

SHORT COMMUNICATION

Novel missense mutation in *DLL4* in a Japanese sporadic case of Adams–Oliver syndrome

This article has been corrected since Advance Online Publication, and a corrigendum is also printed in this issue.

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Adams–Oliver syndrome (AOS, OMIM; 100300) is a rare genetic disease characterized by aplasia cutis congenita, terminal transverse limb defects and cutis marmorata with vascular anomalies such as congenital heart defects. The etiology of this syndrome has remained largely unknown but defective Notch signaling during vascular formation has been suggested. Here we describe a sporadic Japanese newborn case with clinically diagnosed AOS. Trio whole-exome sequencing identified a *de novo*, novel, heterozygous missense mutation in the *Delta-like 4 ligand* gene (*DLL4* c.572G>A, p.Arg191His) in the patient. *DLL4* functions as a requisite ligand for NOTCH1 receptor, which is essential for vascular formation. Amino acid substitution of Arg191 to His was predicted by molecular models to interfere with direct binding between *DLL4* and NOTCH1. *DLL4* has recently been identified as a causative gene of an autosomal dominant type of AOS with milder symptoms. The case described here showed gradual recovery from skull defects after birth and no psychomotor developmental delay has been observed. This is the second report of an AOS case with *DLL4* mutation, and the phenotypic characteristics between the two cases are compared and discussed. *Journal of Human Genetics* (2017) 62, 851–855; doi:10.1038/jhg.2017.48; published online 27 April 2017

INTRODUCTION

Adams–Oliver syndrome (AOS, OMIM; 100300) was first reported by Adams and Oliver in 1945.¹ AOS is an extremely rare disease, with an estimated incidence of 1/225 000. Various clinical manifestations have been reported, including aplasia cutis congenita of the scalp, terminal transverse limb defects, and cutis marmorata with vascular anomalies such as congenital heart defects.² The most common defects are terminal limb malformations (84% of cases), including osseous syndactyly, rudimentary bones, or completely absent digits. Congenital cutis aplasia, the second-most-common defect (75% of cases), usually occurs over the posterior parietal region. Underlying bone defects can be present, and tortuous veins can occur on the posterior scalp.^{3,4}

Six causative genes for AOS have been identified, namely *ARHGAP31* (AOS type 1),⁵ *RBPJ* (AOS type 3),⁶ *NOTCH1* (AOS type 5),^{7,8} *DOCK6* (AOS type 2),⁹ *EOGT* (AOS type 4)¹⁰ and *DLL4*.¹¹ Other genes are still to be identified because many patients with AOS do not exhibit mutations in any of the known causative genes. *RBPJ*, *NOTCH1* and *EOGT* are involved in the Notch signaling pathway, which has an important role in stabilization of arterial endothelial fate and angiogenesis.¹² Consistent with this, malformation of vasculature

and defects in the remodeling of vessels in the early fetal phase are considered common features of this syndrome. Of the six known causative genes, mutations in *ARHGAP31*, *RBPJ*, *NOTCH1* and *DLL4* are inherited in an autosomal dominant manner, and those in *DOCK6* and *EOGT* are inherited in an autosomal recessive manner. Patients carrying *DOCK6* mutations show the most severe phenotype. Mutations in *DLL4* have been recently identified in several families,¹¹ with these patients showing relatively mild phenotypes compared with autosomal recessive cases. Here we report the first Japanese case of AOS with a novel missense variant in *DLL4*. All procedures were reviewed and approved by the Institutional Review Board of Kobe University School of Medicine and were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. We performed the analysis after obtaining written informed consent from the parents of the patients.

CLINICAL REPORT

A Japanese family, including the proband, was investigated in this study (Figure 1a). The proband, the first child of non-consanguineous, healthy parents, was born at 34 weeks, 4 days gestation with a birth

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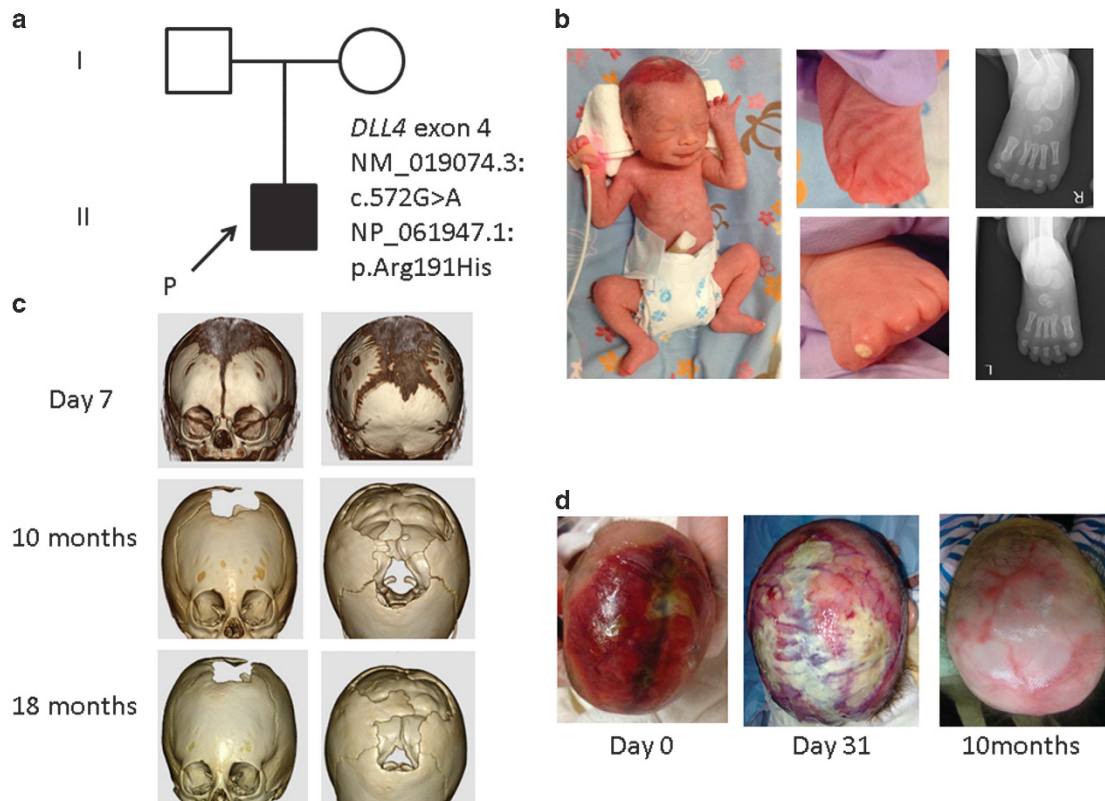


Figure 1 Family pedigree and the clinical features of the case. (a) Family pedigree. Squares, males; circles, females; open shape, unaffected; filled shape, affected; P, proband. (b) Patient's clinical features. Left, Aplasia cutis congenita; middle upper, symbrachydactyly of right toe; right upper, X-ray of the right toe; middle lower, hypoplastic nails; right lower, X-ray of the left toe. (c) Three dimensional representation of the patient's scalp by computed tomography. Left, front view; right, upper views. Wide area of bone defect around frontal fontanelle (day 7 after birth) and gradual ossification were seen at 10 and 18 months after birth. (d) Aplasia cutis congenita. Left, day 0; middle, day 31; right, 10 months after birth. Skin bleeding diminished gradually and crust formation was complete around 10 months after birth.

weight of 1334 g (−2.7 s.d.), height of 39.6 cm (−2.2 s.d.), and occipitofrontal circumference of 26 cm (−2.5 s.d.). During pregnancy, intrauterine growth restriction and single umbilical cord artery were present. The patient was transferred to the newborn intensive care unit (NICU) soon after birth, presenting with bleeding around aplasia cutis congenita of the skull (Figure 1b). Limb abnormalities, such as bilateral syndactyly of the lower extremities and brachydactyly and cutis marmorata were also observed (Figure 1b). Clinical presentation suggested a diagnosis of typical AOS. No congenital heart defect was detected by echocardiogram. Cranial computed tomography indicated a wide range of bone defects around the anterior fontanelle (Figure 1c, upper), but brain MRI was normal (not shown).

In the first 10 months after birth, bleeding symptoms around aplasia cutis congenita of the skull gradually improved with treatment, including petroleum jelly application and gauze protection (Figure 1d). Genetic analysis was conducted for further diagnosis. Firstly, chromosomal analysis showed a normal karyotype. Next, genomic DNA was obtained from peripheral blood leukocytes. Comparative genomic hybridization and single-nucleotide polymorphism array analysis (180 K, SurePrintG3, Agilent Technologies, Santa Clara, CA, USA) were performed on DNA from the proband using an Agilent DNA Microarray Scanner BA (Agilent Technologies). This analysis discovered no pathogenic copy-number variations. For next-generation sequencing (NGS) analysis of the patient, we first used an Illumina TruSight One sequencing panel on a MiSeq platform according to the manufacturer's instructions (Illumina, San Diego, CA, USA). However, no pathogenic sequence variations were

identified. Therefore, we conducted whole-exome sequencing on the proband and his parents using Illumina Nextera Rapid Capture Exome on a HiSeq platform. For data analysis, we used VariantStudio (ver. 2.1.46; Illumina) and Integrative Genomics Viewer software (ver. 2.3.32; Broad Institute, Cambridge, MA, USA). We identified a *de novo*, novel, heterozygous missense mutation in the *Delta-like 4 ligand* gene (*DLL4* c.572G>A, p.Arg191His) on chromosome 15q15.1 in the patient (Figure 2a). This variant was within a conserved region and had not previously been reported or registered in the human genome Human Genome Variation browser. The *in silico* mutation analysis tools, SIFT, PolyPhen-2 and Mutation Taster (<http://mutationtaster.org>) produced scores of 0 (deleterious), 1.0 (damaging) and 'disease causing', respectively. This variant was confirmed by direct sequencing and it was determined that the missense mutation was not transmitted from the parents (Figure 2b). At 18 months of age, no intellectual disability or psychomotor developmental delay was displayed. Brain CT showed a wide range of skull defects around the anterior fontanelle, but the extent of ossification gradually increased, as shown by brain CT on day 0 and at 10 months (Figure 1c). The patient still required a custom-made head protector and had difficulty using eating utensils because of his malformed digits.

DISCUSSION

Involvement of the Notch signaling pathway in vascular development has been demonstrated by both gain- and loss-of-function mutations in humans, mice and zebrafish.¹³ *DLL4* is implicated as an essential

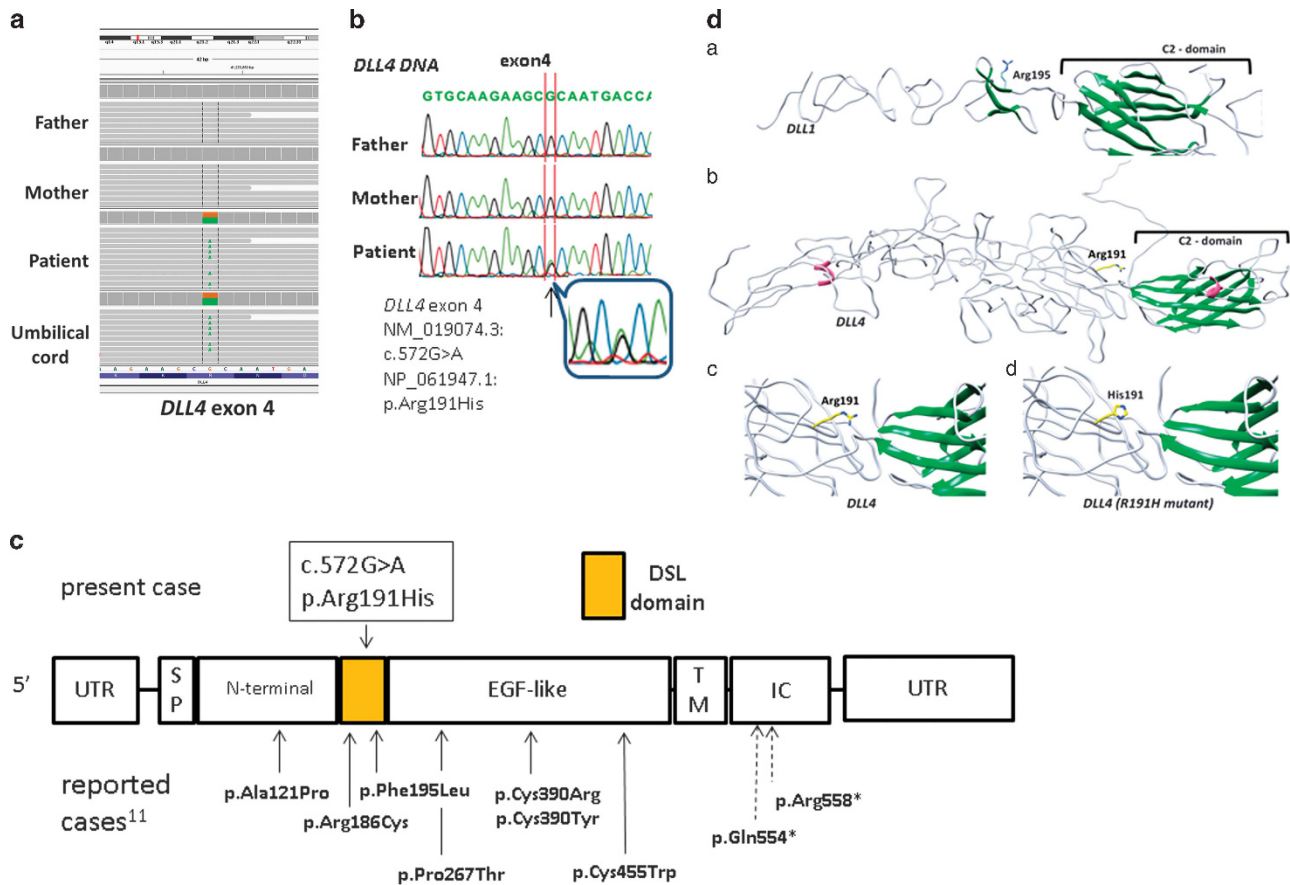


Figure 2 Genetic analysis of the proband. (a) Integrative Genomics Viewer picture of the father (upper lane), mother (second lane), patient (third lane), and the patient's umbilical cord (bottom lane). The pathogenic heterozygous *DLL4* variant (c.572G>A, p.Arg191His) was detected only in the proband's genome. (b) Sanger sequencing of the pathogenic *DLL4* variant. (c) The domain containing identified variants within *DLL4*. Orange rectangle shows DSL domain. (d) Structure of human notch ligand delta-like 1 (DLL1) and delta-like 4 (DLL4) showing conserved arginine. Full length sequence of DLL4 (*Homo sapiens*), which consists of 665 amino acid residues, was taken from the NCBI data bank. DLL4 shows good pairwise sequence similarity (using BLAST) with the crystal structure of DLL1 (Protein Database (PDB) ID 4XBM).¹⁸ Notch receptor (PDB ID 4CC0).¹⁹ These two PDB structures were chosen as a template to model the structure of DLL4 by MODELLER9v8.²⁰ The structure of the R191H mutant was generated in a similar way to that for wild type. Ten models were generated and the top scoring model (DOPE Score) was analyzed and used for figures. Figures were prepared in Chimera. (a) The overall structure of DLL1 (PDB ID: 4XBM) showing the C2 domain and Arg195. (b) Modeled DLL4 structure showing Arg191 in a similar position to that of Arg195 in DLL1. Close-up view of Arg191 in the DLL4 model (c) and of His191 in the R191H mutant (d). The DLL1 structure is used to build up a model of *DLL4*.

modulator of arteriogenesis and angiogenesis albeit together with NOTCH1.¹² In mice, *DLL4* is specifically expressed in arterial endothelial cells, and haploinsufficiency of *DLL4* is embryonic lethal due to defective arterial and vascular development.¹⁴ *DLL4* also regulates the formation of normal vessel sprouting and branching in the mouse retina and *DLL4* expression in human is also predominantly observed in vascular endothelium.¹⁵ It has also been suggested that *DLL4* is essential only at times of active vascularization, but is less important for maintaining normal vessel architecture.¹⁶ *DLL4* contains five domains, an extracellular N-terminal domain, a Delta/Serrate/Lag-2 (DSL) domain, EGF-like domains, a membrane penetrating domain and an intracellular domain (Figure 2c). Structural analysis has shown that the DSL domain in *DLL4* binds to the EGF-like domains in NOTCH1, the interaction being controlled by the mutual interaction between the glycosylated EGF-like domains in NOTCH1 and the N-terminal domain in *DLL4*.¹⁷ The patient described in this paper carries a heterozygous missense mutation in *DLL4*, resulting in an Arg to His substitution at codon 191 within the DSL domain. This arginine is located at the surface of the DSL domain in *DLL4*. Moreover, Arg191 is well conserved in various species, suggesting these sites have

important roles in binding the EGF-like domain 11 in NOTCH1.¹⁷ Although *DLL4* has not yet been crystalized, BLAST analysis showed that human *DLL4* has good pairwise sequence similarity with Notch ligand Delta-like 1 (DLL1)¹⁸ (Figures 2d(a) and d(b)). Arg195 of DLL1 is located within the DSL domain and is also conserved, and is crucial for the interaction with the receptor (Figure 2d(a)).¹⁸ It is thus expected that Arg191 in *DLL4* and Arg195 have similar roles in ligand binding. Therefore, Arg191 in *DLL4* is suggested to have a key role in binding the Notch receptor.¹⁹ By modeling the *DLL4* structure and the R191H mutant using MODELLER9v8,²⁰ the side chain of histidine was shown to be shorter compared to that of arginine (Figures 2d(c) and d(d)). Therefore, the Arg191His mutation might lead to weakening of the electrostatic interaction between *DLL4* and the receptor and it may have reduced access to receptor binding site residues, resulting in the AOS phenotype. The present case is likely to be caused by a loss-of-function mutation in the DSL domain.

In AOS, although rare, complications such as cardiac anomaly, and neurological symptoms such as epilepsy and cortical dysplasia have been reported.^{11,16} We compared the phenotypes of our case and nine previously observed families¹¹ as shown in Table 1. We could not find

Table 1 Genotypes and phenotypes of AOS patients with *DLL4* variants

<i>DLL4</i> structural domain	Variant	Clinical features			
		Scalp defects	Limb defects	Cardiovascular features	Other
N-terminal (MNNL)	c.361G>C, p.Ala121Pro	ACC	Rt. short distal phalanges, middle finger, syndactyly of the index finger, bil.	Truncus arteriosus, ventricular septum defect	Growth hormone deficiency
DSL	c.556C>T, p.Arg186Cys	ACC	—	Normal	—
	c.572G>A, p.Arg191His (present case)	ACC	—	Normal	—
	c.583T>C, p.Phe195Leu	ACC	Bil. synbrachydactyly hypoplastic toe nails	Normal	—
EGF-like	c.799C>A, p.Pro267Thr	ACC, delayed ossification	—	—	—
	c.1168T>C, p.Cys390Arg	ACC, delayed ossification	Hypoplastic toe nails	Normal	Cutis marmorata
	—	Bald area on scalp	—	—	Cutis marmorata
Intracellular	—	Bald area on scalp	Brachydactyly of fingers and toes, syndactyly of 2nd and 3rd toes	—	—
	—	Bald area on scalp	—	—	—
	—	Bald area on scalp	—	—	—
	—	Bald area on scalp	—	—	—
	—	Bald area on scalp	—	—	—
	—	Bald area on scalp	—	—	—
	—	Bald area on scalp	—	—	—
ACC	c.1169G>A, p.Cys390Tyr	ACC	—	—	—
	c.1365C>G, p.Cys455Trp	ACC	—	—	—
	—	ACC	—	—	—
ACC	c.1660C>T, p.Gln544*	—	Short distal phalanges	—	—
	—	ACC	—	—	—
ACC	—	ACC	Brachydactyly, rt. syndactyly of 2nd and 3rd toes	Normal	—
	—	ACC	—	Tricuspid insufficiency, ventricular septum defect	—
ACC	c.1672C>T, p.Arg558*	ACC with underlying skull defect	Lt brachysyndactyly, rt. missing toes	Normal	—
	—	ACC with underlying skull defect	—	—	—
ACC	—	ACC with underlying skull defect	Rt. brachysyndactyly, lt. severe brachysyndactyly	Normal	Small kidneys, mild hypertension
	—	ACC with underlying skull defect	—	—	—

Abbreviations: —, Unknown; bil., bilateral; lt, left; rt, right.

any distinct phenotype–genotype correlations with the mutated domains of the *DLL4* protein in these cases because of limited numbers. However, four cases, including our case, who carry mutations in the DLS domain seemed to have milder symptoms compared with patients with mutations in other domains. This was not conclusive, owing to limited sample size. Therefore, further analysis will be necessary to confirm genotype–phenotype correlations in AOS with *DLL4* mutations. In our case, ossification of the skull was remarkable, suggesting that mutation in *DLL4* has less effect after birth, leading to the milder phenotype of the case.

We report here a novel *de novo* pathogenic mutation in *DLL4* in a sporadic AOS case. This genetic analysis was conducted because a precise diagnosis was desired by the parents, to obtain information on the patient's natural history and prognosis, and for their future family planning. NGS was used to provide precise AOS information and the genetic information required for informed genetic counseling.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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