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# Identification of a New Candidate Locus for Ebstein Anomaly in 1p36.2

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**Established Facts**

- Deletions in 1p36 have previously been identified in patients with Ebstein anomaly (EA).
- All reported cases of EA with 1p36 deletions share *PRDM16* loss.

**Novel Insights**

- We report the first case of EA with a proximal 1p36 deletion not spanning *PRDM16*.
- We propose the existence of an additional proximal locus which can lead to EA when deleted.

**Keywords**
Ebstein anomaly · 1p36 deletion syndrome · *PRDM16* · *RERE* · *SKI* · *UBE4B*

**Abstract**
Ebstein anomaly (EA) is a rare congenital heart defect (CHD) with a poorly characterized genetic etiology. However, some EA patients carry deletions in 1p36, all of which have been reported to carry distal deletions and share loss of the *PRDM16* gene, which is currently considered the most likely candidate for EA development in this region. Here, we report a patient with an 11.96-Mb proximal 1p36 deletion, without loss of *PRDM16*, who presented with EA and a proximal deletion phenotype. This finding suggests that *PRDM16* loss is not required for the development of EA in 1p36 deletions and that the loss of an additional proximal locus in 1p36 is also likely associated with EA. Our data suggest that a distal locus containing the *SKI* gene and a proximal locus containing the CHD-associated genes *RERE* and *UBE4B* are the most probable etiological factors for EA in patients with 1p36 deletion syndrome.


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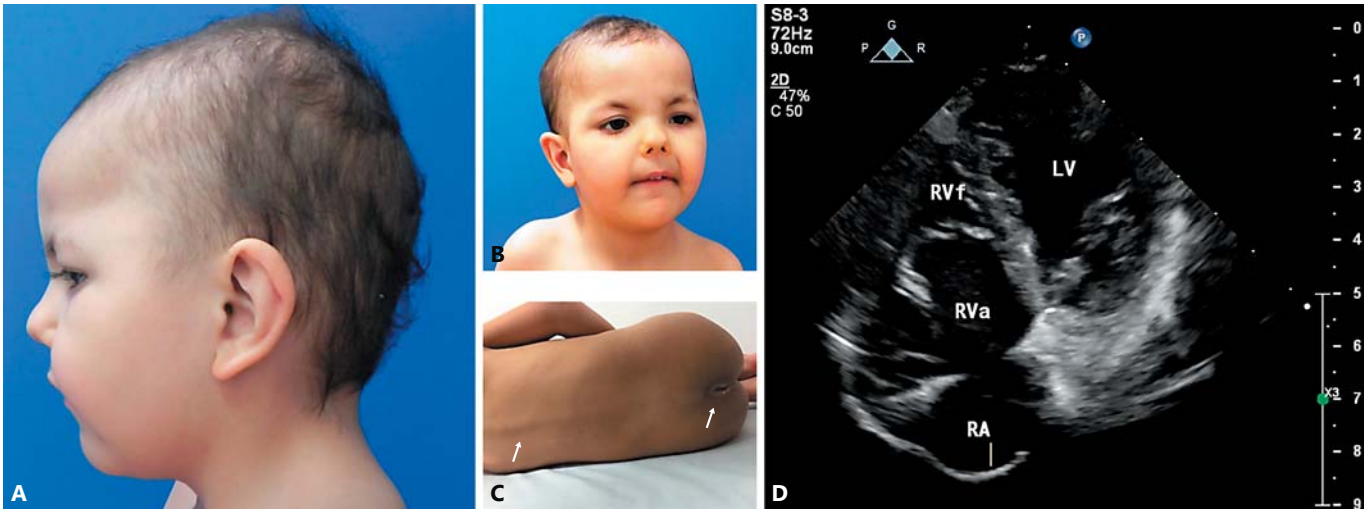


**Fig. 1. A**, **B** Phenotypic characteristics of the patient. The phenotype of the patient is consistent with reported proximal deletions in 1p36, showing trigonocephaly, midface hypoplasia, spaced and medially sparse arched eyebrows, marked superciliary ridges, telecanthus, hypoplastic and anteverted nostrils, low and wide nasal bridge, retrognatia, downturned lip commissures, ogival palate, low-set ears with folded left helix, and asymmetric palpebral fissures. **C** Sacral dimple and hypochromic lines (arrows). **D** Echocardiogram showing Ebstein anomaly type B. RA, right atrium; RVa, atrialized right ventricle; RVf, right ventricle function; LV, left ventricle.

Subtelomeric 1p36 deletion gives rise to a common syndrome, with an incidence of 1:5,000–1:10,000 [Heilstedt et al., 2003], characterized by intellectual disability, abnormal language and behavior, craniofacial features, seizures, ocular abnormalities, hearing loss, structural heart defects, and hypotonia [Shimada et al., 2015]. The size of 1p36 deletions is variable with a mean size of 5 Mb (calculated according to data from DECIPHER). Several studies have attempted to map the chromosomal regions in 1p36 associated with diverse phenotypes in patients with 1p36 deletion syndrome, focusing mainly on distal deletions [Shimada et al., 2015]. However, Kang et al. [2007] described 5 patients with an unusual phenotype carrying interstitial deletions extending from 1p36.23 to 1p36.11 and proposed a distinct proximal 1p36 deletion syndrome. Here, we report an unusual case with proximal 1p36 deletion syndrome presenting with Ebstein anomaly (EA) and discuss the role of haploinsufficiency in *UBE4B* and *RERE* as a likely additional causative defect for EA.

## Case Report and Results

The index case is a 4-year-old boy, born to healthy nonconsanguineous parents, after 40 weeks of pregnancy in which polyhydramnios, maternal hypothyroidism, and vaginal leucorrhoea were recorded. At the time of birth, his father was 33 and his mother 37 years old. Both stated having an additional healthy child from a previous relationship. At birth, weight and height were 2,760 g (3rd percentile) and 51 cm (39th percentile), respectively. The patient presented with cyanosis, generalized hypotonia, and a swallowing disorder which required orogastric tube feeding, although an endoscopic study showed only a severe gastroesophageal reflux. An echocardiogram identified EA type B with moderate tricuspid valve insufficiency, a perimembranous ventricular septal defect, peripheral pulmonary stenosis, and a bicuspid aortic valve (Fig. 1D). He presented with supraventricular tachycardia, which was easily controlled. Brain ultrasound was normal, tomography showed overriding cranial sutures and MRI revealed a diffuse subarachnoid space highlighting augmentation. Renal and abdominal echography proved normal. He was discharged from the hospital at 2 months. At the age of 1 year, he was hospitalized because of bacterial pneumonia and gastroenteritis; during his stay, 6 febrile seizures occurred with normal EEG. He received an ophthalmologic evaluation due to marked photophobia, finding keratoconus in the left eye and thin tortuous retinal vessels. Genetic studies revealed a negative MLPA result for 22q11 deletion and ruled out Mowat-Wilson syndrome by sequencing *ZEB2*.

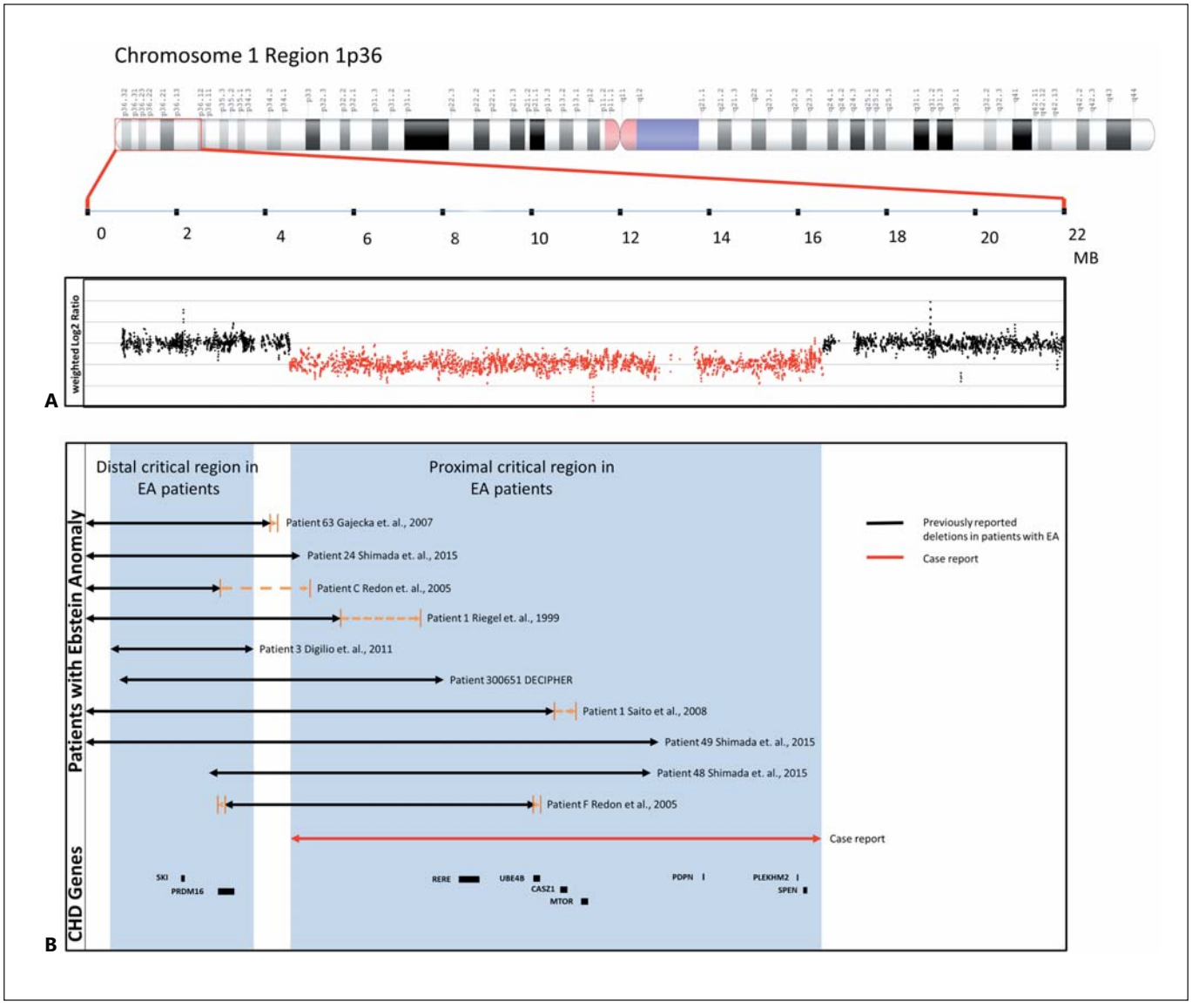


**Fig. 2.** Genotype-phenotype correlation analysis of 1p36 deletions in patients with Ebstein anomaly (EA). **A** The patient shows an 11.95-Mb deletion in chromosome 1 identified by cytogenetic microarray analysis. **B** Reported 1p36 deletions in patients with EA (black lines), including patients with loss of only the EA-associated distal locus, loss of both the distal and proximal EA-associated loci, and loss of only the proximal EA-associated locus as well as our patient (red line). Below, the location of genes in the 1p36 region are shown which are expressed in heart tissue and are associated with heart development, heart physiology, and/or congenital heart defect (CHD).

Physical examination at age 4 years showed failure to thrive, a weight of 11.9 kg (0.4 percentile), height of 94 cm (0.7 percentile), and a head circumference of 47 cm (1.2 percentile). He also presented with distinctive features (Fig. 1A, B), accompanied by hypotonia, pectus excavatum, marked linea alba which continues into the torso, hypochromic lines in the back that do not cross the midline, left cryptorchidism, short and broad phalanx of the fingers, broad palms, shortened halluces, and a sacral dimple (Fig. 1C). A psychological interview and the Wechsler intelligence scale for toddlers (WPPSI-III) showed a marked delay in developmental milestones and a total intelligence quotient of 44 points (0.1 percentile).

To identify potential chromosomal abnormalities, cytogenetic microarray analysis was performed using the Affymetrix cytoscan 750K Human Genome CNV+SNP

array with 550,000 CNV non-polymorphic probes and 200,000 SNP probes with reference to genome version GRCh37 (hg19). The array identified an 11.95-Mb deletion in chromosome 1, with breakpoints at positions 4,629,407 and 16,586,999, affecting bands 1p36.32 to 1p36.13, and including 221 genes, with breakpoints located near distal and proximal nondeleted probes C-6MMTB and C-6WWZQ, respectively (Fig. 2A). Two additional microduplications were identified, but not considered to be clinically relevant: the first one of 104 kbp in chromosome 17q21.31 (chr17:44188450–44292742), involving the genes *KIAA1267* and *LOC644246* and the second one of 181 kbp in chromosome 6p21.2 (chr6:36795665–36976418), involving the genes *CPNE5*, *PPIL1*, *C6orf89*, *PI16*, *MTCH1*, and *FGD2*. A 163-kbp microdeletion in chromosome 8p11.22 (chr8:39226335–39388919), involving the genes *ADAM5P* and *ADAM3A* was also identified.

A search in Medline (https://www.ncbi.nlm.nih.gov/pubmed/), ISCA (retrieved from https://decipher.sanger.ac.uk), and DECIPHER (https://decipher.sanger.ac.uk) [Firth et al., 2009] (with 1p36 CNV losses classified as likely pathogenic or definitely pathogenic) was performed to assemble a database of deletions (ranging from 1 to 22,000,000 bp) and phenotypes from EA patients with 1p36 deletions reported from 1999 to 2017 to identify overlapping regions associated with EA (Fig. 2B). Reports without deletion coordinates or with low-resolution data or from nonpublic databases that could not be verified were excluded.

## Discussion

The phenotype of the patient in this report is consistent with the previous description of proximal 1p36 deletion syndrome, including the presence of pre- and postnatal growth deficiency (mostly postnatal), feeding difficulties, arched eyebrows and the absence of characteristics associated with distal 1p36 deletions such as hearing loss and straight eyebrows [Kang et al., 2007].

In the literature and database search, EA was found in only 10/302 patients with 1p36 deletions. Our patient showed loss of the proximal region of 1p36, but did not show loss of either the critical region (CR) in reported cases with EA or *PRDM16* (Fig. 2), the gene proposed by Shimada et al. [2015] in the distal region to be associated with EA. *PRDM16* has been found in the region of minimal genomic overlap in individuals with 1p36 deletion syndrome, left ventricular noncompaction, and dilated cardiomyopathy, which was further assessed by Arndt et al. [2013] in 2 cohorts of nonsyndromic patients with this type of cardiomyopathy, independently, detecting 3 mutations including a truncation mutant, a frameshift null mutation, and a single missense mutant. Hence, this is the first reported case of a patient with a proximal deletion presenting with EA (Fig. 2). The distal breakpoint in the identified deletion is more than 1 Mb away from the region shared by all other reported EA cases with 1p36 deletions, making it unlikely that this deletion affects a regulatory region for genes contained in the previously reported CR. This finding suggests that *PRDM16* loss is not required for the development of EA in 1p36 deletion syndrome, and that loss of an additional proximal locus in 1p36 is also likely associated with EA.

Our data suggest that delimiting 2 separate CRs in 1p36 is required to explain the cardiac phenotype identified in EA patients with 1p36 deletions. Therefore, it is likely that there are 2 independent loci where haploinsufficiency can contribute to the development of EA, as has been proposed by Jordan et al. [2015] and Zaveri et al. [2014] for different forms of congenital heart defects (CHD). The distal CR is delimited by the patient described by Digilio et al. [2011] and contains both *PRDM16* and *SKI* gene, the latter previously associated with CHD in animal models [Doyle et al., 2013] and human genetic studies [Zhu et al., 2013; Cunnington et al., 2014; Shimada et al., 2015; Zeglinski et al., 2016; Wu et al., 2017] (Fig. 2). Patients with heterozygous missense mutations and in-frame deletions in *SKI* show a multisystemic connective tissue disorder, called Shprintzen-Goldberg syndrome, characterized by a marfanoid habitus, craniosynostosis, severe skeletal muscle hypotonia, intellectual disability, and cardiovascular abnormalities such as mitral valve prolapse and aortic dilation [Carmignac et al., 2012; Schepers et al., 2015]. The proximal CR is delimited by the deletion reported here. It is important to note that the proximal CR does not contain *PRDM16* and suggests that this gene is not necessary for the development of EA in patients with 1p36 deletions (Fig. 2).

The deletion of this case contains approximately 221 genes, including genes which are highly expressed in heart tissue and have been implicated in heart physiology or cardiac development in human, animal, or cell culture models [Jordan et al., 2015], specifically *KCNAB2*, *RNF207*, *UTS2*, *RERE*, *UBE4B*, *CASZ1*, *MTOR*, *NPPA*, *NPPB*, *PLEKHM2*, *FBLIM1*, *ZBTB17*, *HSPB7*, *ARHGEF19*, *NBPF1*, *PDPN*, and *SPEN*. However, only 7 of these genes have been linked to congenital heart disease

in humans or mouse models and are more likely to be associated with cardiac phenotypes in 1p36 deletion patients, namely *RERE*, *UBE4B*, *CAZ1*, *MTOR*, *PDPN*, *PLEKHM2*, and *SPEN* (Fig. 2). With the exception of *PDPN*, *PLEKHM2*, and *SPEN*, hemizygosity has been reported for all of these genes in patients with EA, including 2 (*UBE4B* and *RERE*) which have been implicated in the development of CHD through the regulation of myocardial development [Kaneko-Oshikawa et al., 2005; Kim et al., 2013] and are likely responsible for EA in these patients (Fig. 2).

*Ube4b* is involved in mouse cardiac development, and expressed in the heart muscle during embryogenesis and adulthood. *Ube4b*-knockout mice show enlarged hearts associated with pericardial effusion, indicative of congestive heart failure and reduced trabeculation as well as an undeveloped and compact myocardial layer with increased cardiomyocyte apoptosis during embryogenesis. Deletion of *UBE4B* leads to a misregulation of genes such as *GATA6* which have been implicated in the regulation of myocardial differentiation during cardiogenesis [Kaneko-Oshikawa et al., 2005]. No human phenotype of *UBE4B* haploinsufficiency has been described.

On the other hand, mice carrying null and hypomorphic alleles of *Rere* had several abnormalities including CHD. *Rere*-null mice die of cardiac failure between 9.5 and 11 weeks of embryogenesis, while heterozygous mice carrying a hypomorphic allele have a high frequency of cardiovascular malformations [Kim et al., 2013]. Fregeau et al. [2016] reported 10 cases of *RERE* de novo mutations, in which patients presented with neurodevelopmental disorders, hypotonia, structural eye defects, and genitourinary defects; 40% had CHD, most commonly ventricular septal defect.

Alternatively, a single patient with tetralogy of Fallot and bicommissural aortic valve led Zaveri et al. [2014] to propose an additional third region associated with CHD, from 12,726,755 to 20,540,759 bp, affecting the genes *PDPN* and *SPEN* (previously known as *MINT*), which have been shown to be associated with CHD in mouse models [Kuroda et al., 2003; Mahtab et al., 2009]. Although the patient reported here presents a different form of CHD, his deletion shows significant overlap with this proposed third region, so we cannot rule out the involvement of these genes in this patient's phenotype. Furthermore, CHD associated with 1p36 deletions may be caused by positional effects brought on by changes in the chromosomal architecture, as suggested by Redon et al. [2005]. According to this hypothesis, haploinsufficiency of specific genes contained within the observed deletions may not be causative for the observed phenotypes. Instead, the altered chromosomal environment may result in changes in gene expression of targets located distant to the deletion.

In conclusion, this report represents the first case of a patient with EA carrying a proximal 1p36 deletion, which does not show overlap with the previous CRs reported in other EA patients, suggesting the presence of an additional proximal locus associated with EA. Further studies of the genes contained in these regions in patients with nonsyndromic forms of EA and functional studies are required to determine their role in the pathogenesis of this cardiac defect. Likewise, identification of additional patients with EA carrying proximal 1p36 deletions can further define the proximal region associated with the disease.


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## Statement of Ethics

This research was ethically conducted in accordance with the World Medical Association Declaration of Helsinki. The research protocol was approved by the local institutional ethical review board of the Fundación Cardioinfantil, Instituto de Cardiología. Informed consent was obtained from the parents.

## Disclosure Statement

The authors have no conflicts of interest and no financial relationships relevant to this article to disclose.

## References


Arndt AK, Schafer S, Drenckhahn JD, Sabeh MK, Plovie ER, et al: Fine mapping of the 1p36 deletion syndrome identifies mutation of *PRDM16* as a cause of cardiomyopathy. Am J Hum Genet 93:67–77 (2013).

Carmignac V, Thevenon J, Adès L, Callewaert B, Julia S, et al: In-frame mutations in exon 1 of *SKI* cause dominant Shprintzen-Goldberg syndrome. Am J Hum Genet 91:950–957 (2012).

Cunnington RH, Northcott JM, Ghavami S, Filomeno KL, Jahan F, et al: The Ski-Zeb2-Meox2 pathway provides a novel mechanism for regulation of the cardiac myofibroblast phenotype. J Cell Sci 127:40–49 (2014).

Digilio MC, Bernardini L, Lepri F, Giuffrida MG, Guida V, et al: Ebstein anomaly: genetic heterogeneity and association with microdeletions 1p36 and 8p23.1. Am J Med Genet Part A 155A:2196–2202 (2011).

Doyle AJ, Doyle JJ, Bessling SL, Maragh S, Lindsay ME, et al: Mutations in the TGF-β repressor SKI cause Shprintzen-Goldberg syndrome with aortic aneurysm. Nat Genet 44: 1249–1254 (2013).

Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, et al: DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am J Hum Genet 84:524–533 (2009).

Fregeau B, Kim BJ, Hernández-García A, Jordan VK, Cho MT, et al: De Novo mutations of *RERE* cause a genetic syndrome with features that overlap those associated with proximal 1p36 deletions. Am J Hum Genet 98:963–970 (2016).

Gajecka M, Mackay KL, Shaffer LG: Monosomy 1p36 deletion syndrome. Am J Med Genet Part C Semin Med Genet 145C:346–356 (2007).

Heilstedt HA, Ballif BC, Howard LA, Kashork CD, Shaffer LG: Population data suggest that deletions of 1p36 are a relatively common chromosome abnormality. Clin Genet 64: 310–316 (2003).

Jordan VK, Zaveri HP, Scott DA: 1p36 deletion syndrome: an update. Appl Clin Genet 8:189–200 (2015).

Kaneko-Oshikawa C, Nakagawa T, Yamada M, Yoshikawa H, Matsumoto M, et al: Mammalian E4 is required for cardiac development and maintenance of the nervous system. Mol Cell Biol 25:10953–10964 (2005).

Kang SH, Scheffer A, Ou Z, Li J, Scaglia F, et al: Identification of proximal 1p36 deletions using array-CGH: a possible new syndrome. Clin Genet 72:329–338 (2007).

Kim BJ, Zaveri HP, Shchelochkov OA, Yu Z, Hernández-García A, et al: An allelic series of mice reveals a role for *RERE* in the development of multiple organs affected in chromosome 1p36 deletions. PLoS One 8:e57460 (2013).

Kuroda K, Han H, Tani S, Tanigaki K, Tun T, et al: Regulation of marginal zone B cell development by MINT, a suppressor of Notch/RBP-J signaling pathway. Immunity 18:301–312 (2003).

Mahtab EAF, Vicente-Steijn R, Hahurij ND, Jongbloed MRM, Wisse LJ, et al: *Podoplanin* deficient mice show a RhoA-related hypoplasia of the sinus venosus myocardium including the sinoatrial node. Dev Dyn 238:183–193 (2009).

Redon R, Rio M, Gregory SG, Cooper RA, Fiegler H, et al: 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 (2005).

Riegel M, Castellan C, Balmer D, Brecevic L, Schinzel A: Terminal deletion, del(1)(p36.3), detected through screening for terminal deletions in patients with unclassified malformation syndromes. Am J Med Genet 82:249–253 (1999).

Saito S, Kawamura R, Kosho T, Shimizu T, Aoyama K, et al: Bilateral perisylvian polymicrogyria, periventricular nodular heterotopia, and left ventricular noncompaction in a girl with 10.5 –11.1 Mb terminal deletion of 1p36. Am J Med Genet A 146:2891–2897 (2008).

Schepers D, Doyle AJ, Oswald G, Sparks E, Myers L, et al: The SMAD-binding domain of *SKI*: a hotspot for de novo mutations causing Shprintzen-Goldberg syndrome. Eur J Hum Genet 23:224–228 (2015).

Shimada S, Shimojima K, Okamoto N, Sangu N, Hirasawa K, et al: Microarray analysis of 50 patients reveals the critical chromosomal regions responsible for 1p36 deletion syndrome-related complications. Brain Dev 37: 515–526 (2015).

Wu X, Li R, Fu F, Pan M, Han J, et al: Chromosome microarray analysis in the investigation of children with congenital heart disease. BMC Pediatr 17:117 (2017).

Zaveri HP, Beck TF, Hernández-García A, Shelly KE, Montgomery T, et al: Identification of critical regions and candidate genes for cardiovascular malformations and cardiomyopathy associated with deletions of chromosome 1p36. PLoS One 9:e85600 (2014).

Zeglinski MR, Davies JJL, Ghavami S, Rattan SG, Halayko AJ, Dixon IM: Chronic expression of Ski induces apoptosis and represses autophagy in cardiac myofibroblasts. Biochim Biophys Acta 1863:1261–1268 (2016).

Zhu X, Zhang Y, Wang J, Yang JF, Yang YF, Tan ZP: 576 kb deletion in 1p36.33-p36.32 containing *SKI* is associated with limb malformation, congenital heart disease and epilepsy. Gene 528:352–355 (2013).