

Novel EPHB4 mutation in capillary malformation-arteriovenous malformation syndrome 2 (CM-AVM2): the first genetic study in Asians

Yuanbo Li

Shanghai Jiao Tong University

Yi Sun

Shanghai Jiao Tong University

Xiaojing Zeng

Zhejiang University

Yun Liu

Fudan University School of Basic Medical Sciences

Fatao Liu

Shanghai Jiao Tong University

Xiaoxi Lin (✉ linxiaoxi@126.com)

Shanghai Jiaotong university

Ren CAI

Shanghai Jiao Tong University <https://orcid.org/0000-0002-6025-0170>

Hui Chen

Shanghai Jiao Tong University

Brief report

Keywords: Capillary malformation-arteriovenous malformation 2, EPHB4, novel mutation, Asians, China

Posted Date: August 18th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-60236/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Capillary malformation-arteriovenous malformation syndrome (CM-AVM), a rare vascular malformation, is autosomal dominant and characterized by hereditary capillary malformations (CMs) and potential fast-flow vascular malformation underlying CMs in around one-third of patients, affecting approximately 0.3-0.5% of newborns. CM-AVM was first discovered in association with germline RASA-1 mutation and termed CM-AVM type 1 in 2003. Later, germline EPHB4 mutations were detected in RASA-1-negative CM-AVMs, also known as CM-AVM type 2. Although there have been recent genetic studies confirming the molecular basis of CM-AVM, few have focused on Asian populations, leading to the lack of comprehensive molecular profiling. In this study, we report a female patient diagnosed as CM-AVM2 with a novel stop-gain mutation causing loss of function of the EPHB4 gene. As far as we know, this is the first genetic report of germline EPHB4 loss-of-functional mutation related CM-AVM 2 in Asians, extending our understanding of CM-AVM.

Introduction

Capillary malformation-arteriovenous malformation (CM-AVM) syndrome, a vascular malformation, is known as a autosomal dominant disease that is present in 100,000 individuals¹. CM-AVM is characterized with hereditary capillary malformations (CMs). Around one-third of affected individuals are associated with fast-flow vascular malformation lesions, including arteriovenous malformation (AVM) and arteriovenous fistula (AVF). Capillary malformations (CMs) affected approximately 0.1-0.3% of newborns and are the most common type of vascular malformations². CMs present as cutaneous, flat, sporadic, unifocal, irregular, pink, red to purple patches anywhere in the body, particularly affecting the head and neck³. Although no proliferation was noted, CMs may gradually become darker and thicker with increasing age and do not regress spontaneously. In contrast, the atypical CM of CM-AVM is usually multiple, round to oval, small (1-2 cm) and pink to red⁴. In 2003, Eerola and Colleagues detected heterozygous loss-of-function RASA-1 mutations in around 50% patients with inherited CMs and named this entity CM-AVM1. Later in 2017, Amyere et al. identified EPHB4 mutations in patients with RASA-1-negative CM-AVMs and named it CM-AVM2.

Although there have been recent genetic studies confirming the molecular basis of CM-AVM, few have focused on Asian populations. In this report, we described one female patient with CM-AVM 2 on the left arm and germline EPHB4 gene (c.1093C>T) mutation. As far as we know, this is the first report of germline EPHB4 loss-of-functional mutation related CM-AVM 2 in Asians, extending our understanding of CM-AVM.

Methods

This study was authorized and approved by the Ethics Committee of Shanghai 9th People's Hospital Affiliated to Shanghai Jiaotong University School. We obtained oral and written informed consent from the patient's parents prior to the study. Detailed clinical data of the patient and her family were recorded.

Case Presentation

A three-year-old girl presented at our clinic with sporadic and multifocal erythema on her left arm, left shoulder and chest. According to her parents, the erythema is firstly apparent three month after birth on her left arm, and progressively extended to the left shoulder and chest area. The erythema was scattered with various shapes and sizes (maximum diameter was 3 cm) (Figure 1a). Physical examination revealed higher skin temperature of the erythema than that of the adjacent normal skin. Besides, a mass with detectable palpation was found in the axillary lesion (Figure 1a, arrow). Family history disclosed that the proband's grandfather had a single patch of erythema on his right forearm that had not proliferated for several decades (Figure 1b). Neither erythema nor vascular disease was found in other family members of the proband.

Enhanced CT demonstrated multiple well-defined nodular and lamellar masses in the axillary area. The masses were homogeneously enhanced after reinforcement. Besides, The vessels in the medial left arm were enlarged (Figure 1c,d). Considering the harm of anesthesia and radiation, digital subtraction angiography (DSA) examination was not performed on the patient.

To determine the genetic and histological alterations in this patient, we performed minimally invasive needle biopsy (D=2 mm, all layer biopsy) on the erythema lesion under local anesthesia (Penles and 1% xylocaine). One tissue specimen was stained with hematoxylin-eosin for histological analysis. Then, we performed targeted next-generation sequencing (NGS) in peripheral blood specimens as well as in tissue specimens.

Next-generation sequencing (NGS)

The targeted NGS gene panel was designed according to the ISSVA classification.⁶ We designed a series of capture RNA baits in order to target the exons and the boundaries of the exons/introns of EPHB4. The SureSelect XT kit reagents (Agilent Technologies, Santa Clara, CA) were used for the Illumina adaptors. Then we use the Quantitative PCR (KAPA Biosystems, Wilmington, MA) to verify the concentration of the indexed samples. The indexed sample was sequenced using the MiSeq instrument (Illumina, San Diego, CA) with 2150 paired-end reads. Variants were analyzed by using the Integrative Genomic Viewer.

Sanger sequencing

Gene EPHB4 was PCR-amplified to track the 7:100402906 AGT>A, c.2714_2715delAC, p.His905fs through the tissue samples as well as peripheral blood samples. We use the Big Dye Terminator v3.1 Cycle Sequencing Kit and an ABI 3730 Genetic Analyzer (Life Technologies, Carlsbad, CA) to sequence the amplicon fragments with M13 primer bidirectionally (forward and reverse). Then Mutation Surveyor (SoftGenetics, State College, PA) was used to compare the target sequences to the EPHB4 reference sequence. Primer sequences are available upon request.

Results

Histological study showed capillary hyperplasia in the and dermis (Fig.1e, HE, 100x).

Molecular analysis showed that a germline stop-gain mutation (c.1093C>T, p.Arg365*) in the EPHB4 gene mutation was identified in the samples from peripheral blood of the proband. The same mutation was also detected in the tissue sample of the proband. According to family history of erythema, physical examination of potential fast-flow lesions, CT results and genetic study revealing germline EPHB4 mutation, the patient was diagnosed with capillary malformation-arteriovenous malformation syndrome type 2 (CM-AVM2).

Discussion

Our proband had multifocal, progressive, pink, irregular erythema on the left arm left shoulder and chest. The erythema was consistent with atypical CMs in CM-AVM. The suspicion of CM-AVM further deepened according to physical examination that revealed the higher skin temperature in the erythema and detectable palpation of the mass located in the axillary lesion, and family history of inherited erythema. Enhancement CT demonstrated potential invasion of fast-flow lesions underlying the erythema. Moreover, the suspicion of CM-AVM was then confirmed with definite diagnosis at the molecular level based on detection of germline loss-of-functional mutation of EPHB4.

EPHB4 belongs to the Eph transmembrane receptor tyrosine kinase family, and mainly expressed in the vein endothelial cell membrane and forms a bidirectional signaling pathway with its ligand, EFNB2, which usually expressed in the arterial endothelial cell membrane⁷. Bidirectional signaling plays an essential role in primary angiogenesis and arteriovenous differentiation⁸⁻¹⁰. Elena Groppa et al. discovered that activation of EPHB4 reduced the phosphorylation of ERK1/2 and the proliferation of endothelial cells, while blockade of EPHB4 caused activation of ERK1/2 and sprouting angiogenesis, which was regulated by ephrinB2 reverse signaling¹¹. Thus, the stop-gain mutation of EPHB4 results in an increase in ERK1/2 phosphorylation and spouting angiogenesis. In this case, the mutation of EPHB4 was stopped, resulting in loss-of-function mutation of EPHB4 gene. The MAPK/ERK pathway plays an essential role in various processes, including proliferation, differentiation, migration¹², and apoptosis in a variety of cells¹². Furthermore, RASA1 is the downstream effector of EPHB4 in endothelial cells¹³. Together, both mutations of RASA1 and EPHB4 lead to similar atypical erythema and high-flow vascular malformations. In addition, the “two-hit” hypothesis is that the occurrence of a disease requires the inactivation of two alleles, which was proposed to explain the AVM present in 30% of CM-AVM caused by germline mutation from the parent and second mutation in somatic cells^{4,5,14,15}. However, we did not find any additional mutations in EPHB4 in this study to support this hypothesis.

EPHB4 mutation related CM-AVM2 was firstly reported and named in 2017⁵. According to the literature, CM-AVM usually manifests as atypical CMs that are cutaneous, multifocal, pink to purple in color, round to oval in shape, 1-3 cm in diameter, and are surrounded by pale halos^{4,16,17}. Although the CMs of CM-AVM2 have similar characteristics compared with those of CM-AVM1, they are slightly different. Telangiectasia and bier spots usually presented in CM-AVM2 but not in CM-AVM1, and the pale central

region can present in large CM in CM-AVM2⁵. In addition, AVM was discovered in the armpit of our patient, and fast-flow vascular malformation can occur in CM-AVM2 and CM-AVM1, but the risk of anomalies in the central system is lower than that in CM-AVM1⁵. Molecular diagnosis when necessary, combined with detailed clinical history as well as physical examination, is important for differential diagnosis of CM-AVM, risk assessment of fast-flow lesions, as well as monitoring of complications including heart failure, seizures, and life-threatening hemorrhage.

Molecular analysis provides new management strategies beyond precautions preventing AVM formation and complication with bleomycin injection¹⁸. Kawasaki et al demonstrated that loss-of-functional mutation in EPHB4 or RASA-1 result in downstream activating of mTORC1, overgrowth of vascular and the development of CM-AVM.¹³ mTOR inhibitor may be able to slowdown the progress of CM-AVM, and to some extent, lead to clinical improvement. Yu et.al performed targeted therapy on CM-AVM2 patient with topical sirolimus 1% cream for 4 weeks and reached limited improvement¹⁹. Future EPHB4 modulators may provide additional options for CM-AVM patients with germline EPHB4 mutation^{20,21}.

In conclusion, we report, to the best of our knowledge, the first case of CM-AVM type 2 with a novel germline EPHB4 mutation in East Asia. The molecular mechanism of CM-AVM2 may be that the mutation results in the loss of EPHB4 function, raising the reversal of the EPHB4/ephrinB2 bidirectional signaling pathway and inducing angiogenesis. Moreover, the occurrence of AVM requires a second mutation to cause a complete loss of EPHB4 function.

Conclusion

In this case, we report one female patient with a novel germline EPHB4 loss-of- function mutation as well as multiple CMs and AVMs, consistent with the diagnosis of CM-AVM2. According to our knowledge, this is the first description of CM-AVM2 with a germline EPHB4 mutation in Asians. This finding would extending our understanding of the angiogenesis of CM-AVM type 2, especially in Asian populations.

Abbreviations

Capillary malformation-arteriovenous malformation (CM-AVM)

Capillary malformations (CMs)

Next-generation sequencing (NGS)

Declarations

Ethical Approval and Consent to participate Ethics Committee approval was obtained from the Institutional Ethics Committee of Shanghai 9th People's Hospital, Shanghai JiaoTong University School of Medicine to the commencement of the study. Written informed consent to participate was obtained.

Consent for publication Written informed consent for publication was obtained.

Availability of supporting data All data used during the study appear in the submitted article.

Competing interests The authors declare there is no conflicts of interest regarding the publication of this paper.

Funding This study has received funding by Clinical Research Plan of SHDC (16CR1007A), Clinical Research Program of 9th People's Hospital, Shanghai Jiao Tong University School of Medicine (JYLJ001) and Clinical Research Project of Multi-Disciplinary Team, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine (201701001).

Authors' contributions

Conception and design of study: Ren Cai, Xiaoxi Lin and Hui Chen

Acquisition of data Yuanbo Li, Yi Sun, Xiaojing Zeng

Analysis and/or interpretation of data: Yun Liu, Fatao Liu

Acknowledgements We thank for the patient and her family for their noble action to share their clinical information.

References

- 1 Revencu, N. *et al.* Parkes Weber syndrome, vein of Galen aneurysmal malformation, and other fast-flow vascular anomalies are caused by RASA1 mutations. *Hum Mutat***29**, 959-965, doi:10.1002/humu.20746 (2008).
- 2 Jacobs, A. H. & Walton, R. G. The incidence of birthmarks in the neonate. *Pediatrics***58**, 218-222 (1976).
- 3 Mills, C. M., Lanigan, S. W., Hughes, J. & Anstey, A. V. Demographic study of port wine stain patients attending a laser clinic: family history, prevalence of naevus anaemicus and results of prior treatment. *Clin Exp Dermatol***22**, 166-168 (1997).
- 4 Eerola, I. *et al.* Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations. *Am J Hum Genet***73**, 1240-1249 (2003).
- 5 Amyere, M. *et al.* Germline Loss-of-Function Mutations in EPHB4 Cause a Second Form of Capillary Malformation-Arteriovenous Malformation (CM-AVM2) Deregulating RAS-MAPK Signaling. *Circulation***136**, 1037-1048, doi:10.1161/CIRCULATIONAHA.116.026886 (2017).
- 6 Blei, F. ISSVA Classification of Vascular Anomalies. (2017).

- 7 Bai, J., Wang, Y.-J., Liu, L. & Zhao, Y.-L. Ephrin B2 and EphB4 selectively mark arterial and venous vessels in cerebral arteriovenous malformation. *J Int Med Res***42**, 405-415, doi:10.1177/0300060513478091 (2014).
- 8 Sawamiphak, S. *et al.* Ephrin-B2 regulates VEGFR2 function in developmental and tumour angiogenesis. *Nature***465**, 487-491, doi:10.1038/nature08995 (2010).
- 9 Wang, Y. *et al.* Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis. *Nature***465**, 483-486, doi:10.1038/nature09002 (2010).
- 10 Wang, H. U., Chen, Z. F. & Anderson, D. J. Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell***93**, 741-753 (1998).
- 11 Groppa, E. *et al.* EphrinB2/EphB4 signaling regulates non-sprouting angiogenesis by VEGF. *EMBO Rep***19**, doi:10.15252/embr.201745054 (2018).
- 12 Sun, Y. *et al.* Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis. *J Recept Signal Transduct Res***35**, 600-604, doi:10.3109/10799893.2015.1030412 (2015).
- 13 Kawasaki, J. *et al.* RASA1 functions in EPHB4 signaling pathway to suppress endothelial mTORC1 activity. *J Clin Invest***124**, 2774-2784, doi:10.1172/JCI67084 (2014).
- 14 Cai, R., Liu, F., Liu, Y., Chen, H. & Lin, X. RASA-1 somatic "second hit" mutation in capillary malformation-arteriovenous malformation. *J Dermatol***45**, 1478-1480, doi:10.1111/1346-8138.14676 (2018).
- 15 Revencu, N. *et al.* mosaic mutations in patients with capillary malformation-arteriovenous malformation. *J Med Genet***57**, 48-52, doi:10.1136/jmedgenet-2019-106024 (2020).
- 16 Larralde, M., Abad, M. E., Luna, P. C. & Hoffner, M. V. Capillary malformation-arteriovenous malformation: a clinical review of 45 patients. *Int J Dermatol***53**, 458-461, doi:10.1111/ijd.12040 (2014).
- 17 Boon, L. M., Mulliken, J. B. & Vikkula, M. RASA1: variable phenotype with capillary and arteriovenous malformations. *Curr Opin Genet Dev***15**, 265-269 (2005).
- 18 Jin, Y. *et al.* Treatment of early-stage extracranial arteriovenous malformations with intralesional interstitial bleomycin injection: a pilot study. *Radiology***287**, 194-204 (2018).
- 19 Yu, J., Streicher, J. L., Medne, L., Krantz, I. D. & Yan, A. C. EPHB4 Mutation Implicated in Capillary Malformation–Arteriovenous Malformation Syndrome: A Case Report. *Pediatric Dermatology***34** (2017).
- 20 Garber, K. Of Ephs and Ephrins: Companies Target Guidance Molecules in Cancer. *Journal of the National Cancer Institute***102**, 1692-1694 (2010).

Figures



Figure 1

Clinical manifestations, histological analysis and genetic mutation of proband and her grandfather. (a) The multiple atypical CMs on the left arm, shoulder and chest of the proband. Arrow, a mass with detectable palpation in the axillary lesion. (b) The erythema on the right forearm of the proband's grandfather. (c, d) Enhanced CT demonstrated multiple well-defined nodular and lamellar masses in the

axillary area. The masses were homogeneously enhanced after reinforcement. (e) Histological study showed capillary hyperplasia in the and dermis (HE, 100 \times).