109.07	6.01	103.45	5.14	104.17	47.97	99.32	43.50	108.78	10.85	102.75	18.59
	ANS–PNS	57.56	4.14	54.24	3.22	52.78	24.52	50.70	22.29	55.51	5.88	52.80	8.16
	A–Cd	94.03	5.45	88.42	4.53	93.62	43.10	88.68	38.81	96.55	5.08	91.10	13.71
	RGn–Pog	17.38	2.32	15.77	1.66	15.62	7.36	14.93	6.68	17.54	1.74	16.04	2.70
	Ai–Me	22.06	3.26	20.55	2.55	26.24	12.38	24.08	10.88	27.50	3.03	25.75	5.32
	Pog–Cd	127.35	6.74	117.28	5.07	133.21	61.30	120.64	52.91	129.84	6.01	121.26	18.22
	Gn–Cd	130.06	6.77	119.71	5.24	136.28	62.71	123.36	54.09	133.51	6.00	124.43	22.46
	Me–Go	83.24	5.42	78.43	4.39	80.10	36.94	74.00	32.53	82.51	5.13	78.50	14.40
	Gn–Go	86.66	5.34	81.91	4.62	84.40	38.92	78.36	34.42	87.22	5.16	83.01	15.19
	S–Ba	50.36	3.99	46.85	3.15	51.64	23.82	48.07	21.14	54.79	5.87	49.83	9.40
	S–PNS	52.22	3.18	48.53	3.44	55.65	25.57	50.88	22.33	56.59	6.00	52.35	8.15
	S–Go	87.06	7.20	75.38	5.82	88.82	40.84	79.41	35.04	94.85	6.61	84.01	13.08
	Cd–Go	65.45	6.32	56.20	4.96	70.34	32.42	61.87	27.46	71.79	5.10	64.08	10.42
	Ar–Go	54.53	6.30	45.07	5.01	54.14	25.11	46.65	20.91	55.96	4.99	49.29	8.51

Sciences (SPSS, version 23.0; IBM Corporation, Armonk, NY, USA) was used to perform regression analysis. A P value of <0.05 was considered as a suggestive association. After the Bonferroni adjustment to accommodate the concern of multiple testing, the established α was 0.00062 (0.05/81). The statistical results from the three subject sets were then combined using the inverse variance method.

Results

The derived allele (T) frequency of the rs13317 SNP in the 3′ untranslated region of *FGFR1* (position 38,411,996 on chromosome 8) was 0.817 in the Egyptian subjects, while 0.624 and 0.576 in the Japanese and Korean subjects, respectively. The measurements obtained from lateral and posteroanterior cephalograms are summarized in Tables 2–4. In the Egyptian subjects, multiple regression analysis controlling for sex and GM (Table 5) found that the number of T alleles of rs13317 was suggestively associated with the Zy (R)–Zy (L) ($P = 0.031$) and ZA (R)–ZA (L) ($P = 0.032$) distances obtained from the posteroanterior cephalograms (Fig. 1). In the measurements obtained from lateral cephalograms, the derived allele showed a significant negative association with the N–Or (*z*) depth ($P = 0.00040$) and a suggestive association with the N–Cd ($P = 0.023$) and

Table 4 Summary of the angle measurements obtained from lateral cephalograms

Category	Measurement	Egyptians ($n = 191$)				Japanese ($n = 211$)				Koreans ($n = 226$)			
		Male ($n = 92$)		Female ($n = 99$)		Male ($n = 42$)		Female ($n = 169$)		Male ($n = 132$)		Female ($n = 94$)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2D angle, LAT	N–As–Is	149.71	7.49	153.94	8.72	153.34	70.30	155.17	68.67	150.98	19.40	153.87	23.01
	N–A–Pog	1.67	6.42	5.83	6.54	−1.18	8.78	5.38	8.16	2.70	5.93	5.38	5.40
	N–S–A	42.72	3.27	42.85	3.16	46.64	21.47	47.18	20.69	46.25	2.97	46.39	7.22
	N–S–Gn	66.35	4.36	68.52	4.35	68.34	31.52	72.19	31.70	69.61	3.98	70.78	12.96
	N–Po–A	35.09	2.54	35.33	2.56	38.81	17.87	38.65	16.98	38.37	2.63	38.47	6.02
	N–Po–Gn	63.64	4.31	65.11	4.55	65.72	30.32	68.01	29.87	66.34	3.85	66.97	12.30
	N–S–Ba	132.43	5.95	134.07	6.35	129.10	59.14	131.99	57.60	130.29	12.49	131.21	23.48
	A–N–Rh	36.32	5.62	32.68	4.87	28.07	13.85	24.97	11.48	25.80	4.37	24.34	5.65
	A–N–B	1.52	2.93	3.25	2.87	0.05	4.00	3.16	3.72	2.15	2.56	3.27	2.31
	S–N–Rh	119.06	6.90	115.03	6.82	111.24	51.28	106.16	46.50	107.67	5.99	105.70	16.34
	S–N–KR	55.23	3.71	53.63	4.06	58.74	27.08	56.60	24.84	57.87	3.75	56.53	8.96
	S–N–ANS	87.23	5.00	87.28	4.91	87.82	40.31	85.93	37.57	85.38	3.99	85.21	12.89
	S–N–A	82.74	4.29	82.35	4.30	83.17	38.18	81.19	35.48	81.87	3.87	81.35	12.16
	S–N–B	81.22	4.24	79.10	4.07	83.12	38.29	78.03	34.25	79.72	7.94	78.09	11.84
	Cd–Go–Me	120.62	7.20	122.88	7.31	126.31	58.00	126.11	55.19	116.62	6.17	117.89	21.77
	Ar–Go–Me	125.91	7.29	128.43	7.49	132.38	60.77	131.59	57.59	123.07	6.61	123.75	22.83
	Go–Me–Ai	64.14	6.11	65.79	5.79	72.88	33.62	73.38	32.41	77.26	6.21	77.44	14.63
	Me–Ai–Ii	153.27	8.84	154.92	8.13	168.83	77.49	167.05	73.91	165.20	16.39	166.53	30.06
	(Frankfurt plane)–(S–N)	4.13	3.54	6.00	4.15	6.14	3.96	7.56	4.42	6.16	3.11	6.79	3.54
	(Frankfurt plane)–(N–Pog)	86.04	3.82	85.44	4.08	89.89	41.32	86.18	37.73	86.81	3.19	85.68	13.00
	(Frankfurt plane)–(Go–Me)	29.18	6.44	32.39	6.21	31.00	15.25	33.96	16.00	26.86	5.69	29.95	7.91
	(Frankfurt plane)–(S–Gn)	62.21	4.30	62.52	4.49	62.20	28.72	64.63	28.41	63.44	3.48	64.00	12.00
	(Frankfurt plane)–(occlusal plane)	8.17	4.86	10.17	5.23	8.91	6.61	11.96	6.62	9.55	3.80	11.57	4.83
	(A–B)–(N–Pog)	2.89	4.25	4.82	3.78	0.58	5.52	4.66	4.83	4.12	3.73	5.53	3.37
	(N–A)–(As–Is)	24.79	6.33	21.41	7.48	23.95	12.51	21.81	11.72	25.73	6.35	22.92	6.56
	(As–Is)–(Ai–Ii)	128.30	9.93	126.98	10.67	131.69	61.04	129.12	58.08	127.33	18.37	128.04	20.82
	(occlusal line)–(Ai–Ii)	22.02	7.74	23.09	6.68	16.14	11.11	18.34	11.45	19.47	7.27	19.32	7.28
	(occlusal line)–(As–Is)	29.68	6.34	29.93	6.95	32.17	15.77	32.73	15.90	33.20	6.40	32.64	7.26
	(occlusal line)–(PNS–ANS)	3.67	4.75	6.57	4.16	7.16	5.42	9.85	5.55	6.79	3.52	8.71	3.95
	(occlusal line)–(Go–Me)	21.16	4.41	22.22	4.57	22.09	10.86	22.00	10.41	17.41	3.58	18.39	5.41
	(Ai–Ii)–(Go–Me)	89.14	8.24	89.13	7.31	95.96	44.66	93.67	41.99	88.04	10.66	89.09	17.08
	(Ai–Ii)–(N–B)	25.40	7.26	28.36	7.09	24.31	12.74	25.89	13.46	24.77	7.30	25.78	7.27
	(S–N)–(Ai–Ii)	55.83	8.07	50.74	8.00	58.81	28.48	52.12	24.95	54.93	9.52	52.31	10.97
	(S–N)–(Go–Me)	33.31	6.76	38.39	6.12	37.14	17.90	41.35	19.10	33.02	6.33	36.72	8.31
	(S–N)–(As–Is)	107.53	7.00	103.76	7.96	107.12	49.45	102.99	45.95	107.57	14.62	104.27	16.33
	(PNS–ANS)–(As–Is)	26.01	6.91	23.36	7.41	25.01	12.98	22.95	11.98	26.32	6.96	23.92	7.32

Me–Go ($P = 0.037$) distances. Moreover, rs13317 was suggestively associated with the Frankfurt plane and Me–Go angle ($P = 0.039$) and with the Frankfurt plane and the occlusal plane angle ($P = 0.0082$) (Fig. 1). In the Japanese subjects, the N–Or (z) depth and the Cd–Go–Me angle showed a suggestive association. In the Korean subjects, rs13317 was suggestively associated with the RO (R)–Or (R) height, the Go (R)–Go (L) and Ai–Me distances, the N–Or (z) depth, the Ai–Ii and N–point B angle, and the S–N and Ai–Ii angle.

The combined analysis revealed that only the N–Or (z) depth was significantly associated with rs13317 ($P =$

Table 5 The association tests between *FGFR1* rs13317 and measurements by multiple regression analysis

Measurements	Egyptians				Japanese				Koreans				Combined		
	B	SD	*t*	*P*	*B*	SD	*t*	*P*	*B*	SD	*t*	*P*	*B*	SD	*P*
RO (R)–Or (R) (*y*)	0.239	0.690	0.350	7.3.E − 01	−0.042	0.266	−0.160	8.7.E − 01	0.575	0.267	2.160	3.2.E − 02*	0.264	0.182	1.5.E − 01
LO (R)–LO (L)	−0.699	0.624	−1.120	2.6.E − 01	−0.537	0.349	−1.540	1.3.E − 01	−0.436	0.323	−1.350	1.8.E − 01	−0.510	0.222	2.1.E − 02*
Zy (R)–Zy (L)	1.342	0.617	2.180	3.1.E − 02*	0.064	0.411	0.160	8.8.E − 01	−0.275	0.397	−0.690	4.9.E − 01	0.145	0.259	5.8.E − 01
ZA (R)–ZA (L)	1.252	0.580	2.160	3.2.E − 02*	−0.033	0.417	−0.080	9.4.E − 01	−0.134	0.402	−0.330	7.4.E − 01	0.181	0.259	4.8.E − 01
Go (R)–Go (L)	1.366	0.741	1.840	6.7.E − 02	0.607	0.548	1.110	2.7.E − 01	1.335	0.586	2.280	2.4.E − 02*	1.042	0.352	3.1.E − 03*
N–Rh (*z*)	0.182	0.391	0.470	6.4.E − 01	0.000	0.354	0.000	1.0.E + 00	0.584	0.272	2.150	3.3.E − 02*	0.324	0.189	8.6.E − 02
N–Or (*z*)	−2.118	0.582	−3.640	4.0.E − 04**	−0.715	0.340	−2.100	3.7.E − 02*	−0.589	0.292	−2.020	4.5.E − 02*	−0.830	0.207	6.2.E − 05**
N–Cd	−1.131	0.491	−2.300	2.3.E − 02*	0.259	0.331	0.780	4.3.E − 01	−0.209	0.313	−0.670	5.1.E − 01	−0.190	0.206	3.6.E − 01
Ai–Me	0.036	0.362	0.100	9.2.E − 01	0.057	0.324	0.170	8.6.E − 01	0.791	0.254	3.110	2.1.E − 03*	0.400	0.175	2.2.E − 02*
Pog–Cd	−1.122	0.665	−1.690	9.3.E − 02	−0.429	0.753	−0.570	5.7.E − 01	−0.595	0.461	−1.290	2.0.E − 01	−0.698	0.339	3.9.E − 02*
Me–Go	−1.273	0.605	−2.100	3.7.E − 02*	0.394	0.549	0.720	4.7.E − 01	0.181	0.425	0.430	6.7.E − 01	−0.101	0.294	7.3.E − 01
Cd–Go–Me	1.031	0.955	1.080	2.8.E − 01	−1.501	0.704	−2.130	3.4.E − 02*	0.343	0.642	0.540	5.9.E − 01	−0.193	0.425	6.5.E − 01
(Frankfurt plane)–(Go–Me)	1.691	0.814	2.080	3.9.E − 02*	−1.376	0.740	−1.860	6.5.E − 02	0.895	0.572	1.560	1.2.E − 01	0.434	0.396	2.7.E − 01
(Frankfurt plane)–(occlusal plane)	1.780	0.665	2.680	8.2.E − 03*	−0.743	0.544	−1.370	1.7.E − 01	0.600	0.404	1.490	1.4.E − 01	0.441	0.291	1.3.E − 01
(Ai–Ii)–(N–B)	0.128	0.944	0.140	8.9.E − 01	−0.809	0.871	−0.930	3.5.E − 01	1.284	0.641	2.000	4.6.E − 02*	0.452	0.453	3.2.E − 01
(S–N)–(Ai–Ii)	−0.346	1.089	−0.320	7.5.E − 01	0.606	1.212	0.500	6.2.E − 01	−1.827	0.787	−2.320	2.1.E − 02*	−0.901	0.564	1.1.E − 01

Sex and GM were used as covariates. The results that had a suggestive association ($P < 0.05$) in any of the analyses are shown.

0.000062) even after the Bonferroni correction. A suggestive association was observed in four distance measurements: LO (R)–LO (L), Go (R)–Go (L), Ai–Me, and Pog–Cd.

Discussion

To date, few published studies have focused on the effect of *FGFR1* SNPs on normal craniofacial variations. Coussens and van Daal [5] have showed that the *FGFR1* SNP rs4647905 was significantly associated with a decreased cephalic index. Furthermore, Gòmez-Valdès et al. [11] have reported that rs4647905 was associated with a transversely narrow and elongated anteroposterior head (dolichocephaly), suggesting that *FGFR1* variants play an essential role in the pattern and timing of the closure of skull sutures. Another study using three-dimensional (3D) facial imaging and surface-based morphometry has revealed that the *FGFR1* rs13267109 SNP was associated with variations in some facial traits including the facial breadth in the midface region, and the orbital projection, brow ridges, and nose [13]. Our previous study on Japanese and Korean subjects have also indicated that *FGFR1* SNPs, rs13317 and rs6996321, were associated with a shape component representing midface protrusion/recession and the wideness of cheekbones and orbits [18]. Furthermore, a previous study on samples of Han Chinese revealed that the SNP rs13317 was significantly associated with mandibular prognathism [14].

In the current study, we investigated the impact of the *FGFR1* rs13317 SNP on normal craniofacial morphogenesis in 191 healthy Egyptian subjects in addition to the East Asian subjects that had been previously examined. Our analyses confirmed that rs13317 was significantly associated with the N–Or depth in the Egyptian subjects as well as in the Japanese and Korean subjects. A smaller N–Or (*z*) depth represents a relative recession of the midface and a relative protrusion of the cheekbones, which is associated with the T allele of rs13317.

In contrast, we did not find any statistically robust result for the association between rs13317 and the wideness of cheekbones and orbits in the present study. Although the previous study using principal component analysis (PCA) showed the association of rs13317 with a shape component representing midface protrusion/recession and the wideness of cheekbones and orbits in the East Asian subjects [18], any direct evidence for the association with the wideness of cheekbones (Zy (R)–Zy (L)) and orbits (Or (R)–Or (L)) was not observed in the same subjects. This may suggest the difficulty in the

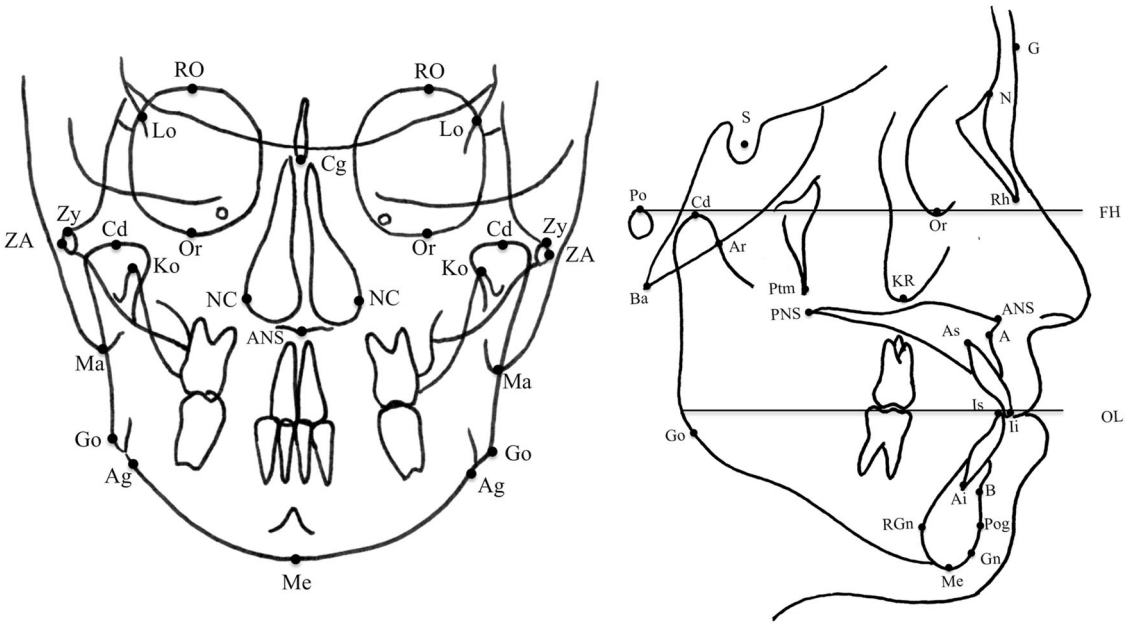

Fig. 1 Lateral and posteroanterior cephalometric tracing showing the craniofacial measurements associated with the *FGFR1* SNP rs13317. RO roof of the orbit, Lo latero orbitale, Or orbitale, Cg neck of crista galli, Zy zygion, ZA zygomatic arch, Cd condylion, Ko koronoid, Ma mastoidare, ANS anterior nasal spine, NC nasal cavity, PNS posterior nasal spine, Ar articular, Po porion, Go gonion, Ag antegonion, Me menton, S sella turcica, G glabella, N nasion, Ba basion, Ptm pterygomaxillary fissure, Rh rhinion, KR key ridge, A point A, B point B, Is incision superius, Ii incision inferius, As apex superius, Ai apex inferius, Pog pogonion, Gn gnathion, RGn retrognathion, FH Frankfurt plane, OL occlusal plane

interpretation of multivariate analysis such as PCA. Instead, we observed a suggestive positive association of the T allele of rs13317 with the Zy (R)–Zy (L) and ZA (R)–ZA (L) distances and a suggestive negative association with the N–Cd and Mo–Go distances in the Egyptian subjects, representing facial characteristics of wider cheekbones and shallower facial depth. In addition, the combined analysis showed that the T allele suggestively had a negative association with LO (R)–LO (L) and a positive association with Go (R)–Go (L), which increases the difficulty in our interpretation. We need to increase the simple size to obtain more robust results. However, we may also need to consider the possibility that the effects of the allele is differently expressed among individuals and populations.

The facial characteristics that we identified as being associated with rs13317 were similar to those of patients with Pfeiffer syndrome, which is caused by dysfunctional mutations of FGFRs. The incidence of Pfeiffer syndrome is approximately 1/100,000 and affects males and females equally. Types I, II, and III have been defined principally on the basis of calvarial and mid-facial severity with Type I the most mild and Type III the most severe. The vast majority of mutations that cause craniosynostosis syndromes are associated with the FGFR2, which cause quite variable and non-specific phenotypes. Calvarial deformations and subsequent variations in facial morphology that observed in Pfeiffer syndrome are a secondary effect of craniosynostosis [3, 4, 21]. Premature fusion of the coronal suture primarily affects the anterior cranial fossa and greater wing of the sphenoid, and hence displaces the periorbital structures from their normal position. Although a reasonable correlation between clinical descriptions and mutations has emerged, identification of these mutations has necessitated some reappraisal of the rather confusing clinical classification of the craniosynostosis disorders. Therefore, abnormalities resulting from mutations in *FGFR2* gene are a good example of a disorder that is better classified by mutation rather than phenotype [3, 11]. A single missense mutation in *FGFR1* (P252R) can cause Pfeiffer syndrome and phenocopies several Pfeiffer syndrome mutations in *FGFR2* [22]. Moreover, recent studies have identified 22 nonrecurring loss-of-function point mutations in *FGFR1* [23–25]. The pathologic mechanism of Pfeiffer syndrome may also explain the morphological difference associated with common polymorphisms in *FGFR1*: It is possible that some polymorphisms in *FGFR1* have a mild influence on the timing of suture closure of some bones and then on potential growth differences. Rs13317 or associated polymorphisms could have a relatively direct effect on the N–Or (*z*) depth and a secondary effect on the position of the cheek and temporal bones, which can cause a slight increase in the facial width [26–29]. Because the mandibular deformity usually seen in craniosynostosis syndromes is caused by cranial base abnormalities and is probably not intrinsic to

the mandible [26, 27], the effect of rs13317 on mandibular measurements could be indirect and thus prone to yielding inconsistencies in results among the examined populations.

FGFR1 SNPs, including rs13317, that have been reportedly associated with certain traits are only polymorphic markers, and their functional effects have not been verified. Therefore, supposing that the same functional variant is shared among different populations, the results of association studies using surrounding polymorphisms can differ because of differences in the linkage disequilibrium structure among populations used in the studies. To verify that the focal SNP is responsible for the focal trait, additional functional experiments to test the activity of the variant should be performed.

Cephalometry used in this study is a method to measure a series of angles and linear measurements projected onto two-dimensional radiographs. Therefore, this method is limited in its ability to evaluate the 3D shape. Especially, since establishing landmarks on cheekbones in the lateral cephalogram is difficult (only Or and KR in this study), evaluation of the 3D shape of cheekbones is limited. Moreover, due to inherent geometric magnification, distortion, and superimposition of the craniofacial structures on the cephalometric radiograph, a reliable and accurate evaluation of these structures in patients with severe anomalies such as craniofacial syndromes can be difficult [30–32]. For that reason, the best way to describe and quantify the patterns of facial shape variation is the use of the 3D digitization technologies such as computed tomography (CT), magnetic resonance imaging (MRI), or optical surface scanning. The use of such 3D images in combination with the methodology of geometric morphometrics will overcome the problem of the inaccurate point identification related to the conventional 2D cephalometry and will allow the results of the statistical analysis of shape to be visualized as deformations of shapes [33].

In conclusion, our analyses of Egyptian, Japanese, and Korean subjects revealed a strong negative association of the derived (T) allele of the rs13317 SNP with the N–Or (z) depth, indicating that this allele is associated with a relative protrusion of the cheekbones. Such facial variation are similar to those seen in Pfeiffer syndrome, which is essentially caused by craniosynostosis from a dysfunctional mutation in *FGFR1*. These findings help understand the roles and functions of *FGFR1* variants in normal human skull variations.

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