Original Article

Clinical Auditory Phenotypes Associated with *GATA3* Gene Mutations in Familial Hypoparathyroidism-deafness-renal Dysplasia Syndrome

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Abstract

Background: Hypoparathyroidism-deafness-renal dysplasia (HDR) syndrome is an autosomal dominant disorder primarily caused by haploinsufficiency of GATA binding protein 3 (*GATA3*) gene mutations, and hearing loss is the most frequent phenotypic feature. This study aimed at identifying the causative gene mutation for a three-generation Chinese family with HDR syndrome and analyzing auditory phenotypes in all familial HDR syndrome cases.

Methods: Three affected family members underwent otologic examinations, biochemistry tests, and other clinical evaluations. Targeted genes capture combining next-generation sequencing was performed within the family. Sanger sequencing was used to confirm the causative mutation. The auditory phenotypes of all reported familial HDR syndrome cases analyzed were provided.

Results: In Chinese family 7121, a heterozygous nonsense mutation c.826C>T (p.R276*) was identified in *GATA3*. All the three affected members suffered from sensorineural deafness and hypocalcemia; however, renal dysplasia only appeared in the youngest patient. Furthermore, an overview of thirty HDR syndrome families with corresponding *GATA3* mutations revealed that hearing impairment occurred earlier in the younger generation in at least nine familial cases (30%) and two thirds of them were found to carry premature stop mutations.

Conclusions: This study highlights the phenotypic heterogeneity of HDR and points to a possible genetic anticipation in patients with HDR, which needs to be further investigated.

Key words: GATA binding protein 3; Genetic Anticipation; Hypoparathyroidism-deafness-renal Dysplasia Syndrome

Introduction

Hypoparathyroidism-deafness-renal dysplasia (HDR) syndrome (MIM 146255), also known as Barakat syndrome,[1] is a rare autosomal dominant disorder named from a triad of hypoparathyroidism, sensorineural deafness, and renal dysplasia.[2] The individuals affected by HDR syndrome have various heterogeneous clinical characteristics. Sensorineural deafness could be the most common clinical feature, while hypoparathyroidism and renal dysplasia were described by various expressions[3-5] and even could be asymptomatic, making a timely diagnosis of HDR syndrome more important.

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GATA binding protein 3 (*GATA3*), a gene belonging to the family of zinc finger transcription factors and binding to the

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[A/T] GATA [A/G] consensus sequence, is the only reported gene responsible for this unusual developmental disease. Located on chromosome 10p15, *GATA3* contains two N-terminal transactivating domains (TA1 and TA2) and two C-terminal zinc finger domains (ZnF1 and ZnF2), as shown in Figure 1. To date, more than fifty *GATA3* mutations related with both sporadic and familial HDR syndrome have been reported, and *GATA3* haploinsufficiency has been considered as the underlying mechanism.[6,7] Compared with sporadic cases, familial cases provide us the opportunity to explore the inheritance pattern and to consider the possible genetic anticipation in patients with HDR.

In the present study, we identified a nonsense mutation in *GATA3*[6] in a hearing impaired Chinese family with various clinical features of HDR syndrome by using targeted capture and next-generation sequencing (NGS). In addition, auditory phenotype in familial HDR syndrome associated with *GATA3* mutation was analyzed by reviewing previous literatures.

Methods

Patients

A 7-year-old boy (proband) came from Chinese family 7121, a three-generation family with a segregating autosomal dominant hearing loss (HL) as shown in Figure 2, and four family members were recruited and gave written consent. This study was approved by the Ethics Committee of Chinese People's Liberation Army General Hospital.

Clinical evaluations for parathyroid glands, renal, and auditory phenotypes

Their medical histories were collected by a questionnaire. Physical examination, otoscopy, immittance testing, pure tone audiometric examination, and speech audiometry were performed on the three affected members to evaluate the auditory conditions. The diagnosis of sensorineural hearing impairment was made according to the World Health Organization criteria available at http://www.who.int/. The degrees of HL were categorized as mild (26–40 dB HL), moderate (41–60 dB HL), severe (61–80 dB HL), and profound HL (>80 dB HL). A computed tomography (CT) scan for the temporal bone of both ears was also performed on the proband.

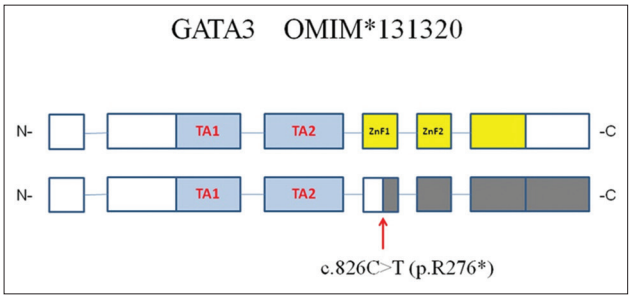

Figure 1: Structure map of *GATA3* gene: *GATA3* contains 6 exons and the arrow denotes the mutation identified in family 7121 located within exon4; *GATA3*: GATA binding protein 3. N: N-terminus; TA: Transactivating domains; ZnF: Zinc fingers domains; C: C-terminus.

Peripheral blood and urine samples were collected to measure the parathyroidal and renal function. Biochemical laboratory tests included serum calcium, magnesium, phosphorus, and intact parathyroid hormone (iPTH) levels, plasma creatinine, and carbamide levels, whereas urinalysis, renal ultrasound, and nuclear examinations were applied to detect the renal anomalies.

Targeted sequencing and variation analysis

Genomic DNA was extracted from peripheral blood sample from the three affected members and one unaffected member. After the examination of DNA quality, Beijing Genomics Institute built the DNA libraries by following the Illumina's protocol, and then 307 deafness-related genes [Supplementary Table 1] including exons, splicing sites, and their flanking introns were captured by using a custom probe and sequenced by Illumina HiSeq2000 (Illumina, San Diego, CA, USA), which had been previously described.[8]

The paired-end reads generated by sequencing were aligned to NCBI37/hg19 assembly by the Burrows-Wheeler Alignment Tool (version 0.7.10, http://bio-bwa.sourceforge.net/), and variant calling was performed by Genome Analysis Toolkit (version 3.3-0, https://software.broadinstitute.org/gatk/index.php).

Variants with allele frequencies higher than 5% in the 1000 Genomes Project and the local database were excluded. Splicing site, frameshift, and nonsense variants would be taken into further consideration. Moreover, SIFT (http://sift.jcvi.org/) and PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) softwares were used to evaluate the pathogenic possibility of missense variants. Sanger sequencing was performed to establish the co-segregation of the candidate gene mutations with the phenotype in the family members. A three-dimensional structure of GATA3 was built by Swiss-model (http://swissmodel.expasy.org/workspace/) and then visualized by Swiss-PdbViewer (version 4.1, http://spdbv.vital-it.ch/).

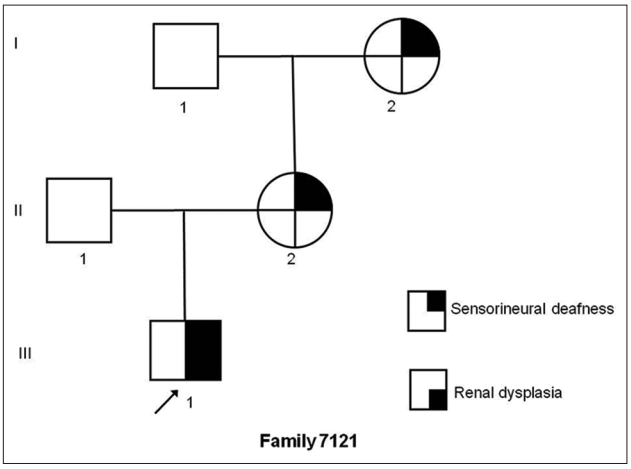

Figure 2: Pedigree of a family with hypoparathyroidism-deafness-renal syndrome. The arrow denotes proband.

Analysis of familial cases and related mutations

Literature review was performed by searching EMBASE and PUBMED databases. The genotypes and auditory phenotypes of these familial HDR syndrome cases were summarized. Then, a comprehensive inter- and intra-family comparison of clinical deafness characteristics was performed.

Results

Mutation detection and analysis

All the three hearing-impaired family members were identified to carry out the same *GATA3* mutation. The heterozygous c.826C>T (NM_002051.2) is a nonsense mutation located within exon4 that resulted in a premature termination codon (R276*) predicted to lead to *GATA3* haploinsufficiency [Figures 1 and 2]. Co-segregation of this mutation with the disease was confirmed by using Sanger sequencing as shown in Figure 3. The normal member among the siblings did not have the mutation, while the other three affected members were carrying the same nonsense mutation. Moreover, the absence of this mutation in the 1000 Genomes Project and 1751 ethnicity-matched normal hearing individuals further supported the pathogenicity.

Clinical description

As shown in Table 1 and Figure 4, the three affected members in family 7121 had early-onset sensorineural deafness. The average hearing thresholds in the better ears of proband and his mother (II2) were 56 and 45 dB HL, respectively, belonging to moderate HL according to the grades of hearing impairment from the World Health Organization. However, grandmother of proband (I2) had profound hearing impairment with the average hearing threshold of 85 dB HL. The proband and his mother (II2) could communicate without any difficulty because their hearing disturbances were not severe. Temporal bone CT scans performed on the proband were normal.

The results of biochemistry tests are summarized in Table 1. Clinically, they all had no symptom for hypoparathyroidism, but the assessment showed hypocalcemia, lower iPTH level, and mild hyperphosphaturia. In contrast, the urinalysis of all the affected members revealed no abnormalities and indicated a normal renal function.

However, nephrosonography showed that the proband had left renal agenesis while the other two affected family members had normal bilateral kidneys without any detectable

Table 1: Genetic and clinical characteristics in family 7121

Member number	Gender	Age at diagnosis (years)	Genotype	Sensorineural deafness		Hypoparathyroidism			Renal hypoplasia	
			c. 826C>T (p.R276*)	Age of onset (years)	Audiometry	Calcium (mmol/L)	Phosphorus (mmol/L)	Intact parathyroid hormone (pg/ml)	Nephrosonography	Creatinine (μmol/L)
I-2	Female	52	Positive	20	Profound	2.1	2.23	17.8	Normal	74
II-2	Female	31	Positive	19	Moderate	1.92	1.33	17.1	Normal	66
III-1	Male	7	Positive	5	Moderate	1.69	1.41	16.8	Left renal agenesis	54
II-1	Male	33	Negative	–	Normal	–	–	–	–	–
Normal range	–	–	–	–	–	2.02–2.6	0.81–1.55	15–65	–	45–110

*Represents the stop of coding.

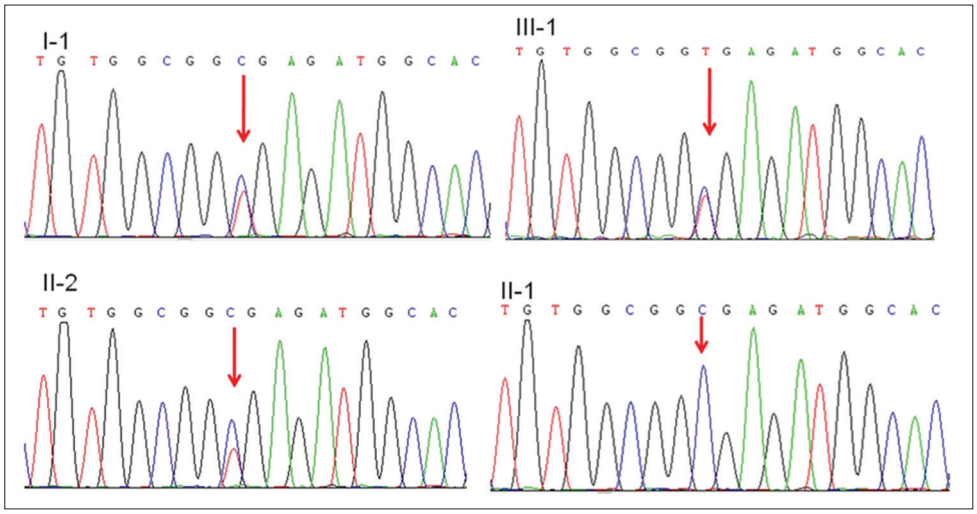

Figure 3: Sanger sequencing results and the co-segregation of the mutation with the phenotype in the family members with hypoparathyroidism-deafness-renal syndrome. Red arrows denote *GATA3* mutation c.826C>T (p.R276*). *GATA3*: GATA binding protein 3.

abnormality. Then, nuclear medical examination on the proband showed the normal renal function further.

Overview of familial hypoparathyroidism-deafness-renal dysplasia syndrome

The reported familial cases of HDR syndrome were summarized in Table 2 by different mutations. A total of 30 families carrying various *GATA3* abnormalities contained missense/nonsense mutations, small deletions and insertions (indels), splicing, and gross deletions. All the corresponding onset time and laterality of HL observed in the familial cases are shown in Table 2. Remarkably, nine parent-child pairs were proved to have hearing impairment earlier more than a decade or more severe in the younger generation, which was observed in 30% of all familial cases. There was a significant difference in the types of the mutations in these

Table 2: Review of genotype and auditory phenotypes in familial hypoparathyroidism-deafness-renal syndrome

Type	Exon	DNA	Protein	Relationship	Deafness	Diagnosis time (years or as denoted)	Reference
Missense/nonsense	2	c. 64C>T	p.Gln22*	Mother female†	B	Adult	[7]
				Son male†	B	2	
		c. 149delT	p.Phe51Leufs*144	Mother female†	B	16	[9]
				Daughter female†	B	7	
		c. 515C>A	p.S172*	Father male†	B	Birth	[10]
				Son male†	B	Birth	
	3	c. 708delC	p.Ser237Alafs*29	Mother female†	L > R	3	[7]
				Daughter female†	B	2.5	
				Son male†	B	Birth	
		c. 404-405insC	p.Ala136Glyfs*168	Father male	B	7	[7]
				Daughter female	B	3	
				Sister female	B	8	
				Father male	B	Childhood	[11]
				Daughter female	B	3	
		c. 682G>T	p.Gln228*	Mother female	B	<25	[12]
				Son male	B	4	
		c. 736delGinsAT	p.G246Mfs57*	Mother female	NM	NM	[13]
				Daughter female	L	NM	
	4	c. 823T>A	p.W275R	Mother female	NM	Childhood	[14]
				Daughter female	NM	Childhood	
		c. 827C>G	p.R276P	Mother female	B	Childhood	[15]
				Daughter female	B	Childhood	
				Daughter female	B	Childhood	
		c. 826C>T	p.R276*	Mother and Son	NM	Unknown	[6]
				Grandmother female	B	20	This study
				Mother female†	B	19	
				Son male†	B	5	
		c. 856A>G	p.N276D	Father male	B	Infancy	[16]
				Daughter female	B	Infancy	
				Daughter female	B	Infancy	
		c. 883-886delAACG	p.Asn295Aspfs*60	Mother female†	B	38	[7]
				Daughter female†	B	7	
				Son male†	B	5	
			p.R299Q	Mother female†	B	41	[17]
				Daughter female†	B	<27	
	5	c. 942T>A	p.C318S	Father male	B	NM	[18]
				Son male	B	Elementary school	
				Daughter female	B	NM	
		c. 1514CA>C	p.Asn320Lys	Mother female†	B	24	[12]
				Daughter female†	B	4	
	6	c. 1059A>T	p.R353S	Mother female	B	Childhood	[19]
				Daughter female	B	5	
				Son male	B	4	
		c. 1099C>T	p.R367*	Mother female	NM	Possible childhood	[14]
				Daughter female	NM	Possible	

Contd...

Table 2: Contd...

Type	Exon	DNA	Protein	Relationship	Deafness	Diagnosis time	Reference
Small indel	3	c. 431delG	p.Gly144Alafs*51	Mother female	B	6	[20]
				Daughter female	B	2	
	3	c. 478delG	p.Asp160Thrfs*35	Father male	B	Childhood	[19]
				Son male	B	10	
				Son male	B	17	
	3	c. 604delC	p.Arg202Valfs*4	Mother female	B	<30	[12]
				Son male	B	3	
	3	c. 709insC	p.Ser273Glnfs*67	Mother female	B	NM	[21]
				Daughter female	L	NM,	
	4	c. 901delCinsAACCCT	p.Leu301Asn*57	Father male	B	Childhood	[14]
				Daughter male	B	27	
				Daughter male	ABRnormal	2months	
Small insert	2	c. 255_256insGTGC	p.Arg86Valfs*219	Father male	NM	NM	[22]
				Son male	B	NM	
	3	c. 523-528dup	p.Gln178Profs*19	3 generations 5 people	B	All childhood	[23]
Splicing	Intron4	IVS4+2T>GCTTACTTCCC		Mother female	B	Children	[19]
				Daughter female	B	2	
	Intron4	IVS4+4_19del		Mother female	B	Infancy	[16]
				Son male	B	Infancy	
	Intron5	IVS5+1G>C		Son male†	B	1	[24]
				Father male†	NO	NO	
				Grandmother†	NO	NO	
Gene deletions	–	250 kb deletion	Deletion of one allele	Uncle male†	B	Adulthood	[6]
				Brother male†	B	1	
				Niece female†	B	At birth	
				Niece female	B	5	

*Represents the stop of coding, †Hearing impairment occurred earlier at least a decade or more severe in parent-child pairs. B: Bilateral; R: Right ear; L: Left ear; NM: Not mentioned; ABR: Auditory brainstem response; NO: No existence of deafness.

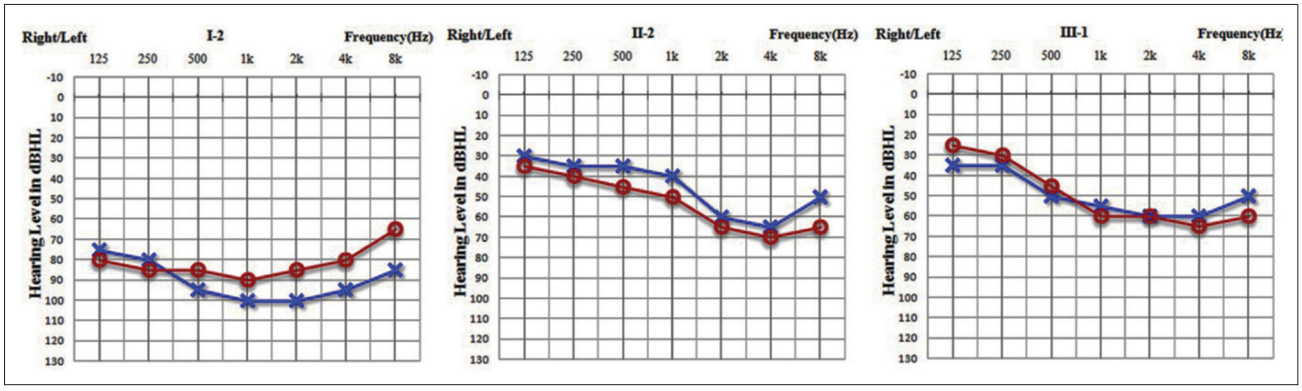

Figure 4: Pure-tone audiograms of the three affected family members with *GATA3* mutation p.R276*: blue represents left ear, red represents right ear. HL: Hearing loss; *GATA3*: GATA binding protein.

nine familial cases, indicating a high proportion of premature stop mutations as much as 66.7%.

Discussion

In the present study, a heterozygous *GATA3* nonsense mutation c.826C>T (p. R276*) was identified in a Chinese HDR family 7121 by applying a combination of the target deafness genes capture and NGS. Initially, all the affected members from three generations came to consult for their autosomal dominant hearing disturbances. Then, HDR syndrome was diagnosed precisely and effectively by using advanced genetic testing technology although there was no symptom of hypocalcemia and renal agenesis. The mutation c.826C>T (p.R276*) reported in the present study had been identified in a family 12/99 by Van Esch *et al.*[6] in the year 2000. The identification of R276* in the Chinese family 7121 further ensured its pathogenic possibility in HDR syndrome: (1) sanger sequencing confirmed the co-segregation of this

mutation with the phenotype in Chinese family 7121, and this specific mutation was absent in both the 1000 genomes and 1751 ethnicity-matched controls; (2) the location of mutation c.826C>Tin *GATA3* was visualized clearly and this nonsense mutation resulted in truncating GATA3 protein completely by losing both ZnF1 and ZnF2 domains [Figures 1 and 5]. Notably, the two zinc fingers domains were described to be necessary for GATA3 protein in binding to DNA as well as the stabilization of binding function.[7] Further functional studies demonstrated that cochlear wiring and postsynaptic differentiation were disrupted without normal GATA3 expression.[25,26]

Clinical spectrum of HDR syndrome includes hypoparathyroidism, sensorineural deafness, and renal dysplasia. As previously reported, about 62.3% of the patients had complete clinical triad in HDR syndrome.[27] The patients carrying the same mutation p.R276* in another European family displayed different clinical phenotypes. Contrary to the Chinese family 7121, the two affected members in 12/99 family did not have any renal anomaly.[6] Moreover, clinical features of patients with HDR syndrome were variable even in the same Chinese family 7121, which was also observed in other cases.[18,19,27] In fact, due to the high heterogeneous expression in individuals, it is not easy for clinicians to make a distinction between human nonsyndromic and syndromic hereditary HL such as HDR syndrome. Therefore, NGS technology could be a powerful tool in early diagnosis and appropriate management.

Genetic anticipation is a biological symptom in successive generation, in which the pedigrees appear to have earlier onset or more severity in the disease tendency. Considering the existing ascertainment bias, we insist on the presence of genetic anticipation that only a different decade is significant and reveal that at least 30% of familial cases (a total of 9 families) showed the possible genetic anticipation, which might be one of the characteristics of familial HDR syndrome. This information is especially important for assisting with family planning in genetic consulting. To our knowledge, a number of genetic diseases such as Charcot-Marie-Tooth disease,[28] Lynch syndrome,[29] and familial essential tremor[30] have been recognized with anticipation in the different mechanisms[31,32] including trinucleotide repeat expansion, telomeric dysfunction as well as epigenetic factors. Regarding the study, *GATA3* mutation analysis in Table 2 reflected a high proportion of premature stop mutations in familial patients with the possible genetic anticipation, which might be associated with the potential mechanism.

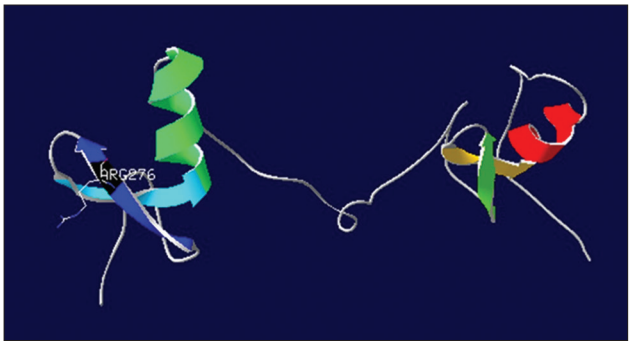

Figure 5: Three-dimensional structure of GATA3 wild-type created by SWISS-MODEL mutation p.R276* causing loss of both ZnF1 and ZnF2 domains. GATA3: GATA binding protein 3.

In conclusion, we have described a three-generation hearing impaired family with *GATA3* nonsense mutation p.R276*, which was identified in Chinese population for the first time by targeted genes capture and NGS technology. An overview of familial cases revealed a decrease in the age at onset of deafness or more severity between generations in 30% of families, indicating the presence of possible anticipation. Further studies are needed to elucidate the molecular mechanisms of this phenomenon in HDR syndrome.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of Interest

There are no conflicts of interest.

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Supplementary Table 1: Targeted captured genes list

Targeted captured gene names						
ABR	*CKB*	*ESR2*	*HOXB1*	*MYH1*	*PDZD7*	*SMS*
ACAN	*CLDN11*	*ESRRB*	*HS6ST2*	*MYH13*	*PHEX*	*SNAI2*
ACTG1	*CLDN14*	*EYA1*	*IFT88*	*MYH14*	*PLDN*	*SOBP*
AIFM1	*CLDN9*	*EYA4*	*IGF1*	*MYH2*	*PMP22*	*SOD1*
AKAP12	*CLIC5*	*FABP4*	*ILDR1*	*MYH3*	*PNOC*	*SORBS1*
ALDH1A2	*CLRN1*	*FAS*	*ITGA8*	*MYH4*	*POU1F1*	*SOX10*
ALMS1	*COCH*	*FBXO2*	*JAG1*	*MYH8*	*POU3F4*	*SOX2*
AP3D1	*COL11A1*	*FGF20*	*JAG2*	*MYH9*	*POU4F3*	*SOX9*
APAF1	*COL11A2*	*FGF3*	*KCNE1*	*MYO15A*	*PROP1*	*SPRY2*
APOA1	*COL2A1*	*FGFR1*	*KCNJ10*	*MYO1A*	*PRPS1*	*ST3GAL5*
AQP4	*COL4A3*	*FGFR2*	*KCNMA1*	*MYO3A*	*PRRX1*	*STRC*
ATF2	*COL4A4*	*FGFR3*	*KCNQ1*	*MYO6*	*PRRX2*	*TAF10*
ATOH1	*COL4A5*	*FIGN*	*KCNQ4*	*MYO7A*	*PTK7*	*TBX1*
ATP2B2	*COL9A1*	*FOXG1*	*KIT*	*NAV2*	*PTPRQ*	*TBX10*
ATP8B1	*COL9A2*	*FOXI1*	*KITLG*	*NAV3*	*RARA*	*TCOF1*
AXIN1	*CPLX1*	*FXN*	*LAMA2*	*NDP*	*RARB*	*TECTA*
BARHL1	*CRYM*	*FZD3*	*LARGE*	*NDRG1*	*RARG*	*TGFA*
BBS1	*DBH*	*FZD6*	*LFNG*	*NEFL*	*RASA1*	*TGFB2*
BBS4	*DDR1*	*GAS7*	*LHFPL5*	*NEU1*	*RDX*	*THRA*
BCR	*DFNA5*	*GATA3*	*LMO4*	*NEURL*	*S1PR2*	*THRB*
BDNF	*DFNB31*	*GBX2*	*LMX1A*	*NEUROD1*	*SCARB2*	*TIMM8A*
BMP4	*DFNB59*	*GFI1*	*LOXHD1*	*NEUROG1*	*SCO1*	*TJP2*
BSN	*DIABLO*	*GIPC3*	*LRIG3*	*NF1*	*SCRIB*	*TMC1*
BSND	*DIAPH 1*	*GJA1*	*LRP2*	*NOTCH1*	*SEMA3E*	*TMEM220*
C17orf48	*DIAPH 3*	*GJB1*	*LRTOMT*	*NOX3*	*SERAC1*	*TMIE*
C1orf125	*DIO2*	*GJB2*	*MAFB*	*NOXO1*	*SERPINB6*	*TMPRSS13*
CACNA1D	*DIO3*	*GJB3*	*MAP1A*	*NR4A3*	*SFTPC*	*TMPRSS3*
CACNB2	*DLX2*	*GJB6*	*MARVELD2*	*NTF3*	*SIX1*	*TNC*
CACNG2	*DLX5*	*GLI3*	*MCOLN3*	*NTN1*	*SIX5*	*TNFRSF11B*
CASP3	*DMD*	*GOT1L1*	*MGAT4B*	*NTRK2*	*SLC12A2*	*TMEM126A*
CCDC50	*DNAH7*	*GPR98*	*MIR182*	*NTRK3*	*SLC12A6*	*TPRN*
CD36	*DNAH9*	*GPSM2*	*MIR183*	*OC90*	*SLC12A7*	*TRIOBP*
CDH23	*DPYS*	*GPX1*	*MIR96*	*OPA1*	*SLC17A8*	*TRPV4*
CDKN1B	*DSPP*	*GRHL2*	*MITF*	*OR2T4*	*SLC19A2*	*TSHR*
CDKN2D	*DVL1*	*GRID1*	*MKKS*	*OTOF*	*SLC1A3*	*TUB*
CEACAM16	*DVL2*	*GRXCR1*	*MON2*	*OTOG*	*SLC26A4*	*TYRP1*
CELSR1	*DVL3*	*GUSB*	*MPV17*	*OTOP1*	*SLC26A5*	*UCN*
CHD7	*EDN3*	*HAL*	*MPZ*	*OTX1*	*SLC30A4*	*USH1C*
EPHB1	*EDNRB*	*HES5*	*MSRB3*	*OTX2*	*SLC4A11*	*USH1G*
HES1	*EGFLAM*	*HGF*	*MSX2*	*PAX2*	*SLC4A7*	*USH2A*
MOS	*EPHB2*	*HMX2*	*MTAP*	*PAX3*	*SLC9A1*	*USP15*
OTOA	*EPHB3*	*HMX3*	*MUC4*	*PCDH15*	*SLCO2B1*	*VANGL2*
chrM	*ERBB4*	*HOXA1*	*MUC6*	*PDE8B*	*SMAD4*	*WFS1*
CHRNA9	*ESPN*	*HOXA2*	*MUTED*	*PDSS1*	*SMPX*	*YME1L1*