Gene 528 (2013) 352–355

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short Communication

576 kb deletion in 1p36.33–p36.32 containing *SKI* is associated with limb malformation, congenital heart disease and epilepsy

Xin Zhu [a], Yi Zhang [a], Jian Wang [b,c], Jin-Fu Yang [b,c], Yi-Feng Yang [b,c], Zhi-Ping Tan [b,c,*]

[a] Department of Gynecology and Obstetrics, Xiangya Hospital of Central South University, China
[b] Department of Cardiothoracic Surgery, the Second Xiangya Hospital of Central South University, China
[c] Clinical Center for Gene Diagnosis and Therapy of State Key Laboratory of Medical Genetics, the Second Xiangya Hospital of Central South University, Changsha, Hunan Province 410011, China

ARTICLE INFO

Article history:
Accepted 2 July 2013
Available online 25 July 2013

Keywords:
1p36 deletion
Shprintzen–Goldberg syndrome
SNP array
Epilepsy
Copy number variation
Congenital heart defect

ABSTRACT

1p36 deletion (monosomy 1p36) is one of the most common terminal deletions observed in humans, characterized by special facial features, mental retardation, heart defects, development delay and epilepsy. Previously, we reported molecular findings in patients with limb, congenital heart disease (CHD) and other malformations with SNP-array. In a syndromic patient of the same cohort, we detected a small deletion of 1p36.33–p36.32 containing *SKI* (Sloan–Kettering Institute protooncoprotein). Recently, dominant mutations in *SKI* were identified to be correlated with Shprintzen–Goldberg syndrome. Retrospective examination revealed this patient with limb malformations, CHD, epilepsy and mild development delay. Together with previous reports, our study suggests that the 1p36.33–1p36.32 deletion encompassing *SKI* may represents a previous undescribed microdeletion disorder.

1. Introduction

1p36 deletion syndrome (monosomy 1p36) is the most common terminal deletion syndrome seen in humans. The main clinical findings of 1p36 deletion are typical craniofacial dysmorphism, developmental delay and mental retardation. Central nervous system malformations (88%), heart defects (71–75%), seizures (44–79%), skeletal anomalies (41%) and other features (e.g. vision problems, hearing loss) are also frequently observed in patients with 1p36 deletion syndrome (Battaglia et al., 2008; Bursztejn et al., 2009; Gajecka et al., 2007; Heilstedt et al., 2003; Zenker et al., 2002). Because of variability in phenotypes, diversity in the sizes and positions of the deletion (Heilstedt et al., 2003), little genotype–phenotype correlations have been found among 1p36 deletion syndrome (Buck et al., 2011). This has led to the hypothesis that the 1p36 deletion syndrome may be constituted by different types of microdeletion disorders (terminal, interstitial or others) with variable causative gene(s) for specific phenotypic features (Heilstedt et al., 2003; Rosenfeld et al., 2010).

Malformations in limb and ear are clinically important because they facilitate the diagnosis of patients with multiple malformations (Biesecker, 2011). In our clinical gene diagnosis center, we have genotyped a cohort of 206 patients with an Illumina SNP-array platform in CHD patients with limb, ear and other malformations (Chen et al., 2012; Luo et al., 2012). In a patient from the same cohort, we identified a small deletion of the 1p36.33–p36.32 region containing *SKI*, *PRKCZ* and *PEX10*. Because no information is available in the literature regarding the pathological consequences caused by these genes, we defined this genomic lesion as copy number variation (CNV) with unknown clinical importance. Recently, *SKI* (Sloan–Kettering Institute protooncoprotein) was identified as causative gene for Shprintzen–Goldberg syndrome (SGS, MIM 182212) (Carmignac et al., 2012; Doyle et al., 2012). Retrospective examination identified this patient with limb malformations, CHD, epilepsy and mild development delay.

We describe here a two-year old boy with multiple malformations and a 576 kb deletion in 1p36.33–p36.32 containing *SKI*. Clinical and genetic findings in all four patients (including this patient) with small 1p36.33–1p36.32 deletions were summarized.

2. Clinical report

The patient was first seen in the Department of Gynecology and Obstetrics of Xiangya Hospital. The baby boy was born to a normal and non-consanguineous Chinese parent. Family history of birth defects was absent. Vaginal bleeding occurred during the first 3 months of pregnancy and medical treatment was required. The patient was born by cesarean section at 40th week of gestation weighing 3,000 g (25th centile) and measuring 45 cm (25th centile) with a head circumference of 33 cm (25 centile). After birth, the patient was observed to have

Abbreviations: CNV, copy number variation; CHD, congenital heart defects; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction; CT, computed tomography; OMIM, Online Mendelian Inheritance in Man; Hg19, human genome 19; ASD, atrial septal defect; DGV, Database of Genomic Variants; SGS, Shprintzen–Goldberg syndrome.

* Corresponding author at: Department of Cardiothoracic Surgery, The Second Xiangya Hospital of Central South University, 139 Renmin Road, Changsha, Hunan 410011, China. Tel.: +86 731 8529 2178; fax: +86 731 8529 2133.
E-mail address: tanzhiping@gmail.com (Z.-P. Tan).

0378-1119/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.07.024

cardiac murmurs and a hypoplastic thumb of left hand. Psychomotor milestones were mildly delayed, sitting without support at 10 months and walking independently at 18 months. Other related information was not remarkable.

At 2 years old, the patient was referred to our in-patient department for surgical treatment of cardiac defects. The boy was diagnosed with secundum atrial septal defect (ASD) by two-dimensional color Doppler echocardiography. The ASD was subsequently surgically repaired. Physical examination showed short stature with a height of 80 cm (3rd centile) and a weight of 12 kg (3rd centile). He had facial features including upslanting palpebral fissures, broad nasal tip, elongated philtrum, a pointed chin and a small mouth (Fig. 1.A). A hypoplastic thumb of left hand (with less function) and bilateral fifth finger clinodactyly were also observed. (Fig. 1.B). Epileptic seizures occurred in early childhood and he has been seizure-free for 6 months. A CT scan revealed slightly enlarged cerebral ventricles.

3. Methods

The Review Board of the Second Xiangya Hospital of the Central South University approved this study. All subjects consented to this study.

3.1. Cytogenetic analysis

Chromosome analysis was performed with the peripheral blood samples of the patient by conventional G-Banded techniques at the level of 550 bands. Five milliliters of peripheral blood was collected and subjected to lymphocyte culture according to standard cytogenetic protocol.

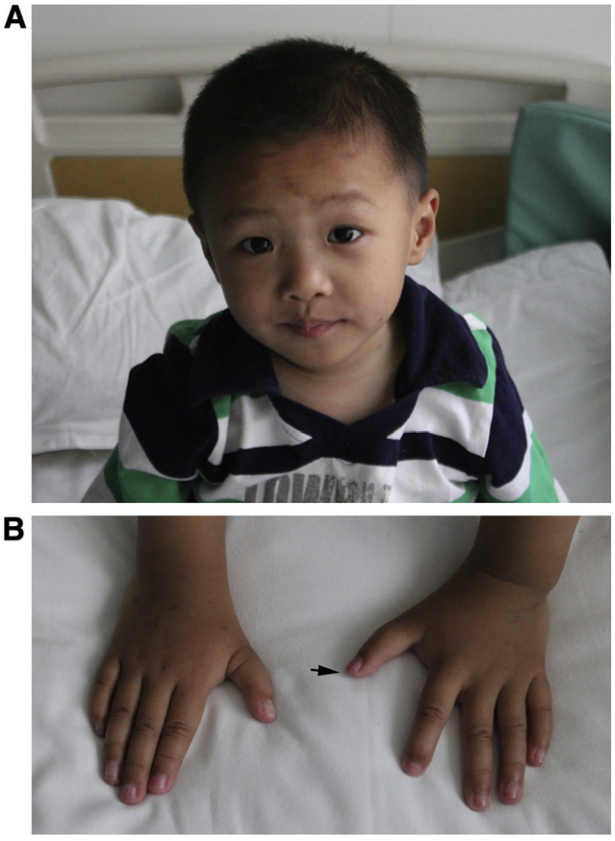

Fig. 1. Clinical features of the proband. (A) Upslanting palpebral fissures, broad nasal tip, elongated philtrum, a pointed chin and a small mouth. (B) A hypoplastic thumb of left hand with less function (arrow) and bilateral fifth finger clinodactyly.

3.2. DNA extraction

The parents of the patient gave written informed consent and genomic DNA was prepared from peripheral blood of the patient and his parents. Genomic DNA was prepared using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) on the QIAcube automated DNA extraction robot (Qiagen, Hiden, Germany).

3.3. SNP array analysis

The Human660W-Quad Chip (Illumina Inc, San Diego, USA) and the Illumina BeadScan genotyping system (Beadstation Scanner) were employed to obtain the signal intensities of probes (SNP) following the manufacturer's instructions. The Illumina GenomeStudio V2011 and Illumina cnvPartition (V2.3.4) softwares were used to analyze the genotypes (human genome build 37/Hg19) and evaluate the experimental quality. The call rates of the samples are greater than 99.5%. We performed a family-based CNV validation with the PennCNV algorithm to obtain authentic CNVs.

3.4. Quantitative PCR validation

To validate variable copy numbers, real-time quantitative PCR (qPCR) were performed using the 7500 Fast Real-Time PCR systems (Applied Biosystems, Foster City, California). For potentially pathogenic CNV, two primer sets were designed within the boundaries of the CNV region. Primer pairs were designed by an online tool (PrimerQuest, IDT) (http://www.idtdna.com/Primerquest/Home/Index). PCR reactions were prepared with the SYBR Premix Ex Taq II PCR reagent kit (TaKaRa Bio, Dalian, China) according to the manufacturer's protocol. Amplification efficiencies were identical and the relative copy number was calculated with the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

4. Results

Karyotype analysis of the patient was apparently normal. We hypothesized that copy number variations may contribute to the multiple birth defects of this boy and employed SNP-array analysis to identify such a change (Human660W-Quad Chip, Beadstation Scanner and GenomeStudio V2011 software). We identified a 576 kb deletion of 1p36.33–1p36.32 (chr1:2,045,453-2,622,423/Hg19), which includes *SKI*, *PRKCZ*, *PEX10* and *PLCH2* (Fig. 2). Real-time quantitative PCR (qPCR) with genomic DNA of the trios (the proband and his parents) confirmed this *de novo* deletion. We compared the clinical and genetic features of this patient with those patients carrying small deletions (<1 Mb) of 1p36.33–1p36.32 containing *SKI* (Table 1).

5. Discussion

Shprintzen–Goldberg syndrome (SGS, OMIM# 182212) is a rare autosomal-dominant disorder with a marfanoid habitus and neurological, cardiovascular and skeleton anomalies. SGS has been previously suspected to be associated with aberrant TGF-β signaling. In consistent with this suggestion, mutations in FBN1 and TGFBR2 have been detected in patients with phenotypes of SGS (Kosaki et al., 2006; Sood et al., 1996). Therefore, it is likely that SGS, Marfan syndrome and Loeys–Dietz syndrome (LDS) share a similar pathogenesis. Recently, *SKI* (Sloan–Kettering Institute protooncoprotein) was found to underlie SGS by two independent groups with exome sequencing (Carmignac et al., 2012; Doyle et al., 2012).

SKI is a conserved protein and functions as a negative regulator of the TGF-β signaling (Luo, 2004). *SKI* locates at chromosome 1p36 and has been proposed to contribute for the phenotypes such as cleft lip/palate in 1p36 deletion syndrome (Colmenares et al., 2002). This hypothesis is also supported by the study of *ski* homozygous mutant

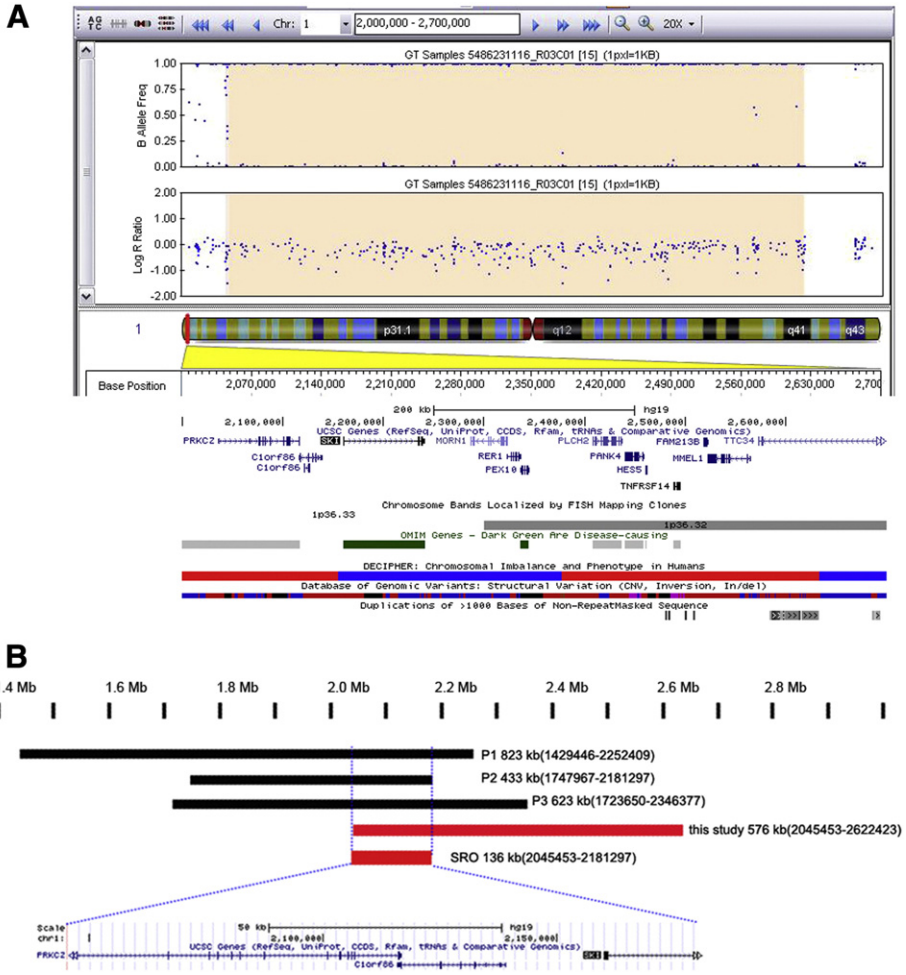

Fig. 2. Human 660w-Quad SNP-array result of the 1p36.33–1p36.32 deletion in the proband. (A) SNP based array shows a 576 kb deletion of 1p36.33–1p36.32 (chr1:2,045,453-2,622,423/Hg19). The ratio of Log R and frequency of B allele are showed in upper panel; (B). The lower panel shows the individuals with 1p36.33–1p36.32 deletions containing *SKI* in the literature. The smallest overlap region (SRO) is indicated. Genomic data have been converted to Hg19. Patients 1, 2 and 3 are Patients 3, 4 and 5 reported by Rosenfeld et al. (2010), respectively.

mice, which show defects in craniofacial patterning, neuroepithelial and skeleton muscle development (Berk et al., 1997).

Four patients (including this study) with small interstitial deletions of 1p36 encompassing *SKI* (<1 Mb) have been reported (Rosenfeld et al., 2010). Interestingly, three of four patients (75%) have seizures (Table 1). The critical region for epilepsy of 1p36 deletion syndrome has been previously defined to 1p36.3 region. Two genes, *KCNAB2* and *GABRD*, are associated with 1p36 seizure phenotype (Heilstedt et al., 2001; Windpassinger et al., 2002). However, neither of these genes is mapped in the critical region of our study (Fig. 2). Therefore it is possible that a different candidate gene in the critical region may contribute to the seizure phenotype (Rosenfeld et al., 2010).

Our proband has a less severe phenotype compared with SGS patients, e.g., the skeleton malformations, arachnodactyly/camptodactyly and joint contracture. It suggests that deletion of *SKI* (loss-of-function) and point mutation of *SKI* (gain-of-function) may result in differences in the severity or types of clinical features. Several other plausible explanations may also account for these findings. The mutant SKI may exert a dominant-negative effect on cell structure and signaling transduction. The missense mutants (poison peptide) impair the biosynthesis as well as the trafficking of wild-type SKI proteins (Clatot et al., 2012). Another explanation is that the gene(s) mapped in the deleted region may have position effect that regulates neighboring gene(s) (Redon et al., 2005). In addition, mitral valve prolapse and aortic root dilation, two important features in cardiovascular systems of SGS patients, are not presented in our patient. Given that aortic root dilation is a progressive disease, it is possible these symptoms might appear when our proband grows older.

In conclusion, we report the clinical and molecular findings in a patient with a de novo 576 kb deletion in interstitial 1p36.33–1p36.32 region (chr1:2,045,453-2,622,423/Hg19). Our study supports the notion that haploinsufficiency of *SKI* underlies the etiology of limb anomalies, CHD, development delay. Together with previous reports, 1p36.33–1p36.32 encompassing *SKI* may represent a previously undescribed disorder.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

We thank the patient and his parents for participating in this study. We thank Prof. Long Ma for critical suggestions and the State Key Laboratory of Medical Genetics of China for technical assistance. We appreciate supports from the National High Technology Research and Development Program of China (863 Program) (2011AA02A112 to Y.Y.F.), and the National Natural Science Foundation of China (81101475 to T.Z.P.).

Table 1
Summary of clinical findings of individuals with small interstitial1p36 deletion containing *SKI*.

Patient reported	1p36 del (%)[a,b]	SGS[c,d]	Small interstitial1p36 deletion containing *SKI*				
			Patient 1[e]	Patient 2[e]	Patient 3[e]	This study	Total
Sex			F	M	M	M	1 F/3 M
Age			17.5y	4.5y	14y	2y	
Size of deletion (kb)			623	433	823	570	
Dysmorphic features							
Broad nasal tip	38	UK	+	+	+	+	4/4
Pointed chin	63–100	UK	−	+	+	+	3/4
Midface hypoplasia	51–100	UK	+	+	−	+	3/4
Deep-set eyes	73–100	UK	−	+	+	−	2/4
Low-set ears	49	29/29	+	−	−	+	2/4
Large anterior fontanelle	74–77	UK		+			1/4
Clinodactyly	60	UK	−	−	−	+	1/4
Brachydactyly	54–80	UK	+	−	−	−	1/4
Neurological							
Development delay	100	29/29	+	+	+	+	4/4
Seizures	44–79	UK	−	+	+	+	3/4
Intellectual disability	95–100	29/29	+	+	+	−	3/4
Speech delay	79–100	UK	+	+	+	−	3/4
Behavior disorder	47–55	UK	−	−	+	−	1/4
Neonatal hypotonia	92–95	UK	−	−	+	−	1/4
Cardiovascular							
Heart defects	71–75	UK	−	−	−	+, ASD	1/4
Cardiomyopathy	23–31	UK	−	−	−	−	0/4
Visual	44–52	UK	−	−	+	−	1/4
Hearing loss	28–77	UK	−	−	−	−	0/4
Obesity	UK	UK	+	+	+	−	3/4
Other malformations							
High palate	UK	29/29	+	−	−	−	1/4
Kidney abnormalities	22	UK	−	−	+	−	1/4

Abbreviations: M, male; F, female; y, year; ASD, atrial septal defect; UK, unknown.
[a,b]Based on Gajecka et al. (2007)[a] and Battaglia et al. (2008)[b].
[c,d]Based on Doyle et al. (2012)[c] and Carmignac et al. (2012)[d].
[e]Based on Rosenfeld et al. (2010)[e]. Patients 1, 2 and 3 are Patients 3, 4 and 5 reported by Rosenfeld et al. (2010)., respectively.

References

Zenker, M., et al., 2002. Monosomy 1p36—a recently delineated, clinically recognizable syndrome. Clin. Dysmorphol. 11, 43–48.
Heilstedt, H.A., et al., 2003. Physical map of 1p36, placement of breakpoints in monosomy 1p36, and clinical characterization of the syndrome. Am. J. Hum. Genet. 72, 1200–1212.
Gajecka, M., Mackay, K.L., Shaffer, L.G., 2007. Monosomy 1p36 deletion syndrome. Am. J. Med. Genet. C: Semin. Med. Genet. 145C, 346–356.
Battaglia, A., et al., 2008. Further delineation of deletion 1p36 syndrome in 60 patients: a recognizable phenotype and common cause of developmental delay and mental retardation. Pediatrics 121, 404–410.
Bursztejn, A.C., Bronner, M., Peudenier, S., Gregoire, M.J., Jonveaux, P., Nemos, C., 2009. Molecular characterization of a monosomy 1p36 presenting as an Aicardi syndrome phenocopy. Am. J. Med. Genet. A 149A, 2493–2500.
Buck, A., du Souich, C., Boerkoel, C.F., 2011. Minimal genotype–phenotype correlation for small deletions within distal 1p36. Am. J. Med. Genet. A 155A, 3164–3169.
Rosenfeld, J.A., et al., 2010. Refinement of causative genes in monosomy 1p36 through clinical and molecular cytogenetic characterization of small interstitial deletions. Am. J. Med. Genet. A 152A, 1951–1959.
Biesecker, L.G., 2011. Polydactyly: how many disorders and how many genes? 2010 update. Dev. Dyn. 240, 931–942.
Chen, J.L., Yang, Y.F., Huang, C., Wang, J., Yang, J.F., Tan, Z.P., 2012. Clinical and molecular delineation of 16p13.3 duplication in a patient with congenital heart defect and multiple congenital anomalies. Am. J. Med. Genet. A 158A, 685–688.
Luo, H., et al., 2012. Duplication of 8q12 encompassing CHD7 is associated with a distinct phenotype but without duane anomaly. Eur. J. Med. Genet. 55, 646–649.
Doyle, A.J., et al., 2012. Mutations in the TGF-beta repressor SKI cause Shprintzen–Goldberg syndrome with aortic aneurysm. Nat. Genet. 44, 1249–1254.
Carmignac, V., et al., 2012. In-frame mutations in exon 1 of SKI cause dominant Shprintzen–Goldberg syndrome. Am. J. Hum. Genet. 91, 950–957.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402–408.
Sood, S., Eldadah, Z.A., Krause, W.L., McIntosh, I., Dietz, H.C., 1996. Mutation in fibrillin-1 and the Marfanoid-craniosynostosis (Shprintzen–Goldberg) syndrome. Nat. Genet. 12, 209–211.
Kosaki, K., et al., 2006. Molecular pathology of Shprintzen–Goldberg syndrome. Am. J. Med. Genet. A 140, 104–108 (author reply 109-110).
Luo, K., 2004. Ski and SnoN: negative regulators of TGF-beta signaling. Curr. Opin. Genet. Dev. 14, 65–70.
Colmenares, C., et al., 2002. Loss of the SKI proto-oncogene in individuals affected with 1p36 deletion syndrome is predicted by strain-dependent defects in Ski−/− mice. Nat. Genet. 30, 106–109.
Berk, M., Desai, S.Y., Heyman, H.C., Colmenares, C., 1997. Mice lacking the ski proto-oncogene have defects in neurulation, craniofacial, patterning, and skeletal muscle development. Genes Dev. 11, 2029–2039.
Heilstedt, H.A., et al., 2001. Loss of the potassium channel beta-subunit gene, KCNAB2, is associated with epilepsy in patients with 1p36 deletion syndrome. Epilepsia 42, 1103–1111.
Windpassinger, C., Kroisel, P.M., Wagner, K., Petek, E., 2002. The human gamma-aminobutyric acid A receptor delta (GABRD) gene: molecular characterisation and tissue-specific expression. Gene 292, 25–31.
Clatot, J., et al., 2012. Dominant-negative effect of SCN5A N-terminal mutations through the interaction of Na(v)1.5 alpha-subunits. Cardiovasc. Res. 96, 53–63.
Redon, R., et al., 2005. Tiling path resolution mapping of constitutional 1p36 deletions by array-CGH: contiguous gene deletion or "deletion with positional effect" syndrome? J. Med. Genet. 42, 166–171.