JCI The Journal of Clinical Investigation

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J Clin Invest. 2023. https://doi.org/10.1172/JCI166497.

Research Letter In-Press Preview Vascular biology

A seed sequence variant in miR-145-5p causes multisystem smooth muscle dysfunction syndrome

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The authors have declared no conflicts of interest exist

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Results:

Multisystemic smooth muscle dysfunction syndrome (MSMDS, OMIM #613834) is an ultrarare smooth muscle myopathy(1). Cases are monogenic from missense variation at arginine 179 of the *ACTA2* gene(1, 2). Herein we describe a case of MSMDS associated with a single nucleotide variant in the gene *MIR145*.

Fetal ultrasound revealed polyhydramnios, enlarged abdomen and bladder, and prune belly syndrome. Profound gastrointestinal dysmotility was identified during infancy. His cerebrovascular disease began with frontal cortex and watershed strokes at approximately 2.5 years of age. Straightening of cerebral arteries and flattening of the genu of the corpus callosum and pons was observed. During school age he had multiple strokes consistent with arterial ischemic and watershed infarctions. Severe progressive steno-occlusive disease developed, worse in the anterior circulation (Figure 1A&B). The vascular anatomy also showed straightening and decreased caliber of the terminal internal carotid artery, consistent with described cases of MSMDS(3, 4). Thoracic aortic imaging has been normal.

A thoracic aortic aneurysm/dissection panel was negative, including analysis of *ACTA2*. Quad genome sequencing was negative; however, research-based analysis revealed a de novo single nucleotide variant in *MIR145* (NR_029686.1:n.18C>A)(Figure 1C). This variant is absent from gnomAD, has a CADD score of 20.9, and *MIR145* is enriched in tissues with high smooth muscle cell (SMC) content(5), (Supplemental Figure 1).The *MIR145* transcript is processed into two microRNAs, with the variant position at nucleotide 3 of miR-145-5p.

To determine if the miR-145-5p variant could mediate the observed patient phenotype of smooth muscle dysfunction, we undertook molecular analysis. Cases of MSMDS to date have been caused by recurrent missense variants in the *ACTA2* gene altering arginine 179(2). These variants impair smooth muscle actin function, resulting in a cellular state resembling a loss of protein function. The miR145-5p variant is located within the seed sequence (nts. 2-8), the portion of a microRNA that stalls lateral diffusion of the RISC complex and promotes stable interactions with complementary RNAs (Figure 1C). We hypothesized that mutant miR145-5p may not be able to target 3' UTRs that mediate proper SMC function and may thus result in cellular changes similar to *ACTA2* R179 variants. To assess this possibility, we exposed human vascular SMCs to either an siRNA targeting miR145-5p, wild type miR145-5p, or a mutant version of miR145-5p with the patient variant. Indeed, transfection of either an siRNA against miR145-5p or mutant miR145-5p induced a notable decrease in the expression of several cytoskeletal proteins including transgelin, calponin, and importantly, smooth muscle actin (Figure 1D and Supplemental Figure 2).

Cellular models of the *ACTA2* R179H mutation demonstrate global filamentous actin cytoskeletal deficiency(6). Transfection of either siRNA against miR145-5p or the mutant miR145-5p induced a phenotype characterized by deficient F-actin, whereas treatment with wild type miR145-5p enhanced stress fiber formation (Figure 1E). Therefore we next performed RNA-seq analysis including mRNAs and miRNAs in patient skin fibroblasts and compared them to wild type skin fibroblasts. Principal component analysis of differentially expressed genes (DEGs) substantially differentiated the patient's fibroblasts from control fibroblasts

(Supplemental Figure 2). Furthermore, pathway analysis of DEGs was significant for categories related to “hsa04810: regulation of actin cytoskeleton” (Supplemental Figure 3). Hybridization analysis and miR RNA-seq demonstrated a decrease in expression of miR145-5p in the presence of mutant miR145-5p (Supplemental Figure 1 & 4), consistent with impairment in a positive feedback loop for *MIR145* expression(5).

In conclusion, genetic variation in the *MIR145* gene expands the possible loci associated with MSMDS and further confirms the syndrome as a disorder of failed SMC development and function, although discovery of further cases will be necessary to confirm our findings. To our knowledge this is the first patient reported with a monogenic vascular disease caused by a mutation in a non-coding gene.

Acknowledgements:

We would like to thank the patient and has family for their participation in this study and his home medical team for referring him to the UDN study.

This work was supported by the Caitlin and Rich Hill Family Fund for Undiagnosed Diseases, and the NIH Common Fund under award number U01HG007690 (D.A.S., L.C.B.). M.E.L. is supported by the Toomey fund for Aortic Dissection Research, P.L.M. is supported by R01NS117575, and P.L.M. and M.E.L. are supported by NIH R01NS125353. C.L.L.C. is supported by NIH K01HL164687.

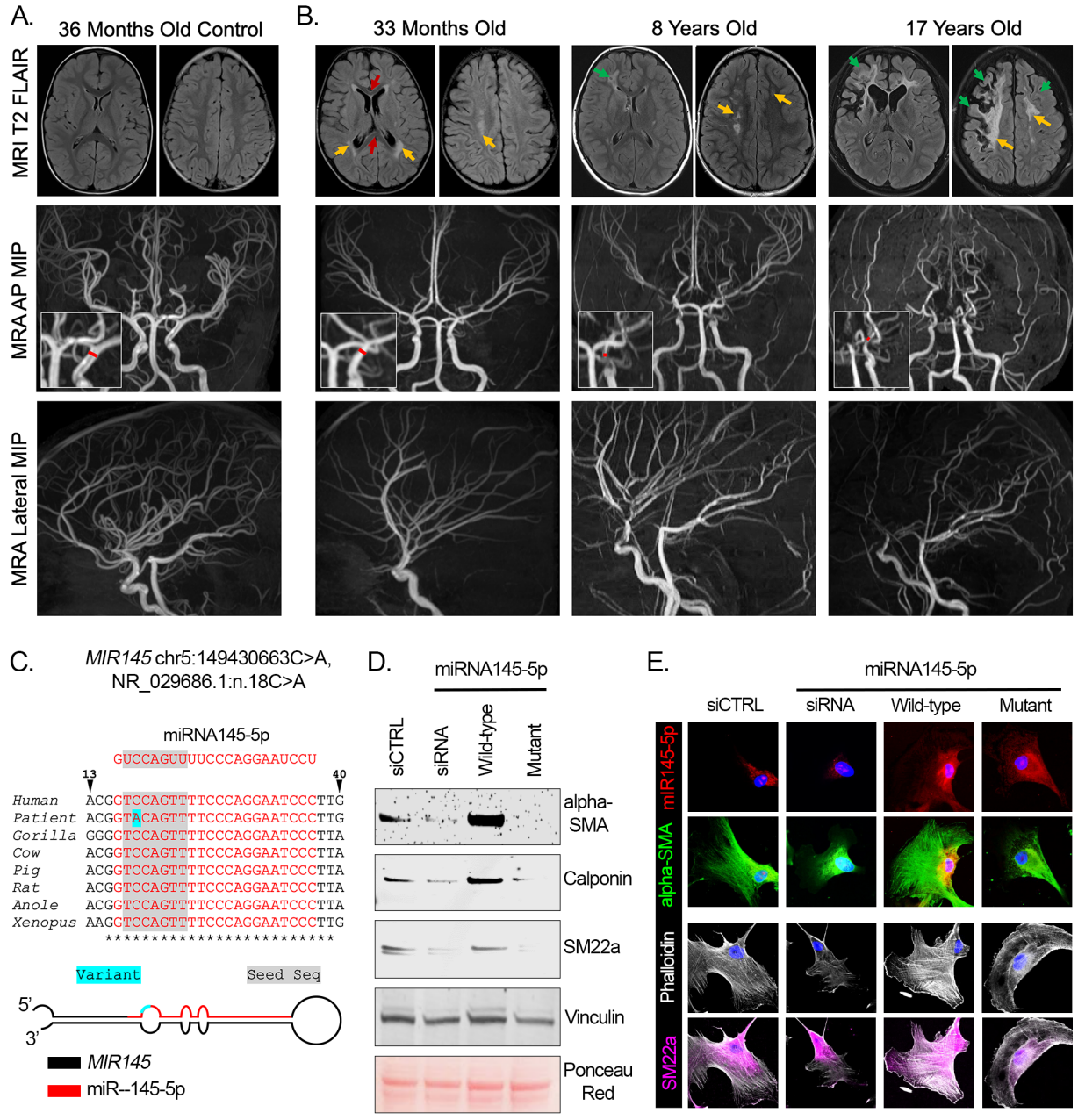

Figure 1. Representative MRI T2-weighted FLAIR and maximum intensity projection (MIP) of MRA (anterior-posterior (AP) and lateral MIP) images (A.) from a 36 month-old control and (B.) three different time points of patient with miRNA145-5p mutation. Note increased angulation of the forceps of the corpus callosum (red arrows) and significant bilateral periventricular and right watershed white matter injury (yellow arrows) at age 33 months, which progressed to bilateral white matter (yellow arrows) and right frontal ischemic infarctions (green arrows) by 8 years of age. Additional arterial ischemic infarctions occurred through 17 years of age. Vascular anatomy showed straightening and decreased caliber of terminal internal carotid artery (ICA) and basal cerebral arteries with progression of the relative stenosis of the terminal ICA (red bar) when compared to its petrous segment. (C.) Variant in the *MIR145* gene shown as primary structure

in multiple species comparison and in secondary structure. (D.&E.) Vascular SMCs transduced with indicated miRNAs, analyzed by western blot and immunofluorescence demonstrate that the mutant version of miRNA-145-5p fails to mediate contractile protein expression or induce stress fiber formation similar to WT miRNA-145-5p. *Sequence conservation across species

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