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A candidate gene approach to identify modifiers of the palatal phenotype in 22q11.2 deletion syndrome patients

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Abstract

Objective—Palatal anomalies are one of the identifying features of 22q11.2 deletion syndrome (22q11.2DS) affecting about one third of patients. To identify genetic variants that increase the risk of cleft or palatal anomalies in 22q11.2DS patients, we performed a candidate gene association study in 101 patients with 22q11.2DS genotyped with the Affymetrix genome-wide human SNP array 6.0.

Methods—Patients from Children's Hospital of Philadelphia, USA and Wilhelmina Children's Hospital Utrecht, The Netherlands were stratified based on palatal phenotype (overt cleft, submucosal cleft, bifid uvula). SNPs in 21 candidate genes for cleft palate were analyzed for genotype-phenotype association. In addition, TBX1 sequencing was carried out. Quality control and association analyses were conducted using the software package PLINK.

Results—Genotype and phenotype data of 101 unrelated patients (63 non-cleft subjects (62.4%), 38 cleft subjects (37.6%)) were analyzed. A Total of 39 SNPs on 10 genes demonstrated a *p*-value ≤0.05 prior to correction. The most significant SNPs were found on FGF10. However none of the SNPs remained significant after correcting for multiple testing.

Conclusions—Although these results are promising, analysis of additional samples will be required to confirm that variants in these regions influence risk for cleft palate or palatal anomalies in 22q11.2DS patients.

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Appendix A. Supplementary data: Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijporl.2012.10.009.

Keywords

22q11.2 deletion syndrome; Cleft palate; Candidate gene

1. Introduction

The 22q11.2 deletion syndrome (22q11.2DS) is a common microdeletion syndrome that occurs in between one in 4000 and 6000 live births [1,2]. It encompasses the phenotypes previously known as DiGeorge syndrome, velocardiofacial syndrome, conotruncal anomaly face syndrome, many cases of the autosomal dominant Opitz G/BBB syndrome, and Cayler cardiofacial syndrome (asymmetric crying facies). Patients with 22q11.2DS have a range of findings, including palatal abnormalities (overt cleft palate, submucosal cleft palate (SMCP), bifid uvula, velopharyngeal insufficiency, and vascular ring), conotruncal heart disease, characteristic facial features, immune deficiency, psychiatric problems, and learning difficulties. Structural palatal abnormalities are found in approximately one third of patients with 22q11.2DS [3]. About 16% percent have a submucosal cleft, 11% have an overt cleft, and 5% have a bifid uvula.

The 22q11.2DS is a contiguous gene deletion syndrome, which can be inherited in an autosomal dominant manner. However, over 90%of patients have a de novo deletion. The majority of individuals have a similar 3 megabase deletion on 22q11.2. Remarkable inter- and intra-familial clinical variability complicates genotype– phenotype correlations [4]. Possible mechanisms causing phenotypic variability may be modifier genes on the remaining allele of 22q11.2, elsewhere in the genome, epigenetic events, or chance.

Currently a large study is being carried out by the international 22q11.2 Consortium in an attempt to identify genetic modifiers of the 22q11.2DS phenotype. The study is using a genome wide single nucleotide polymorphisms (SNPs) association scan of 1000 DNA samples. The present analysis describes a search for potential modifiers of palatal features using a candidate gene approach in 107 samples selected from the larger study. Possible association between SNPs in these candidate genes and palatal features was investigated.

2. Materials and methods

2.1. Study subjects

The DNA samples described in this paper were obtained from studies concerning 22q11.2DS at the Children's Hospital of Philadelphia, USA and the Wilhelmina Children's Hospital in Utrecht, The Netherlands. The presence of the 22q11.2 deletion, prior to enrollment in this study, was confirmed using fluorescence in situ hybridization (FISH) or multiplex ligation-dependent probe amplification (MLPA) [5]. The current study was approved by the Institutional Review Board (IRB) at both centers, as well as by the Albert Einstein College of Medicine IRB in New York where genotyping was carried out (Genomics Core).

2.2. Phenotype data

Information on the presence of palatal abnormalities was obtained through database and chart review from both the Department of Clinical Genetics and the Department of Plastic Surgery at the Children's Hospital of Philadelphia and the Department of Medical Genetics at the Wilhelmina Children's Hospital in Utrecht. A total of 223 charts were reviewed (177 from Philadelphia and 46 from Utrecht). If no reliable clinical data could be obtained (i.e. when specialists did not agree, or when insufficient data was available) patients were excluded from analysis. Patients from both hospitals were stratified into two groups based on phenotype: “non-cleft” and “cleft” (overt cleft palate, submucosal cleft palate, bifid uvula).

2.3. Selection of candidate genes

One of the important genes in the typically deleted region is TBX1. Animal models of *Tbx1*, specifically those homozygous for a null allele, have shown a role of TBX1 in many of the physical anomalies that are found in 22q11.2DS, including cleft palate [6– 9]. This implies that variants in the single copy of TBX1 that is present in patients with 22q11.2DS may have an effect on the development and/or severity of palatal abnormalities. Consequently, TBX1 was chosen as the first candidate gene to examine as a modifier of the palatal phenotype.

Recently, studies in mice demonstrated a possible role for Bmp antagonism and the chordin (CHRD) gene as interacting genetically with Tbx1 in mouse models [10]. As a result, CHRD was added as a candidate gene in our study. Other potential genetic modifiers outside of the deleted region in 22q11.2DS were selected based on research providing evidence of linkage or association between a genetic variant and cleft palate in humans. These were interferon regulatory factor 6 (IRF6) [11], transforming growth factor α (TGFA) [12], SATB homeobox 2 (SATB2) [13], small ubiquitin-like modifier 1 (SUMO1) [14], muscle segment homeobox (MSX1) [15,16], estrogen receptor 1 (ESR1) [17], poliovirus receptor-related 1 (PVRL1) [18], and transforming growth factor β3 (TGFB3) [19].

Impaired fibroblast growth factor signaling has been associated with orofacial clefting [20]. Thus, the following genes were included as possible candidate genes: fibroblast growth factor 2 (FGF2),fibroblast growth factor 3 (FGF3), fibroblast growth factor 7 (FGF7), fibroblast growth factor 10 (FGF10), fibroblast growth factor receptor 1 (FGFR1), fibroblast growth factor receptor 2 (FGFR2), and fibroblast growth factor receptor 3 (FGFR3). Lastly, because studies have shown that the risk of facial clefts may be influenced by maternal folate intake [21] common SNPs in genes involved in the folate-homocysteine metabolic pathway were also investigated, namely methylene tetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), and cystathionine beta synthase (CBS).

2.4. Genotype data

Genome wide data for all subjects was acquired using the Affymetrix genome-wide human SNP array 6.0. Genotyping was carried out in the Genomics Core in the Department of Genetics of the Albert Einstein College of Medicine, New York. The array allows for the detection of 906,600 SNPs across the genome. As we used a candidate gene approach, we

investigated the SNPs located in the 21 genes described above including SNPs located 5 kb on either side of each gene (for the total list of SNPs see supplement I).

To evaluate the coverage of the candidate genes that was provided by the SNPs available on the array, data was downloaded for the same gene regions in the Centre d'Etude du Polymorphisme Humain from Utah (CEU) samples from the HapMap database release 22 (http://www.hapmap.org/index/html). This data was analyzed using the Tagger procedure implemented in the Haplo-view software [22]. Table 1 shows the number of SNPs tested for each candidate gene, as well as the number of SNPs in the same regions in the HapMap database with a minor allele frequency >0.05 in the CEU population, the percentage of these SNPs tagged by our genotyped SNPs with an $r^2 > 0.8$, and the average r^2 value between the genotyped SNPs and the tagged SNPs.

2.5. Data analysis

As the means of sample quality control, all individuals with a genotype call rate (defined as the percentage of successful genotyping across the genome) less than 95% were intended to be excluded. However, as none of our samples failed this criterion, none had to be removed.

In order to implement SNP data quality control before statistical analysis, all SNPs with an individual call rate of less than 90% were removed. In addition, SNPs that failed the Hardy–Weinberg Equilibrium (HWE) test at a significance threshold of $p < 0.0001$ and SNPs with a minor allele frequency below 5% were also removed. The total number of SNPs remaining after these quality control measures was 654,469. Out of these markers, the number available for each candidate gene is shown in Table 1. Two genes (CHRD and TBX1) could not be tested for association as there were few SNPs on the array for either locus and after ruling out these, no test SNPs remained after quality control.

2.6. TBX1 sequencing

As TBX1 could no longer be studied using data from the whole genome analysis, Sanger sequencing on TBX1 coding exons and evolutionary conserved non-coding regions within the gene locus was carried out on a subset of patients at the Venter Institute. The sequence of the gene included 5 kb upstream and downstream of the first and last exons, respectively. This generated information on SNPs in selected regions within TBX1 allowing for a more detailed analysis of the gene. The goal was to identify SNPs that alter amino acid sequence or affect splicing or a transcriptional regulatory region.

2.7. Statistical analysis

Genotyping data was exported into a text file format suitable for association analyses using the software package PLINK v1.06 [23] (http://pngu.mgh.harvard.edu/purcell/plink/). One degree-of-freedom chi-square tests of association were performed by comparing SNP allele frequencies among patients with and without palatal anomalies. Empirical *p*-values were calculated by permutation tests for all SNPs in each gene separately, thus providing an effective correction for multiple tests based on the number of SNPs in each gene.

3. Results

3.1. Study population

Genotype and phenotype data were obtained on 107 unrelated patients (Table 2). Most of these were from the Children's Hospital of Philadelphia (94 samples). The remaining 13 samples came from the Wilhelmina Children's Hospital in Utrecht. Six of the subjects with a self-reported ethnicity other than Caucasian (2 Hispanic, 2 African American, 1 Asian, 1 North African) were excluded from statistical analysis. Of the 101 remaining subjects, 38 had a form of palate anomaly (overt cleft palate, submucosal cleft palate, bifid uvula) while 63 subjects did not (for details see Table 2). None of the subjects had cleft lip with or without cleft palate.

3.2. Genetic association analysis

A total of 39 SNPs on 10 genes demonstrated an asymptotic *p*-value ≤0.05 (Table 3). These were CBS, ESR1, FGF3, FGF10, FGFR2, IRF6, MTRR, PVRL1, SATB2, and TGFA. Of these SNPs, 11 SNPs remained significant after correcting for multiple testing for the number of SNPs in each gene by means of permutation analysis. However, this significance was not retained when multiple testing for all genes was accounted for using the Bonferroni correction (threshold for experiment-wise significance $p \leq 0.002$).

3.3. TBX1 resequencing

TBX1 sequence data was obtained for 80 patients from the Children's Hospital of Philadelphia. Of these, 53 had a normal palate (66.3%) and 27 had a cleft phenotype (6 overt cleft, 15 SMCP, 6 bifid uvula; total 33.8%). Twelve SNPs on the TBX1 gene with an MAF > 0.05 were tested for significant differences in allele frequencies between cleft and non-cleft subjects. None of the SNPs demonstrated a *p*-value ≤0.05.

4. Discussion

This report rules out common SNPs in the most promising candidate genes as being major modifiers of the palatal phenotype in 22q11.2DS. It does provide tentative evidence for modest modifiers and suggests a relationship between a number of cleft palate candidate genes and the development of palatal anomalies in 22q11.2DS. The gene with the most significant SNPs associated with cleft palate in our data set is FGF10. A number of studies have shown a role for fibroblast growth factors including FGF10 in orofacial cleft development [20].

In embryology, the formation of the pharyngeal arches plays a central role in the development of the face and neck. The genetic regulation of this craniofacial myogenesis, however, remains relatively unknown [24]. Orofacial musculature can be divided into branchiomeric and non-branchiomeric muscles. Branchiomeric muscles include the masticator muscles, derived from the first arch; muscles of facial expression, derived from the second arch; and muscles of the pharynx and larynx, derived from the third and fourth arches. Non-branchiomeric head muscles include extra-ocular muscles, derived from the anterior mesoderm; and tongue muscles, derived from the hypoglossal cord. A recent study

by Kelly et al. [25] demonstrated that *Tbx1* is an important regulator in the onset of branchiomeric myogenesis and pharyngeal muscle development in the mouse. It is hypothesized that *Tbx1* is required for the transcriptional activation of myogenic determination genes as it showed that *Tbx1* regulates the expression of *Fgf10* in the core of the first pharyngeal arch. Mutations in *Tbx1* resulted in down-regulation of *Fgf10* expression which affected the patterning of cells in the mandibular arch and thus resulted in defects in branchiomeric myogenesis in mice [25].

Another study examining the role of FGF10 in orofacial development was that of Harada et al. that showed the *Fgf*10 null mice exhibited a reduction in cell proliferation in dental epithelium; an effect which could be reversed by adding exogenous FGF10 [26]. Rice and colleagues showed that FGF10 is crucial in mediating tissue-tissue interactions during palate development [27]. Mice lacking *Fgf10* did show initial palatal shelf buds but they did not undergo palatal extension and growth. Finally, Hosokawa and colleagues recently demonstrated how FGF10 signaling in cranial neural crest cells controlled the development of myogenic progenitor cells in tongue formation, a vital structure in palate development [28].

These studies of animal models illustrate both the important role of FGF10 in palate development and the important interaction between FGF10 and TBX1. Unfortunately, the SNPs that were tested in FGF10 in the current report did not retain significance after correction for multiple testing. This may be a due to a number of possible limitations, such as the number of selected SNPs being too low to achieve full gene coverage and/or the small number of analyzed patients.

In summary, in this research report we investigated the association of development of palatal anomaly in 22q11.2DS with variants in known cleft palate genes. Despite the small sample size, some variants showed nominal significance and might act as moderate genetic modifiers. However, although 11 SNPs retained statistical significance after correcting for the number of SNPs tested in each individual gene, none of these were significant after correcting for the total number of genes tested. As this project is part of a larger study being performed by the International 22q11.2 Consortium, additional DNA samples should provide more data in the future. The results from these additional samples will be required to confirm the findings in this report.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Wilson DI, Cross IE, Wren C. Minimum prevalence of chromosome 22q11 deletions. Am J Hum Genet. 1994; 55:A169.
2. Devriendt K, Fryns JP, Mortier G, van Thienen MN, Keymolen K. The annual incidence of DiGeorge/velocardiofacial syndrome. J Med Genet. 1998; 35:789–790. [PubMed: 9733045]
3. McDonald-McGinn DM, Kirschner RE, Goldmuntz E, Sullivan K, Eicher P, Gerdes M, et al. The Philadelphia story: the 22q11.2 deletion: report on 250 patients. Genet Couns. 1999; 10:11–24. [PubMed: 10191425]

4. McDonald-McGinn DM, Tonnesen MK, Laufer-Cahana A, Finucane B, Driscoll DA, Emanuel BS, et al. Phenotype of the 22q11. 2 deletion in individuals identified through an affected relative: cast a wide FISHing net! Genet Med. 2001; 3:23–29. [PubMed: 11339373]
5. Vorstman JA, Jalali GR, Rappaport EF, Hacker AM, Scott C, Emanuel BS. MLPA: a rapid, reliable, and sensitive method for detection and analysis of abnormalities of 22q. Hum Mutat. 2006; 27:814–821. [PubMed: 16791841]
6. Jerome LA, Papaioannou VE. DiGeorge, syndrome phenotype in mice mutant for the T-box gene, Tbx1. Nat Genet. 2001; 27:286–291. [PubMed: 11242110]
7. Lindsay EA, Vitelli F, Su H, Morishima M, Huynh T, Pramparo T, et al. Tbx1 haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. Nature. 2001; 410:97–101. [PubMed: 11242049]
8. Merscher S, Funke B, Epstein JA, Heyer J, Puech A, Lu MM, et al. TBX1 responsible for cardiovascular defects in velo-cardio-facial syndrome. Cell. 2001; 104:619–629. [PubMed: 11239417]
9. Liao J, Kochilas L, Nowotschin S, Arnold JS, Aggarwal VS, Epstein JA, et al. Full spectrum of malformations in velo-cardio-facial syndrome/DiGeorge syndrome mouse models by altering Tbx1 dosage. Hum Mol Genet. 2004; 13:1577–1585. [PubMed: 15190012]
10. Choi M, Klingensmith J. Chordin is a modifier of tbx1 for the craniofacial malformations of 22q11 deletion syndrome phenotypes in mouse. PLoS Genet. 2009; 5:e1000395. [PubMed: 19247433]
11. Zucchero TM, Cooper ME, Maher BS, Daack-Hirsch S, Nepomuceno B, Ribeiro L, et al. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip and palate. N Engl J Med. 2004; 351:769–780. [PubMed: 15317890]
12. Vieira AR. Association between the transforming growth factor alpha gene and nonsyndromic oral clefts: a HuGe review. Am J Epidemiol. 2006; 163:790–819. [PubMed: 16495466]
13. Fitzpatrick DR, Carr IM, McLaren L, Leek JP, Wightan P, Williamson K, et al. Identification of SATB2 as the cleft palate geneon 2q32–q33. Hum Mol Genet. 2003; 12:2491–2501. [PubMed: 12915443]
14. Alkuraya FS, Saadi I, Lund JJ, Turbe-Doan A, Morton CC, Maas RL. SUMO1 haploinsufficiency leads to cleft lip and palate. Science. 2006; 313:175. [PubMed: 16840684]
15. Van den Boogaard MJ, Dorland M, Beemer FA, Ploos van Amstel HK. MSX1 mutation is associated with orofacial clefting and tooth agenesis in humans. Nat Genet. 2000; 24:342–343. [PubMed: 10742093]
16. Fallin MD, Hetmanski JB, Park J, Scott AF, Ingersoll R, Fuernkranz HA, et al. Family-based analysis of MSX1 haplotypes for association with oral clefts. Genet Epidemiol. 2003; 25:168–175. [PubMed: 12916025]
17. Osoegawa K, Vessere GM, Utami KH, Mansilla MA, Johnson MK, Riley BM, et al. Identification of novel candidate genes associated with cleft lip and palate using array comparative genomic hybridisation. J Med Genet. 2008; 45:81–86. [PubMed: 17873121]
18. Avila JR, Jezewski PA, Vieira AR, Orioli IM, Castilla EE, Christensen K, et al. PVRL1 variants contribute to non-syndromic cleft lip and palate in multiple populations. Am J Med Genet A. 2006; 140:2562–2570. [PubMed: 17089422]
19. Lidral AC, Romitti PA, Basart AM, Doetschman T, Leysens NJ, Daack-Hirsch S, et al. Association of MSX1 and TGFB3 with nonsyndromic clefting in humans. Am J Hum Genet. 1998; 63:557–568. [PubMed: 9683588]
20. Riley BM, Mansilla MA, Ma J, Daack-Hirsch S, Maher BS, Raffensperger LM, et al. Impaired FGF signaling contributes to cleft lip and palate. Proc Natl Acad Sci U S A. 2007; 104:4512–4517. [PubMed: 17360555]
21. Van Rooij IA, Ocke MC, Straatman H, Zielhuis GA, Merkus HM, Steegers-Theunissen RP. Periconceptional folate intake by supplement and food reduces the risk of nonsyndromic cleft lip with or without cleft palate. Prev Med. 2004; 39:689–694. [PubMed: 15351534]
22. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]

23. Purcell S, Naele B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. [PubMed: 17701901]

24. Richman JM, Lee SH. About face: signals and genes controlling jaw patterning and identity in vertebrates. Bioessays. 2003; 25:554–568. [PubMed: 12766945]

25. Kelly RG, Jerome-Majewska LA, Papaioannou VE. The del22q11.2 candidate gene Tbx1 regulates branchiomeric myogenesis. Hum Mol Genet. 2004; 13:2829–2840. [PubMed: 15385444]

26. Harada H, Toyono T, Toyoshima K, Yamasaki M, Itoh N, Kato S, et al. FGF10 maintains stem cell compartment in developing mouse incisors. Development. 2002; 129:1533–1541. [PubMed: 11880361]

27. Rice R, Spencer-Dene B, Connor EC, Gritli-Linde A, McMahon AP, Dickson C, et al. Disruption of Fgf10/Fgfr2b-coordinated epithelial–mesenchymal interactions causes cleft palate. J Clin Invest. 2004; 113:1692–1700. [PubMed: 15199404]

28. Hosokawa R, Oka K, Yamaza T, Iwata J, Urata M, Xu X, et al. Dev Biol. 2010; 341:186–195. [PubMed: 20193675]

Table 1

Cleft palate candidate genes included in our study. HapMap SNPs: number of SNPs in the HapMap release 22 in each gene including 5 kb on both sides and with a minor allele frequency >0.05 in the CEU population. Test SNPs: number of SNPs tested in this study (and included in HapMap) and with a minor allele frequency >0.05 in our population. Captured SNPs: number and percentage of HapMap SNPs tagged by the test SNPs with an r^2 >0.8. r^2 mean: average r^2 between test SNPs and tagged HapMap SNPs. CEU: Centre d'Etude du Polymorphisme Humain from Utah; SNP: single nucleotide polymorphism.

Gene	HapMap SNPs	Test SNPs	Captured SNPs		
	N	*N*	*N*	%	r^2 mean
CBS	30	11	13	43.3	0.99
CHRD	9	0	0	0	–
ESR1	257	65	185	72.0	0.98
FGF2	61	17	41	67.2	0.97
FGF3	15	7	12	80.0	0.98
FGF7	49	9	27	55.1	0.97
FGF10	64	21	62	96.9	0.99
FGFR1	28	7	18	64.3	0.95
FGFR2	72	20	34	47.2	0.97
FGFR3	2	2	1	50.0	1.00
IRF6	29	16	27	93.1	0.99
MSX1	5	3	3	60.0	0.99
MTHFR	41	6	16	39.0	0.95
MTR	112	32	111	99.1	0.99
MTRR	48	11	37	77.1	0.95
PVRL1	39	15	36	92.3	0.99
SATB2	89	24	75	84.3	0.99
SUMO1	19	4	1	5.3	1.00
TBX1	8	0	0	0	–
TGFA	151	43	128	84.8	0.99
TGFB3	19	8	14	73.7	0.97

Table 2

Characteristics of study subjects. USA: patients from Children's Hospital of Philadelphia, USA. NL: patients from Wilhelmina Children's Hospital, Utrecht, the Netherlands.

	Study subjects		
	USA (*n* = 94)	**NL (*n* = 13)**	**Total (*n* = 107)**
Male	48	4	52 (48.6%)
Female	46	9	55 (51.4%)
Caucasian	89	12	101 (94.4%)
Non-Caucasian	5	1	6 (5.6%)
Non-cleft phenotype	59	8	67 (62.6%)
Cleft phenotype	35	5	40 (37.4%)
Overt cleft palate	6	1	7 (6.5%)
Submucosal cleft palate	22	3	25 (23.4%)
Bifid uvula	7	1	8 (7.5%)

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Table 3

Results of association analysis of candidate genes with nominal *p* values ≤0.05. SNP: single nucleotide polymorphism. Major allele: most frequent in sample. Minor allele: least frequent in sample. OR: odds ratio. χ^2: chi square statistic. *P*: nominal *p* value. EMP: empirical *p* value after permutation analysis.

Gene (band)	SNP (position)	Role	Major Allele	Minor Allele	Minor Allele Relative Frequency Cases	Minor Allele Relative Frequency Controls	Genotype Count Cases	Genotype Count Controls	OR	χ^2	*P*	EMP
IRF6 (1q32.2)	rs2235372 (208027059)	Near-gene-3	G	A	0.25	0.12	2/15/21	0/15/48	2.47	5.81	0.02	0.06
	rs742215 (208027646)	Near-gene-3	A	T	0.25	0.12	2/15/21	0/15/48	2.47	5.81	0.02	0.06
	rs17317411 (208027937)	Untranslated-3	T	C	0.22	0.08	2/13/23	0/11/52	3.01	7.39	0.007	0.03
	rs17015218 (208034538)	Intron	A	G	0.24	0.10	2/14/22	0/13/50	2.70	6.52	0.01	0.04
	rs17015226 (208036719)	Intron	C	G	0.24	0.10	2/14/22	0/13/50	2.70	6.52	0.01	0.04
	rs6540560 (208048696)	Unknown	C	T	0.24	0.10	2/14/22	0/13/50	2.70	6.52	0.01	0.04
	rs6696825 (208048995)	Unknown	A	G	0.25	0.12	2/15/21	0/15/48	2.47	5.81	0.02	0.06
TGFA (2p13.3)	rs432203 (70618196)	Intron	G	T	0.54	0.37	11/18/8	12/23/28	1.98	5.32	0.02	0.29
	rs377122 (70620533)	Intron	C	T	0.53	0.37	11/18/9	12/23/28	1.87	4.54	0.03	0.47
SATB2 (2q33.1)	rs4673309 (199855654)	Intron	A	G	0.21	0.08	1/14/23	0/10/53	3.10	7.27	0.007	0.04
	rs10497832 (199875181)	Intron	G	C	0.21	0.08	1/14/23	0/10/53	3.10	7.27	0.007	0.04
	rs1374361 (199878979)	Intron	C	T	0.18	0.08	1/12/25	0/10/53	2.62	4.98	0.03	0.14
	rs4675475 (199884288)	Intron	G	T	0.30	0.18	2/19/17	1/21/41	1.94	3.89	0.05	0.28
	rs7569519 (199888067)	Intron	A	C	0.30	0.18	2/19/17	1/21/41	1.94	3.89	0.05	0.28
	rs930616 (199894160)	Intron	A	G	0.30	0.18	2/19/17	1/21/41	1.94	3.89	0.05	0.28
	rs17197938 (199908430)	Intron	C	T	0.12	0.25	0/9/29	2/28/33	0.39	5.38	0.02	0.12
	rs16831370 (199939292)	Intron	C	G	0.01	0.07	0/1/37	0/10/53	0.15	4.04	0.04	0.26
	rs10497836 (199996963)	Intron	T	C	0.39	0.24	4/22/12	2/26/35	2.09	5.57	0.02	0.12
	rs895882 (200011260)	Intron	A	G	0.39	0.24	4/22/12	2/26/35	2.09	5.57	0.02	0.12
	rs13392032 (200017738)	Intron	C	G	0.29	0.17	1/20/17	3/15/45	2.04	4.27	0.04	0.21
MTRR (5p15.31)	rs17184211 (7919106)	Intron	A	T	0.14	0.27	3/5/30	3/28/31	0.45	4.53	0.03	0.23
FGF10 (5p12)	rs17234541 (44344655)	Intron	G	T	0.01	0.11	0/1/37	2/10/51	6.62	0.11	0.01	0.08
	rs17234079 (4497961)	Intron	C	T	0.28	0.10	2/17/19	1/11/51	3.32	10.15	0.001	0.01
	rs10512852 (44429326)	Unknown	T	C	0.01	0.17	0/1/37	3/16/44	0.06	12.25	0.0005	0.005
ESR1 (6q25.1)	rs1543403 (152470397)	Unknown	G	C	0.59	0.44	12/21/5	10/36/17	1.82	4.14	0.04	0.63

Gene (band)	SNP (position)	Role	Major Allele	Minor Allele	Minor Allele Relative Frequency Cases	Minor Allele Relative Frequency Controls	Genotype Count Cases	Genotype Count Controls	OR	χ^2	*P*	EMP
FGFR2 (10q26.13)	rs1649202 (123230615)	Intron	C	T	0.50	0.31	11/16/11	6/26/30	2.26	7.49	0.006	0.06
	rs2278202 (123233187)	Intron	A	G	0.46	0.26	8/19/11	4/25/34	2.41	8.38	0.004	0.04
FGF3 (11q13.3)	rs12577891 (69332822)	Near-gene-3	G	T	0.29	0.17	7/8/23	2/17/44	2.04	4.27	0.04	0.20
	rs10908228 (69336099)	Intron	C	T	0.29	0.17	7/8/23	2/17/44	2.04	4.27	0.04	0.20
PVRL1 (11q23.3)	rs10790332 (119058888)	Intron	T	C	0.36	0.50	5/17/16	13/37/13	0.55	4.02	0.05	0.19
	rs4936492 (119065659)	Intron	G	A	0.36	0.52	5/17/16	15/35/13	0.52	4.93	0.03	0.12
	rs7950059 (119070705)	Intron	C	T	0.38	0.54	6/17/15	15/38/10	0.53	4.75	0.03	0.13
	rs1467051 (119073511)	Intron	G	A	0.35	0.53	5/16/16	13/39/9	0.48	6.10	0.01	0.07
	rs7945395 (119074947)	Intron	A	G	0.34	0.53	6/14/18	14/39/10	0.46	6.87	0.009	0.04
	rs7945424 (119075012)	Intron	G	T	0.33	0.54	5/14/17	14/40/9	0.43	7.84	0.005	0.02
	rs715849 (119080934)	Intron	G	A	0.34	0.52	6/14/18	13/40/10	0.47	6.31	0.01	0.06
CBS (21q22.3)	rs2839631 (43343155)	Unknown	C	T	0.46	0.33	7/21/10	5/31/27	1.77	3.69	0.05	0.22
	rs9325622 (43352770)	Intron	G	A	0.46	0.32	7/21/10	5/30/28	1.84	4.16	0.04	0.19
	rs1789953 (43356005)	Intron	T	C	0.07	0.18	0/5/33	2/19/42	0.32	5.41	0.02	0.10

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