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**EPHA4** haploinsufficiency is responsible for the short stature of a patient with 2q35-q36.2 deletion and Waardenburg syndrome

Chuan Li1, Rongyu Chen1, Xin Fan1, Jingsi Luo1, Jiale Qian1, Jin Wang1, Bobo Xie1, Yiping Shen1,2,3* and Shaoke Chen1*

**Abstract**

**Background:** Waardenburg syndrome type I (WS1), an auditory-pigmentary genetic disorder, is caused by heterozygous loss-of-function mutations in **PAX3**. Abnormal physical signs such as dystopia canthorum, patchy hypopigmentation and sensorineural hearing loss are common, but short stature is not associated with WS1.

**Case presentation:** We reported a 4-year and 6 month-old boy with a rare combination of WS1 and severe short stature (83.5 cm (−5.8SD)). His facial features include dystopia canthorum, mild synophrys, slightly up-slanted palpebral fissure, posteriorly rotated ears, alae nasi hypoplasia and micrognathia. No heterochromia was noticed. He had a normal intelligence quotient and hearing. Insulin-like growth factor-1 (IGF-1) was 52.7 ng/ml, lower than the normal range (55 ~ 452 ng/ml) and the peak growth hormone level was 7.57 ng/ml at 90 minutes after taking moderate levodopa and pyridostigmine bromide. The patient exhibited a good response to human growth hormone (rhGH) replacement therapy, showing a 9.2 cm/year growth rate and an improvement of 1 standard deviation (SD) of height after one year treatment. CMA test of patient’s DNA revealed a 4.46 Mb *de novo* deletion at 2q35-q36.2 (hg19; chr2:221,234,146-225,697,363).

**Conclusions:** **PAX3** haploinsufficiency is known to cause Waardenburg syndrome. Examining overlapping deletions in patients led to the conclusion that **EPHA4** is a novel short stature gene. The finding is supported by the *splotch-retarded* and *epha4* knockout mouse models which both showed growth retardation. We believe this rare condition is caused by the haploinsufficiency of both **PAX3** and **EPH4** genes. We further reported a growth response to recombinant human growth hormone treatment in this patient.

**Keywords:** Chromosomal microarray, 2q35-q36.2, **PAX3**, Waardenburg syndrome, **EPHA4**, Short stature
part or the whole PAX3 gene [3]. These variants may be detected by karyotyping [4,5], FISH [6,7] and MLPA [8,9]. Recently chromosomal microarray (CMA) analysis has allowed for a more accurate delineation of the deletion interval and genes involved, allowing for better genotype-phenotype correlation study.

Case presentation
Here we report a 4-year and 6 month-old Chinese boy with a rare combination of Waardenburg syndrome type 1 and severe short stature. The patient was the first child of a non-consanguineous marriage. He had severe short stature (83.5 cm (−5.8SD)) and poor weight gain (10 kg (−4.1 SD)). Both parents had short stature but are healthy: father’s height is 153 cm (−3.2 SD) and mother’s height is 148 cm (−2.3SD). Patient’s short stature is proportionate. His facial features included dystopia canthorum (W index = 2.31), mild synophrys, slightly up-slanted palpebral fissure, posteriorly rotated ear, alae nasi hypoplasia and micrognathia (Figure 1a and b). He has normal intelligence and hearing. No heterochromia or other pigmentation anomalies was noticed. Physical examination did not find any other abnormalities. The patient met the clinical diagnostic criteria of WS1. Routine blood, urine tests as well as liver and renal function tests were all normal. Endocrine tests for TSH, FT3, FT4, FSH, LH and insulin were normal. Insulin-like growth factor-1 (IGF-1) was 52.7 ng/ml, lower than the normal range (55 ~ 452 ng/ml). The peak growth hormone level was 7.57 ng/ml at 90 minutes after taking moderate levodopa and pyridostigmine bromide. Brain MRI revealed a pituitary gland about 3.2 cm long without abnormal morphology. Bone age based on left hand X-ray is 1.5 years.

The patient underwent recombinant human growth hormone (rhGH) replacement therapy for over a year.

Table 1 Efficacy and side-effect of the rhGH treatment

| Course of GH treatment | HT (cm) | HT SDS (SDS) | Body weight (kg) | Bone age (y) | Drug dose (IU/kg) | IGF-1 (ng/ml) | Side-effect |
|------------------------|---------|--------------|------------------|--------------|------------------|--------------|-------------|
| prior treatment        | 83.5    | −5.80        | 10.0             | 1.5          | -                | 52.7         | -           |
| 1 month of therapy     | 85.3    | −5.47        | 10.0             | -            | 0.11             | 75.8         | N           |
| 3 month of therapy     | 87.0    | −5.17        | 10.5             | -            | 0.11             | -            | N           |
| 6 month of therapy     | 88.3    | −5.09        | 11.0             | 2.0          | 0.10             | 99.1         | N           |
| 7 month of therapy     | 89.5    | −5.13        | 11.5             | -            | 0.10             | -            | N           |
| 8 month of therapy     | 89.9    | −5.24        | 10.5             | -            | 0.11             | -            | N           |
| 11 month of therapy    | 92.2    | −4.83        | 11.5             | -            | 0.12             | -            | N           |
| 13 month of therapy    | 93.3    | −4.76        | 11.0             | 2.0          | 0.12             | 128.0        | N           |
| 15 months of therapy   | 94.8    | −4.70        | 11.5             | -            | 0.12             | -            | N           |
| 9 months out of therapy| 98.1    | −4.80        | 12.0             | -            | 0.12             | -            | N           |
The daily dosage was 0.11 IU/kg. Growth velocity and side-effect profile were monitored for at regular intervals (Table 1). The patient exhibited a good response to rhGH treatment. He showed a 9.2 cm/year growth rate and an improvement of 1 SD of height after one year treatment (Figure 2).

CMA test of patient’s DNA using illumina Human SNP cyto-12 array revealed a 4.46 Mb de novo deletion at 2q35-q36.2 (chr2:221,234,146-225,697,363) (hg19, Figure 3). The deletion involved the whole PAX3 gene which is responsible for the Waardenburg syndrome phenotype and neighboring genes including EPHA4.

We evaluated previously published cases of overlapping deletions with our case’s (Table 2 and Figure 4). We noticed that two thirds of deletion cases reported short stature or growth retardation as one of the phenotypic features when EPHA4 gene was involved in the deletions. The remaining cases did not provide height information. The animal model supported the notion that the EPHA4 deletion is responsible for short stature. The Sp mutant was created by X-ray mutagenesis and characterized by a cytogenetically detectable deletion of band C4 on mouse chromosome 1. Heterozygous mice displayed...
| Case ID | Sex | Age at exam (yrs) | Cytogenetic location of the deletion | stature | WS related features | Developmental issues | Additional features | Year reported or database | Reference |
|---------|-----|------------------|-------------------------------------|--------|--------------------|----------------------|---------------------|------------------------|-----------|
| Case 1  | M   | 22               | 2q35-q36.1 (221107075–222960879)    | NA     | NA                 | NA                   | Abnormal hands      | DECIPHER 282314         | [6]       |
|         |     |                  |                                     |        |                    |                      |                     |                        |           |
| Case 2  | M   | 4                | 2q35-q36.1 (215300000–225200000)    | Smaller than 95% of his age-matched peers | WS1 (DC, CHL, HI)   | MD, ID               | NA                  | 1993       | [14]      |
|         |     |                  |                                     |        |                    |                      |                     |                        |           |
| Case 3  | F   | ?                | 2q35-q36.1 (219971907–224926273)    | Short stature | WS1 (DC, HI, synophrys) | ID               | NA                  | DECIPHER 248718         | [15]      |
|         |     |                  |                                     |        |                    |                      |                     |                        |           |
| Case 4  | F   | 8                | 2q34-q36.1 (213206475–222612545)    | Proportionate short stature | NA | SLD, ID          | Postnatal microcephaly, bifid uvula, heart abnormality | DECIPHER 281765 | [16] |
|         |     |                  |                                     |        |                    |                      |                     |                        |           |
| Case 5  | F   | 5                | 2q34-q36 (209000000–231,000,000)    | Short stature | NA | Normal intelligence | NA                  | 1976       | [17]      |
|         |     |                  |                                     |        |                    |                      |                     |                        |           |
| Case 6  | M   | 6                | 2q35-q36.2 (215300001–226100000)    | <5 percentile | WS3 (DC, HNA, HI, SD) | Mild MD, DD, ID    | Normal hearing, speech and bone age | 1992       | [18]      |
|         |     |                  |                                     |        |                    |                      |                     |                        |           |
| Case 7  | M   | 4                | 2q35-q36.2 (215300001–226100000)    | NA     | WS3 (DC, HNA, HI, HLM, synophrys) blepharophimosis, a bulbous nose, a cupid’s bow upper lip with a short philtrum and high nasal bridge | NA                  | NA                  | 1998       | [19]      |
|         |     |                  |                                     |        |                    |                      |                     |                        |           |
| Case 8  | F   | 4                | 2q35-q36.2 (215300001–226100000)    | NA     | WS3 (DC, HNA, HLM, Synophrys) a cupid’s bow upper lip, a bulbous nose and high nasal bridge | SLD                  | Myelomeningocele; small hands; rhogryposis and camptodactyly partial subluxation | 1998       | [20]      |
|         |     |                  |                                     |        |                    |                      |                     |                        |           |
| Case 9  | M   | 11               | 2q34-q36.2 (209000000–226100000)    | Severe growth retardation | WS1 (DC, BNR, CHL, HNA, WF) | ID                  | NA                  | 1994       | [21]      |
|         |     |                  |                                     |        |                    |                      |                     |                        |           |
| Case 10 | F   | 5                | 2q35-q36 (215300001–231,000,000)    | Short stature | WS3 (CHL, HH, HI, BNR, DC, HNA, synophrys) | Severe DD | NA                  | 1993       | [22]      |
|         |     |                  |                                     |        |                    |                      |                     |                        |           |
| Case 11 | F   | 4                | 2q36 (221,500,001–231,000,000)      | NA     | WS1 (CHL, DC, HH) | -                   | Medial eyebrow flare | 2013       | [23] |
|         |     |                  |                                     |        |                    |                      |                     |                        |           |
| Case 12 | F   | infant           | De novo 2q36 (221,500,001–231,000,000) | Hypertelorism, hypoplastic nasal bridge with prominent nasal tip and anteverted nares. | DD | NA                  | 1993       | [24]      |
|         |     |                  |                                     |        |                    |                      |                     |                        |           |

Not available (NA); Waardenburg syndrome (WS); dystopia canthorum (DC); congenital hearing loss (CHL); hypoplastic nasal alae (HNA); heterochromia idiris (HI); skin depigmentation (SD); developmental delay (DD); motor delay (MD); intellectual disability (ID); broad nasal root (BNR); white forelock (WF); intrauterine growth retardation (IUGR). HLM, HH.
white spotting of the belly, tail and feet, equivalent pig-
mentary features of WS. They also had persistent growth 
retardation throughout their development [10]. The dele-
tion is approximately syntenic to human chr2:218,449,525-
232,459,056 region, both Pax3 and Epha4 were involved 
in the deletion. The other Splotch mutants caused by missense 
mutation (Spd) [11] or splicing mutation (Sp) [12] in Pax3 
gene do not exhibit growth retardation, suggesting genes 
adjacent to Pax3 are responsible for the growth retardation 
phenotype. The most importa
nt evidence came from the 
recent epha4 knockout mouse model. Both heterozygous 
and homozygous epha4 knockout mice showed significant 
postnatal growth retardation in a dose dependent manner 
[13]. So far only one patient has been reported to carry a 
deletion involving only the EPHA4 gene (DECIPHER 
282314). It was a de novo deletion. The available pheno-
type of this patient involves the shortening of digital 
bones such as short 1st metacarpal, short distal phalanx 
of hallux, short distal phalanx of the 2nd finger, short 
distal phalanx of the thumb, short distal phalanx of toe 
and short first metatarsal. All these phenotypes were ab-
sent from either of the parents. The height information 
was not available for this patient. Due to their close prox-
imity on chromosome 2, EPHA4 and PAX3 are often co-
deleted as the cases shown in Figure 4. Most of them had 
reported short stature as a clinical feature [4-6,8,9,14-17]. 
In addition to EPHA4 and PAX3, there are about 10 more 
OMIM genes involved at the deletion interval of our pa-
tient, three are associated with human diseases (AP1S3 is 
associated with the susceptibility to pustular psoriasis-15; 
MRPL44 is likely associated with Combined oxidative phos-
thorylation deficiency 16; CUL3 is associated with Pseudo-
hypoaldosteronism, type IIE). None of these genes or the 
rest of the genes at the interval are known to be associated 
with short stature. EPHA4 is not currently a known disease 
causing gene. Our patient and other similar deletion pa-
tients, as well as the mouse model provide compelling evi-
dence indicating EPHA4 as a novel short stature gene.

**EPHA4** is a member of the EPH family of receptor tyro-
sine kinases. It was demonstrated that EPHA4 interacts 
directly with growth hormone receptor (GHR) and JAK2 [11]. 
They form a ternary complex in human cells [11]. It is concei-
vable that EPHA4 haploinsufficiency causes short stature 
by impairing the growth hormone pathway that regulates 
down-stream effectors such as STAT5B and IGF1. Segrega-
tion of the short stature phenotype with EPHA4 mutations 
will further support the causal relationship.

It is interesting to note that growth hormone injection 
did not improve the growth of the Epha4 homozygous 
knockout mouse. In our patient, growth hormone replace-
ment therapy resulted in significant improvement of 
growth velocity and height. It is not known if heterozygous 
knockout mice will respond to growth hormone or not. 
The response we observed in our patient provided pre-
liminary indication for rhGH treatment in patients with 
EPHA4 deletion.

**Conclusions**
The rare combination of Waardenburg syndrome pheno-
type and short stature observed in our patient can be 
explained by the haploinsufficiency of both PAX3 and 
EPHA4 genes involved within the deletion. Our analysis 
indicated that EPHA4 is a novel short stature gene. Re-
combiant human growth hormone treatment improved 
the height of the patient; suggesting that diagnosis may 
help in determining utility of growth hormone in other in-
dividuals with EPHA4 associated short stature.

**Consent**
We obtained a written consent from patient’s parent for 
utilizing patient’s clinical information, genetic details 
and photos in this publication. The study is approved by 
the internal review board of the Guangxi Maternal and 
Child Health Hospital.
Endnotes

*W* index is defined as $X + Y + a/b$. $a$ = inner canthal distance; $b$ = interpupillary distance; $c$ = outer canthal distance. A *W* index result greater than 1.95 is abnormal.

Abbreviations

PAX3: Paired box 3; WS: Waardenburg syndrome; CMA: Chromosomal microarray; EPHA4: Ephrin Receptor A type 4; SD: Standard deviation; TSH: Thyroid stimulating hormone; FT3: Free triiodothyronine; FT4: Free thyroxine; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; IGF-1: Insulin-like growth factor-1; MR: Magnetic resonance imaging; rGH: Recombinant human growth hormone; EPH: Ephrin; GHR: Growth hormone receptor; JAK2: Janus kinase 2.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

CL, XF, JL and JQ carried out the clinical evaluation and treatment of the patient. RC, JW and BX carried out the chromosomal microarray testing for the patient and his parents. YS and SC conceived of the study, and participated in its design and coordination. YS, SC and CL helped to draft the manuscript. All authors read and approved the final manuscript.

Authors’ information

YS is an ABMG-certified medical geneticist. SC, CL, XF, JQ and JL are board-certified clinical pediatricians. RC, JW and BX are molecular laboratory medical technologist.

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References

1. Arias S, Mota M. Apparent non-penetration for dystopia in Waardenburg syndrome type I, with some hints on the diagnosis of dystopia canthorum. J Genet Hum. 1978;26(2):103–31.
2. Farrer LA, Grundfast KM, Amos J, Amos KS, Asher Jr JH, Beighton P, et al. Waardenburg syndrome (WS) type I is caused by defects at multiple loci, with initial evidence for a high-penetration locus on chromosome 2q. Hum Genet. 1978;45(4):295–301.
3. Pingault V, Ente D, Dastot-Le Moal F, Goossens M, Marlin S, Bondurand N. Molecular characterization of a deletion in a child with deletion (2)(q35q36.2). Am J Hum Genet. 1992;44(3):699–700.
4. Tassabehji M, Newton VE, Leverton K, Turnbull K, Seemanova E, Kunze J, et al. PAX3 gene structure and mutations: close analogies between Waardenburg syndrome and the splotch mouse. Proc Natl Acad Sci U S A. 1993;90(2):532–6.
5. Epstein DJ, Malo D, Vekemans M, Gros P. Molecular characterization of a deletion encompassing the splotch mutation on mouse chromosome 1. Genomics. 1991;10(1):89–93.
6. Vogan KJ, Epstein DJ, Tasler DG, Gros P. The splotch-delayed (Spd) mouse mutant carries a point mutation within the paired box of the PAX-3 gene. Genomics. 1993;7(2):346–94.
7. Lu-Kuo J, Ward DC, Spritz RA. Fluorescence in situ hybridization mapping of 25 markers on distal human chromosome 2q surrounding the human Waardenburg syndrome, type I (WS1) locus (PAX3 gene). Genomics. 1993;16(1):173–9.
8. Milunsky JM, Maher TA, Ito M, Milunsky A. The value of MLPA in Waardenburg syndrome. Genet Test. 2007;11(2):179–82.
9. Matsunaga T, Mutai H, Namba K, Morita N, Masuda S. Genetic analysis of PAX3 for diagnosis of Waardenburg syndrome type I. Acta Otolaryngol. 2013;133(4):345–51.
10. Epstein DJ, Malo D, Vekemans M, Gros P. Molecular characterization of a deletion encompassing the splotch mutation on mouse chromosome 1. Genomics. 1991;10(1):89–93.
11. Vogan KJ, Epstein DJ, Tasler DG, Gros P. The splotch-delayed (Spd) mouse mutant carries a point mutation within the paired box of the PAX-3 gene. Genomics. 1993;7(2):364–94.
12. Epstein DJ, Vogan KJ, Tasler DG, Gros P. A mutation within intron 3 of the PAX-3 gene produces aberrantly spliced mRNA transcripts in the splotch (Sp) mouse mutant. Proc Natl Acad Sci U S A. 1993;90(2):532–6.
13. Ilang X, Miyajima M, Sawada T, Chen Q, Ilida K, Fujishima K, et al. Crosstalk of humoral and cell-cell contact-mediated signals in postnatal body growth. Cell Rep. 2012;2(3):652–65.
14. Warter S, Lausecker C, Pennerath A. A girl with a deletion (2)(q34q36): cytogenetic and clinical observations. Hum Genet. 1976;32(2):225–7.
15. Kirkpatrick SJ, Kent CM, Laxova R, Sekhon GS. Waardenburg syndrome type I in a child with deletion (2)(q35q36.2). Am J Med Genet. 1992;44(3):699–700.
16. Nye JS, Balkin N, Lucas H, Knepper PA, McLone DG, Charrow J. Myelomeningocele and Waardenburg syndrome (type 3) in patients with interstitial deletions of 2q35 and the PAX3 gene: possible digenic inheritance of a neural tube defect. Am J Med Genet. 1998;75(4):401–8.
17. Melnyk AR, Muraskas J. Interstitial deletion of chromosome 2 region in a malformed infant. Am J Med Genet. 1993;45(1):49–51.