A novel *DLL4* missense mutation in a Chinese patient with Adams-Oliver syndrome

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To the Editor: Adams-Oliver syndrome (AOS, including 6 types) was initially reported in a three-generation family by Adams and Oliver in 1945,[1] with an estimated incidence of 1 in 225,000 live births.[2] Approximately 84% of AOS patients have terminal transverse limb defects, including amputations, syndactyly, brachydactyly, or oligodactyly. Congenital cutis aplasia is the second most common defect, occurring in 75% of AOS cases covering the posterior parietal region.[3] Other features include vascular anomalies (20%), cardiovascular diseases (20%), central nervous system anomalies and gastrointestinal malformations.[4] To date, six genes associated with AOS have been identified. Mutations in *DOCK6* (MIM: 614219, AOS type 2) and *EOGT* (MIM: 615297, AOS type 4) have been linked to the autosomal-recessive form of AOS, while mutations in *ARHGAP31* (MIM: 100300, AOS type 1), *RBPJ* (MIM: 614814, AOS type 3), *NOTCH1* (MIM: 616028, AOS type 5) and *DLL4* (MIM: 616589, AOS type 6) cause the autosomal-dominant form. A recent study showed that mutations in *NOTCH1* (about 10%) were the most common cause of AOS in a large European cohort, followed by mutations in *DLL4* (about 6%), *DOCK6* (about 6%), *ARHGAP31* (about 3%), *EOGT* (about 3%), and *RBPJ* (about 2%).[5]

A 3-year-old female patient underwent gene related examination in the McKusick-Zhang Center for Genetic Medicine, Institute of Basic Medical Sciences Chinese Academy of Medical Sciences in 2016. The institutional review board of Peking Union Medical College approved this study. The patient had a 3 cm × 4 cm skin and skull defect at the scalp vertex with mucosal covering at birth. After 4 months, the defect was almost healed with a scar, but hair had not grown in [Figure 1A and 1B]. In addition, the patient showed transverse terminal limb anomalies, such as brachydactyly and hypoplastic nails or absence of nails in both her hands and feet. X-ray images showed the distal phalanx of the 2nd to 5th fingers of both hands were absent [Figure 1C]. Only one phalange or an ossification center of the phalange was found in the 1st to 4th toes of the left foot and the 3rd to 5th toes of the right foot. Phalanges were absent in the 5th toe of the left foot and the 1st to 2nd toes of the right foot [Figure 1D]. Congenital cardiovascular defects were not detected by echocardiogram. The patient did not have other internal organ involvement, and physical and mental development was normal. Amino acid and acylcarnitine profiles test was performed to exclude neonatal genetic metabolic diseases. Her mother denied consanguineous marriages and any history of maternal drug intake, infection, or radiation exposure during pregnancy. In addition, there was no previous family history of skin or limb malformations. Chromosomal analysis showed a 46, XX, inv (9). The inv (9) chromosomal variation likely did not contribute to the AOS phenotype in this patient.

To screen for the pathogenic mutation in genes associated with AOS, we amplified the exons and exon-intron boundaries of *ARHGAP31*, *DOCK6*, *RBPJ*, *EOGT*, *NOTCH1*, and *DLL4* by polymerase chain reaction and sequenced them directly after purification. We identified a novel heterozygous missense mutation, c.1346G>C (p.Cys449Ser), in exon 9 of *DLL4* in the patient [Figure 1E]. This mutation was absent in her unaffected parents and 200 ethnically matched control individuals. In addition, the mutation was not previously reported or registered in the dbSNP150, 1000 genome project, Exome Aggregation Consortium (ExAC), Human Gene Mutation Database (HGMD), or ClinVar databases. The cysteine residue at 449 is located in the epidermal growth factor (EGF)-like 7

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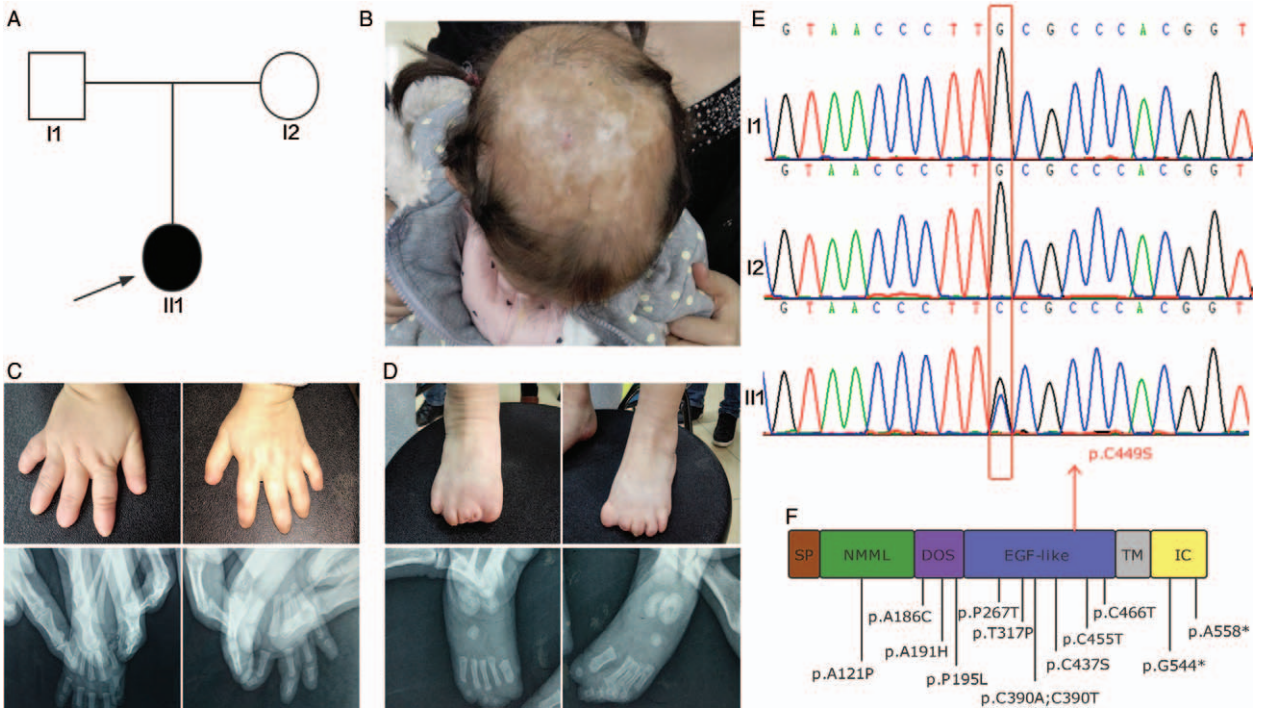

Figure 1: (A) Family pedigree: black circles indicate affected individuals, the arrow indicates the proband. (B) Images showing aplasia cuits congentia. (C and D) Photographs and X-ray images of the hands and feet of the proband, respectively. (E) Genetic analysis of the proband: red square indicates the position of the mutation (c.1346G>C). (F) Schematic map of *DLL4* showing the position of mutations. The red arrow indicates the mutation identified as p.Cys449Ser.

domain of *DLL4* and is highly conserved throughout eight species ranging from rhesus to lamprey, according to the Multiz Alignments. In *silico* bioinformatics tools, including Polyphen-2 (http://genetics.bwh.harvard.edu/pph2), SIFT (http://sift.jcvi.org) and Mutation Taster (http://www.mutationtaster.org), predict that the c.1346G>C *DLL4* mutation is damaging, deleterious and "disease-causing". HSF (http://www.umd.be/HSF3/) predicted that this mutation does not affect normal splicing. *DLL4* maps to chromosome 15q15.1 and has a genomic size of 9728 bps, with eleven coding exons. *DLL4* signaling through the NOTCH1 receptor regulates many different tissue-specific cellular processes, including cell-fate determination and neural and hematopoietic stem cell differentiation.[6] In mice, *DLL4* is expressed in endothelial cells (ECs), specifically in arteries and capillaries, and *DLL4* haploinsufficiency results in embryonic lethality due to defects in arterial and vascular development.[7] In both embryonic and adult tissues, *DLL4* is also expressed predominantly in the vascular endothelium, suggesting that *DLL4* is involved in the regulation of vascular biology.[8] The *DLL4* protein contains five domains: an N-terminal domain with NOTCH ligands (MNNL), a Delta/Serrate/Lag-2 domain (DSL), eight EGF-like domains, a transmembrane domain and an intracellular domain that lacks catalytic motifs [Figure 1F]. The crystal structure of interacting regions of the NOTCH1-DLL4 complex was recently described, showing that NOTCH1 EGF-like repeats 11 and 12 interact with the *DLL4* DSL domain and module at the MNNL domain, respectively.[9]

To date, 14 pathogenic mutations have been identified, including two nonsense mutations and twelve missense mutations. These mutations are distributed throughout the gene, affecting all known structural domains. Among them, eight missense mutations are located in the EGF-like domains, of which six are cysteine-replacing or -creating mutations, followed by three missense mutations in the DSL domain, two nonsense mutations in the intracellular domain and one missense mutation in the MNNL domain[10,11] [Figure 1F]. Other types of mutations have not been reported. Patients with the c.361G>C (p.A121P) mutation in the MNNL domain showed the most serious clinical manifestations, including congenital cutis aplasia, terminal transverse limb defects, cardiovascular defects, and growth hormone deficiency. Patients with mutations in the DLS domain have milder symptoms. However, because of the limited sample size, further analysis will be necessary to confirm genotype-phenotype correlations in AOS with the *DLL4* mutations. Nearly all AOS affected individuals display congenital cutis aplasia, except one with a c.1660C>T (p.G544*) *DLL4* mutation. In contrast, other features, such as terminal transverse limb defects or cardiovascular anomalies, show variable expressivity, suggesting that environmental or other genetic and epigenetic factors may be involved in the clinical manifestation of AOS.

In the present study, we identified a novel heterozygous missense mutation (c.1346G>C, p.Cys449Ser) in *DLL4* in a non-consanguineous Chinese patient. The cysteine replacement mutation is located in the EGF-like 7 domain

of *DLL4* and is expected to affect the formation of disulfide bonds, which may disrupt the *DLL4* structure and lead to loss of function. Because this mutation was not observed in the proband's unaffected parents, we speculate that it is a *de novo* mutation, although it may also be caused by a germline mosaic mutation.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient has given her consent for her images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal her identity, but anonymity cannot be guaranteed.

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Conflicts of interest

None.

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