Postzygotic dominant-negative mutations of *RHOA* cause a mosaic neuroectodermal syndrome

Pierre Vabres^{1,3,*}, Arthur Sorlin^{1,2,*}, Stanislav S Kholmanskikh^{4,*}, Bénédicte Demeer⁵, Judith St-Onge^{1,2,6}, Yannis Duffourd^{1,2}, Paul Kuentz^{1,2,7}, Jean-Benoît Courcet^{1,3}, Virginie Carmignac^{1,2}, Didier Bessis⁸, Odile Boute⁹, Alain Bron³, Guillaume Captier⁸, Esther Carmi⁵, Sophie Dalac³, Bernard Devauchelle⁵, David Geneviève⁸, Catherine Gondry-Jouet⁵, Laurent Guibaud¹⁰, Arnaud Lafon³, Julien Thevenon^{1,3}, William B Dobyns¹¹, Geneviève Bernard⁶, Laurence Faivre^{1,3,+}, M Elizabeth Ross^{4,+}, Jean-Baptiste Rivière^{1,2,6,+}

1 FHU Translad, Dijon, France — **2** Inserm UMR 1231 GAD team, Université Bourgogne Franche-Comté, Dijon, Fr — **3** CHU Dijon Bourgogne, Fr — **4** Center for Neurogenetics, Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, New York, USA — **5** CHU Amiens Picardie, Fr — **6** McGill University, Montreal, Canada — **7** CHRU de Besançon, Fr — **8** CHU de Montpellier, Fr — **9** CHU Lille, Fr — **10** Hospices Civils de Lyon, Fr — **11** Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, Washington, USA; * These authors contributed equally to this work + These authors jointly directed this work.

Introduction

Hypomelanosis of Ito (HI):

- A non-specific manifestation of mosaicism
- A frequent neurocutaneous syndrome
- Unknown genetic cause

We ascertained 5 unrelated individuals with similar features that did not match any known syndrome:

- **HI** and pilar dysplasia following the lines of Blaschko
- **asymmetric facial dysmorphism** (craniofacial microsomia and broad nasal bridge)
- **acral anomalies** (brachydactyly, syndactyly, and broad first toe)
- **teeth anomalies** (oligodontia, microdontia, and conical teeth)
- **ocular anomalies** (microphthalmia, strabismus, and myopia)
- **leukoencephalopathy** with enlarged lateral ventricles
- normal development and intelligence

Material and Methods

- Deep whole exome sequencing (WES) on patient skin (mean coverage 150 X) and parents' blood (trio)
- Targeted ultra-deep sequencing (TUDS) *RHOA* next-generation sequencing of using Nextera XT DNA Library Preparation Kit on DNA from skin biopsy and blood
- Transfected NIH3T3 cells with myc-tagged mutants and wild-type *RHOA* plasmids, for Immunocytochemical and Western blot analysis: comparison of our variants to known constitutively active (CA) and dominant-negative (DN) mutants.

Results

We identified postzygotic variants in the same gene, *RHOA* (Figure 2):

- c.139G>A; p.Glu47Lys in subjects S1 and S2 in WES, and in S3 and S4 in TUDS
- c.211C>T; p.Pro71Ser in WES in S3

Variants were supported by 2.6% up to 30.6% of reads in skin DNA, and absent from blood

Immunocytochemical localization of F-actin stress fibers and microtubules:

- marked cytoskeletal alterations in all transfected cells
- **reduced cell spreading and decreased number of stress fibers, as well as microtubule disorganization, similar to the DN mutant**

Western blot analysis: **reduced levels of phosphorylated downstream effectors of RhoA, also similar to the DN mutant**

Discussion

We have delineated a **clinical and molecular subset of pigmentary mosaicism**, which could be named "***RHOA*-related mosaic ectodermal dysplasia**".

Apart from recent reports of linear hypopigmentation in three patients with *MTOR*-related hemimegalencephaly, no other genes have been implicated in pigmentary mosaic disorders.

RHOA encodes a RAS-related Rho GTPase, RhoA, with a central role in **signal transduction and actin cytoskeleton dynamics**, through regulation of **stress fibers and focal adhesion formation**.

It is a highly conserved protein particularly **intolerant to amino acid substitutions**, and is included in a set of genes **essential to cell viability**, supporting the idea that *RHOA*-related mosaic ectodermal dysplasia should be added to the list of disorders resulting **from lethal mutations surviving only by mosaicism**, which includes Proteus, Sturge-Weber, and other mosaic syndromes. Our data show mosaic mutations can act through a **dominant-negative effect**, whereas variants in other mosaic syndromes are often activating mutations.

As *RHOA* is not linked to the PI3K-AKT-mTOR and RAS-MAPK pathways involved in mosaic developmental disorder, identification of other genes causing pigmentary mosaic syndromes may pinpoint new pathogenesis pathways, which will help enhancing our understanding of their causes.

Our findings pave the way towards elucidating the etiology of pigmentary mosaicism and highlight the role of *RHOA* in human development and disease.

References

- Blomen, V. A. *et al. Science* **350**, 1092–1096 (2015); Canman, J. C. *et al. Science* **322**, 1543–1546 (2008); Happle, R. *J. Am. Acad. Dermatol.* **16**, 899–906 (1987); Lek, M. *et al. Nature* **536**, 285–291 (2016); Mirzaa, G. M. *et al. JAMA Neurol.* **73**, 836–845 (2016); Narumiya, S. *J. Biochem. (Tokyo)* **120**, 215–228 (1996); Narumiya, S. *J. Biochem. (Tokyo)* **120**, 215–228 (1996); Pan, Z. K. *et al. J. Immunol. Baltim. Md* **160**, 3038–3045 (1998); Ruggieri, M. & Pavone, L. *J. Child Neurol.* **15**, 635–644 (2000); Sybert, V. P. *J. Invest. Dermatol.* **103**, 141S–143S (1994); Van Steensel, M. A. M. *J. Pediatr. Genet.* **4**, 144–153 (2015); Wang, T. *et al. Science* **350**, 1096–1101 (2015); Wu, K. Y. *et al. Nature* **436**, 1020–1024 (2005); Zhao, X. *et al. Cancer Res.* **69**, 483–491 (2009)

Figure 1 (up) Main clinical features of affected individuals.

Figure 2 (right) Mutant allele fraction of *RHOA* mutations in the 5 subjects

Figure 3 (left) Cytoskeletal organization and morphology (a-c) and expression level of RhoA and downstream effectors (d)