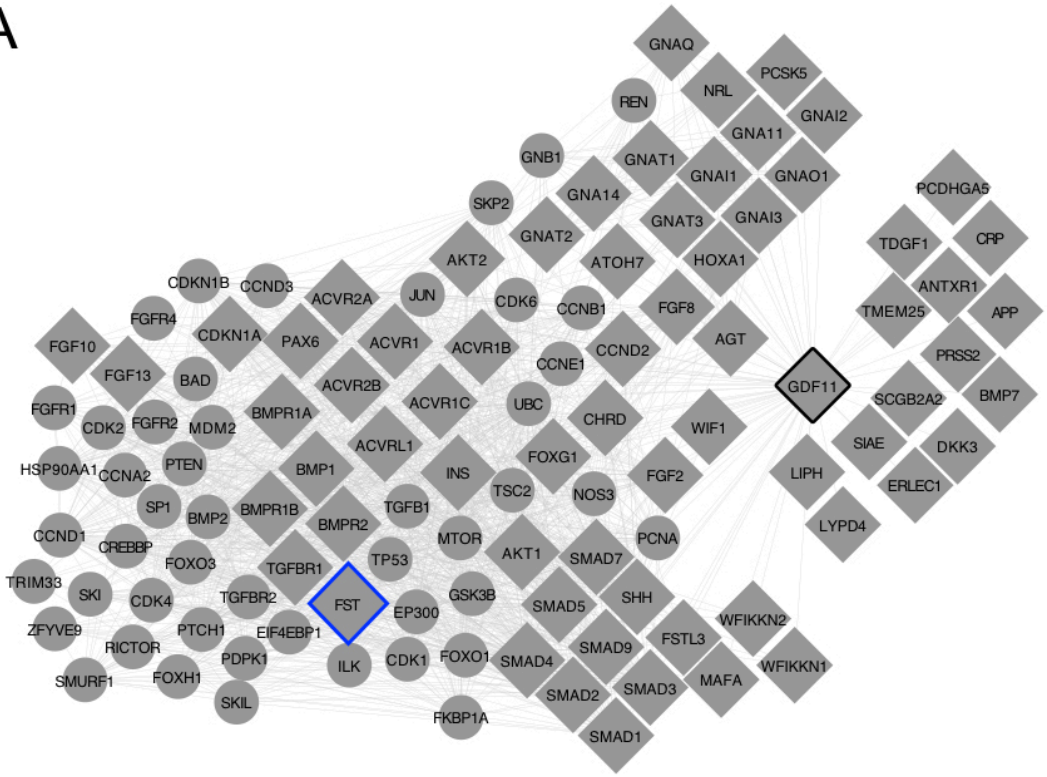
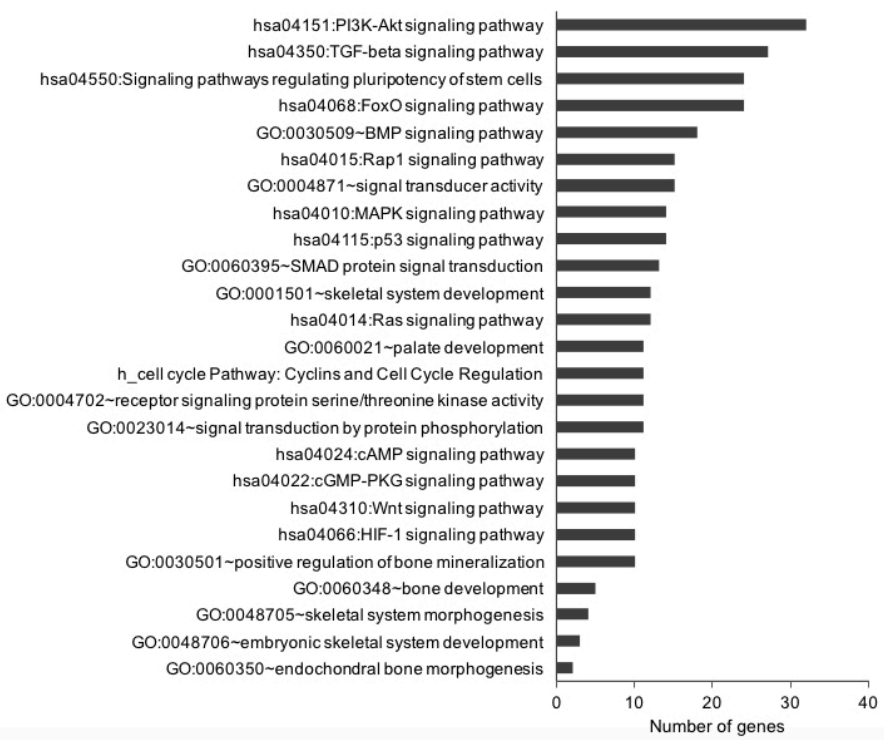
Supp. Figure S1: STRING-based protein-protein interaction network of GDF11. A. Analysis of the human GDF11 protein-interactome in the “String” database revealed 66 direct molecular interactors (diamond shape nodes) and their nearest neighbors (circles). B. Factors in the GDF11 network were analyzed by the bioinformatics tool “DAVID” for potential enrichment of functional categories. For example, the following functional categories relevant to orofacial development were found to be enriched at p-value < 0.05: PI3K-Akt signaling pathway (hsa0415), TGF-beta signaling pathway (hsa04350), BMP signaling pathway (GO:0030509), skeletal system development (GO:0001501), bone development (GO:0060348) and palate development (GO:0060021).

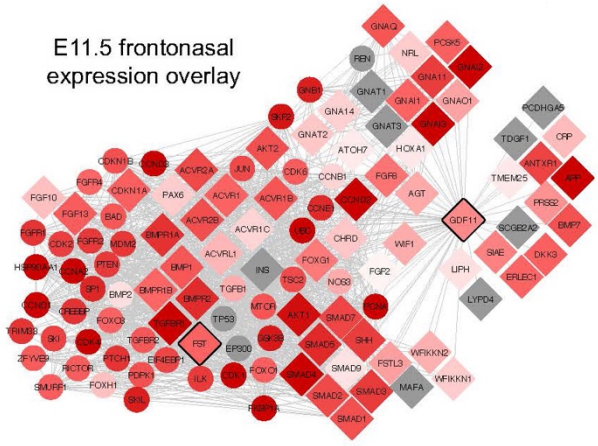
A

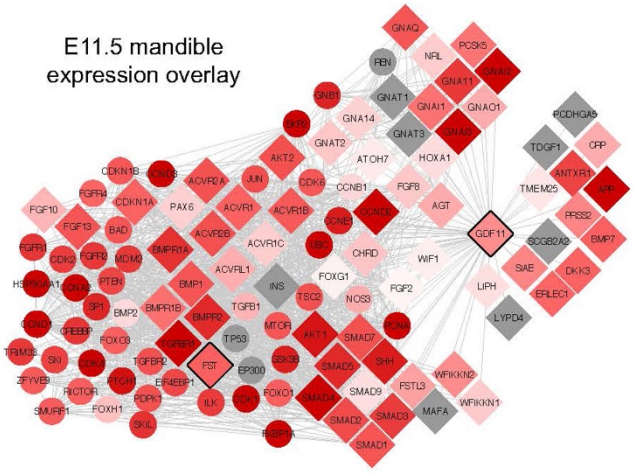

GNAQ
PCSK5
NRL
REN
GNAI2
GNA11
GNB1
GNAT1
GNAI1
GNAO1
PCDHGA5
SKP2
GNA14
GNAT3
GNAI3
TDGF1
CRP
GNAT2
ATOH7
HOXA1
CDKN1B
CCND3
ACVR2A
JUN
AKT2
CDK6
CCNB1
FGF8
ANTXR1
TMEM25
APP
FGFR4
CDKN1A
PAX6
ACVR1
ACVR1B
CCND2
AGT
FGF10
CCNE1
PRSS2
FGF13
BAD
ACVR2B
GDF11
SCGB2A2
BMP7
FGFR1
ACVR1C
UBC
CHRD
WIF1
CDK2
FGFR2
MDM2
BMPR1A
SIAE
DKK3
ACVRL1
FOXG1
HSP90AA1
CCNA2
PTEN
BMP1
INS
FGF2
LIPH
ERLEC1
TSC2
NOS3
SP1
BMP2
BMPR1B
TGFB1
LYPD4
CCND1
BMPR2
MTOR
PCNA
CREBBP
AKT1
SMAD7
FOXO3
TP53
TRIM33
TGFBR1
SKI
CDK4
TGFBR2
FST
GSK3B
SMAD5
SHH
WFIKKN2
ZFYVE9
EP300
FSTL3
PTCH1
EIF4EBP1
SMAD9
RICTOR
WFIKKN1
PDPK1
ILK
CDK1
FOXO1
SMAD4
MAFA
SMURF1
FOXH1
SMAD2
SMAD3
SKIL
FKBP1A
SMAD1

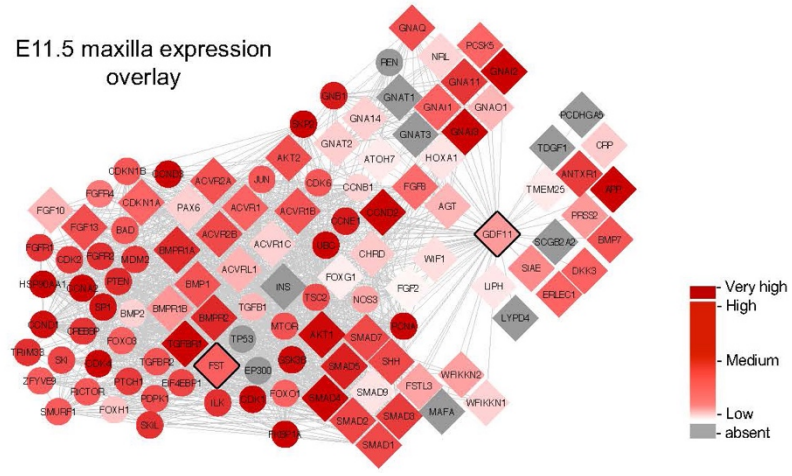
B


hsa04151:PI3K-Akt signaling pathway
hsa04350:TGF-beta signaling pathway
hsa04550:Signaling pathways regulating pluripotency of stem cells
hsa04068:FoxO signaling pathway
GO:0030509~BMP signaling pathway
hsa04015:Rap1 signaling pathway
GO:0004871~signal transducer activity
hsa04010:MAPK signaling pathway
hsa04115:p53 signaling pathway
GO:0060395~SMAD protein signal transduction
GO:0001501~skeletal system development
hsa04014:Ras signaling pathway
GO:0060021~palate development
h_cell cycle Pathway: Cyclins and Cell Cycle Regulation
GO:0004702~receptor signaling protein serine/threonine kinase activity
GO:0023014~signal transduction by protein phosphorylation
hsa04024:cAMP signaling pathway
hsa04022:cGMP-PKG signaling pathway
hsa04310:Wnt signaling pathway
hsa04066:HIF-1 signaling pathway
GO:0030501~positive regulation of bone mineralization
GO:0060348~bone development
GO:0048705~skeletal system morphogenesis
GO:0048706~embryonic skeletal system development
GO:0060350~endochondral bone morphogenesis
0
10
20
30
40
Number of genes

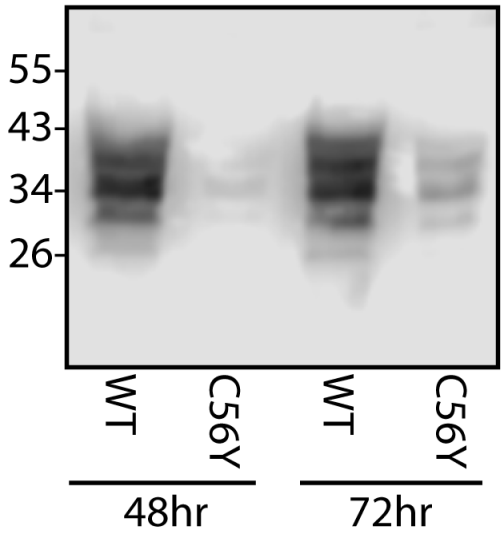
Supp. Figure S2. Gdf11 protein-protein network expression dynamics in mouse embryonic mandibular, maxillary, frontonasal and palatal tissue. Overlay of E11.5 mandibular, maxillary, and frontonasal expression from SysFACE on the Gdf11 protein-protein network. Differing intensities of red represent increasing transcript expression. Gdf11 direct interactors, including FST, exhibit significant expression and enrichment in these tissues.


E11.5 frontonasal
expression overlay


E11.5 mandible
expression overlay


E11.5 maxilla expression
overlay
Very high
High
Medium
Low
absent

Supp. Figure S3. Western blot of WT and mutant Fs288 expression. HEK293T cells were transfected with equal amounts of Fs288 WT or C56Y vector DNA. 48 and 72hrs post transfection conditioned media was collected and analyzed via Western blot under non-reducing conditions with an anti-follistatin antibody.



Supp. Table S1: Rare variants identified by exome sequencing in Family 4527
MAF = minor allele frequency (gnomAD). nd = not detected

gene	Chr	Position	MAF	AA change	Polyphen Score / prediction	Sift Score / prediction	Cadd scaled
LGALS8	1	g.236702344GAA>G	nd	p.E100X	None	None	None
CHRM3	1	g.240071784T>C	$2.05x10^{-6}$	p. S345P	0.199 / benign	0.02 / deleterious	21.4
TNFAIP6	2	g.152235980G>A	$5.22x10^{-5}$	p.G256E	0.005 / benign	0.23 / tolerated	15.49
HARS	5	g.140057542A>G	nd	p.I194T	0.967 / Prob damaging	0.01 / deleterious	28.3
PCDHGA5	5	g.140744853T>C	$4.01x10^{-6}$	p.V319A	0.794 / Poss damaging	0.04 / deleterious	17.99
AIM1	6	g.106967614C>T	$2.47x10^{-3}$	p.T436I	0.303 / benign	0.00 / deleterious	15.65
HBS1L	6	g.135318600A>G	$3.98x10^{-6}$	p.I245T	0.182 / benign	0.23 / tolerated	18.27
TNS3	7	g.47331594G>A	$5.11x10^{-3}$	p.T1296M	0.915 / Prob damaging	0.04 / deleterious	19.72
FOXD4	9	g.117593T>C	nd	p.N176S	0.741 / Poss damaging	0.22 / tolerated	14.95
GDF11	12	g.56143335G>A	nd	p.R298Q	0.969 / Prob damaging	0.01 / deleterious	35
SLC35E3	12	g.69145890A>G	$1.50x10^{-4}$	p.M198V	0.159 / benign	0.00 / deleterious	16.09
PCDH8	13	g.53420234C>T	$1.60x10^{-4}$	p.E780K	0.058 / benign	0.02 / deleterious	27.2
MAN2C1	15	g.75648535G>A	$4.02x10^{-6}$	p.S988L	0.067 / benign	0.12 / tolerated	14.95
ADAMTSL3	15	g.84561509G>A	$3.98x10^{-6}$	p.V446I	0.462 / Poss damaging	0.07 / tolerated	27.6
TM2D3	15	g.102182739G>T	$2.01x10^{-5}$	p.D229E	1.000 / Prob damaging	0.00 / deleterious	15.27
TRPM2	21	g.45815425C>G	$1.13x10^{-3}$	p.I641M	0.109 / benign	0.04 / deleterious	16.1

Supp. Table S2: DOMINO prediction of candidate genes in Family 4527

gene	LDA Score	Class	Probability of being AD
GDF11	2.599	Very likely dominant	0.998
CHRM3	1.368	Very likely dominant	0.911
TNS3	0.956	Likely dominant	0.774
PCDH8	0.284	Either dominant or recessive	0.428
TRPM2	0.240	Either dominant or recessive	0.407
FOXD4	0.147	Likely recessive	0.364
HBS1L	-0.247	Likely recessive	0.223
HARS	-0.325	Likely recessive	0.202
TNFAIP6	-0.501	Very likely recessive	0.163
PCDHGA5	-0.560	Very likely recessive	0.152
AIM1	-0.858	Very likely recessive	0.110
MAN2C1	-0.973	Very likely recessive	0.098
TM2D3	-0.997	Very likely recessive	0.095
CAGE1	-1.016	Very likely recessive	0.094
ADAMTSL3	-1.407	Very likely recessive	0.068
LGALS8	-1.536	Very likely recessive	0.063
SLC35E3	-1.679	Very likely recessive	0.058

Supp. Table S3: Rare variants identified by exome sequencing in Family 22.
MAF = minor allele frequency (gnomAD). nd = not detected

gene	Chr	Position	MAF	AA change	Polyphen Score / prediction	Sift Score / prediction	Cadd scaled
EML4	2	g.42472707C>T	nd	p.R30*	None	None	29.1
ABTB1	3	g.127394882G>A	$7.46x10^{-5}$	p.R82H	0.996 / Prob damaging	0.00 / deleterious	21.8
SERPINI2	3	g.167183441A>T	$2.63x10^{-4}$	p.S167T	0.097 / benign	0.14 / tolerated	19.46
PPEF2	4	g.76809466A>G	nd	p.Y145H	1.000 / Prob damaging	0.00 / deleterious	24.2
C5orf42	5	g.37108485C>G	nd	p.E3109D	0.024 / benign	0.16 / tolerated	22.9
FST	5	g.52778791G>A	nd	p.C56Y	0.822 / Poss damaging	0.00 / deleterious	28.8
IL31RA	5	g.55178902T>G	nd	p.V162G	0.891 / Poss damaging	0.00 / deleterious	20.6
SPARC	5	g.151043709G>C	$1.99x10^{-5}$	p.D274E	0.817 / Poss damaging	0.00 / deleterious	23.9
TENM2	5	g.167474466G>T	$1.28x10^{-5}$	p.Q407H	0.078 / benign	0.01 / deleterious	18.84
RNF20	9	g.104302543A>G	nd	p.D63G	0.000 / benign	0.01 / deleterious	17.91
ENTPD7	10	g.101458425G>GC	nd	p.C382CX	None	None	None
ENTPD7	10	g.101458427G >GAGCCCCCT	nd	p.G383EPPX	None	None	None
NCAPD3	11	g.134079367T>C	$7.87x10^{-5}$	p.D191G	0.199 / benign	0.20 / tolerated	23.5
MYO1A	12	g.57434994C>G	nd	p.E415Q	0.740 / Poss damaging	0.19 / tolerated	21.8
HERC1	15	g.63930974T>A	nd	p.D3968V	0.998 / Prob damaging	0.13 / tolerated	24.4
VMAC	19	g.5905029G>T	$6.61x10^{-5}$	p.R43L	0.008 / benign	0.18 / tolerated	16.09
FFAR2	19	g.35941218G>A	$7.95x10^{-6}$	p.R201H	0.784 / Poss damaging	0.07 / tolerated	20.1
C19orf47	19	g.40832261T>A	nd	p.E228V	0.382 / benign	0.02 / deleterious	23.7
A1BG	19	g.58858937A>G	nd	p.L421P	0.997 / Prob damaging	0.00 / deleterious	16.19
CRYBB2	22	g.25620990G>C	nd	p.V54L	0.966 / Prob damaging	0.01 / deleterious	27.9

Supp. Table S4: DOMINO prediction of candidate genes in Family 22

gene	LDA Score	Class	Probability of being AD
FST	3.300	Very likely dominant	1.000
SPARC	2.120	Very likely dominant	0.991
HERC1	1.804	Very likely dominant	0.974
TENM2	1.680	Very likely dominant	0.962
RNF20	0.885	Likely dominant	0.741
CRYBB2	0.299	Either dominant or recessive	0.436
EML4	-0.027	Likely recessive	0.294
FFAR2	-0.072	Likely recessive	0.278
C19orf47	-0.350	Very likely recessive	0.196
A1BG	-0.512	Very likely recessive	0.161
MYO1A	-0.535	Very likely recessive	0.157
IL31RA	-0.644	Very likely recessive	0.138
PPEF2	-0.654	Very likely recessive	0.137
VMAC	-0.723	Very likely recessive	0.126
NCAPD3	-0.918	Very likely recessive	0.103
SERPINI2	-1.035	Very likely recessive	0.092
C5orf42	-1.200	Very likely recessive	0.080
ABTB1	-1.370	Very likely recessive	0.070
ENTPD7	-1.444	Very likely recessive	0.066

Supp. Table S5: Annotation of *GDF11* and *FST* variants in Families 22 and 4527

	GDF11		*FST*	
Family	4527		22	
Country of origin	USA		Colombia	
Pedigree	Multi-affected AD		Multi-affected AD	
Genomic variation	chr12(GRCh37):g.56143335G>A		chr5(GRCh37):g.52778791G>A	
Exon	Exon 3 of 3		Exon 2 of 6	
cDNA variation	NM_005811.4:c.893G>A		NM_013409.2:c.167G>A	
Effect	Nonsynonymous SNV		Nonsynonymous SNV	
Protein variation	NP_005802.1:p.(Arg298Gln)		NP_037541.1:p.(Cys56Tyr)	
Effect	Missense		Missense	
PFAM domain	RXXR motif		CR-NTD	
Pathogenicity Classification	Likely pathogenic		Likely Pathogenic	
ACMG Evidence Group	PS3, PM1, PM2, PP3		PS3, PM1, PM2, PP3	
RVIS v2	49.76%		82.45%	
ExAC Missense constraint	z = 4.47		z=2.92	
ExAC LoF intolerance	pLi = 0.96		pLI=0.96	
CADD score/prediction	35	Damaging	28.8	Damaging
REVEL score/prediction	0.608	Damaging	0.939	Damaging
PROVEAN score/prediction	-3.2	Damaging	-9.72	Damaging
SIFT score/prediction	0.001	Damaging	0	Damaging
Polyphen2 (HVAR) score/prediction	0.982	Probably damaging	0.766	Probably damaging
Polyphen2 (HDIV) score/prediction	1	Probably damaging	0.979	Probably damaging

Mutation Taster score/prediction	1	Disease causing	1	Disease causing
FATHMM score/prediction	-1.54	Damaging	-7.31	Damaging
LRT score	0	Deleterious	0	Deleterious
Mutation Assessor score/prediction	3.09	Medium	3.065	Medium
VEST3 score/prediction	0.782	Damaging	0.991	Damaging
MetaSVM score/prediction	0.547	Damaging	0.964	Damaging
MetaLR score/prediction	0.695	Damaging	0.991	Damaging
M CAP score/prediction	0.141	Damaging	0.808	Damaging
DANN score/prediction	0.999	Damaging	0.996	Damaging
FATHMM MKL score/prediction	0.97	Damaging	0.996	Damaging
Eigen score/prediction	0.865	Damaging	0.733	Damaging
GenoCanyon score/prediction	1	Damaging	1	Damaging
fitCons score/prediction	0.672	Tolerable	0.726	Damaging
GERP score/prediction	4.95	Conserved	4.8	Conserved
phyloP score/prediction	10.003	Conserved	9.253	Conserved
phastCons score/prediction	1	Conserved	1	Conserved
SiPhy score/prediction	16.072	Conserved	17.834	Conserved
ReVe score/prediction	0.7769	Tolerated	0.9077	Tolerated
dbSNP	-		-	
gnomAD Exome ALL	-		-	
gnomAD genome ALL	-		-	
ExAC ALL	-		-	
ExAC nontcga ALL	-		-	

ExAC nonpsych ALL	-	-
1000genomes ALL	-	-
ESP6500siv2 ALL	-	-
Kaviar AF	-	-
HRC AF	-	-
HRC non1000G AF	-	-
CG69	-	-
COSMIC	-	-
ICGC	-	-
ClinVar	-	-
denovo-db	-	-
GWAS Catalog	-	-
Segmental duplication	-	-

Supp. Table S6: Genotyping of candidate variants in Families 4527 and 22.

Affected individuals = asterisked. Genotypes in grey font indicate heterozygosity in an unaffected individual.

A

Family 4527							
		individual					
gene	**variant**	II.1	II.5	III.1*	III.3	III.8*	III.10
CHRM3	*g.240071784T>C*	TT	TC	TC	TT	TC	TT
TNS3	*g.47331594G>A*	GG	GA	GA	GA	GA	GA
PCDH8	*g.53420234C>T*	CT	CC	CT	CT	CT	CT
TRPM2	*g.45815425C>G*	CG	CC	CG	CC	CG	CG
HARS	*g.140057542A>G*	AG	AA	AG	AG	AG	AA
TM2D3	*g.102182739G>T*	GT	GG	GT	GT	GT	GT
GDF11	*g.56143335G>A*	GG	GG	GA	GG	GA	GG

B

Family 22						
		individual				
gene	**variant**	I.1	II.1*	II.2	III.2*	III.3*
SPARC	*g.151043709G>C*	GC	GC	GG	GC	GC
HERC1	*g.63930974T>A*	TA	TA	TT	TA	TA
TENM2	*g.167474466G>T*	GT	GT	GG	GT	GT
RNF20	*g.104302543A>G*	AG	AG	AA	AG	AG
EML4	*g.42472707C>T*	CT	CT	CC	CT	CT
FST	*g.52778791G>A*	GG	GA	GG	GA	GA