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# VPS51 biallelic variants cause microcephaly with brain malformations: a confirmatory report

Annette Uwineza<sup>1, §</sup>, Jean-Hubert Caberg<sup>2</sup>, Janvier Hitayezu<sup>1</sup>,  
Stephane Wenric<sup>3</sup>, Leon Mutesa<sup>1</sup>, Yoann Vial<sup>4,5</sup>, Séverine Drunat<sup>4,5</sup>,  
Sandrine Passemard<sup>4,5</sup>, Alain Verloes<sup>4,5</sup>, Vincent El Ghouzzi<sup>5</sup>, Vincent  
Bours<sup>2</sup>

<sup>1</sup>Center for Human Genetics, College of Medicine and Health Sciences, University of Rwanda,  
Kigali, Rwanda

<sup>2</sup>Center for Human Genetics, Centre Hospitalier Universitaire, University of Liege, Liege, Belgium

<sup>3</sup>GIGA-Research, Human Genetics Unit, University of Liege, Liege, Belgium;

<sup>4</sup>Department of Genetics, AP HP - Robert Debré University Hospital, Paris, France.

<sup>5</sup> PROTECT, INSERM UMR1141, Université de Paris, Paris, France

## §Correspondence:

Annette Uwineza, MD, PhD

Center of Human Genetics

College of Medicine and Health Sciences

University of Rwanda, Rwanda

Tel: (+250) 788741577; email address: [A.UWINEZA@ur.ac.rw](mailto:A.UWINEZA@ur.ac.rw)

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63 **Abstract**  
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65 Genome-wide linkage analysis and whole exome sequencing undertaken in two siblings with delayed  
66 psychomotor development, absent speech, severe intellectual disability and postnatal microcephaly,  
67 revealed a homozygous intragenic deletion in *VPS51*, which encodes the vacuolar protein sorting-  
68 associated protein, one the four subunits of the GARP and EARP complexes that promotes the fusion  
69 of endosome-derived vesicles with the *trans*-Golgi network (GARP) and recycling endosomes  
70 (EARP). This observation supports a pathogenic effect of *VPS51* variants, which has only been  
71 reported previously once, in a child with microcephaly. It confirms the key role of membrane  
72 trafficking in normal brain development and homeostasis.  
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85 **Keywords:**  
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87 VPS51, GARP, EARP, Golgipathies, Golgi, endosomes, postnatal microcephaly, neurodevelopmental  
88 disorders, Rwanda  
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## Introduction

Neurodevelopmental disorders (NDDs) encompass a wide range of intellectual, behavioral and motor disabilities (van Bokhoven, 2011). High-throughput sequencing has allowed to more readily capture variations causing subtle or atypical phenotypes, especially in NDDs (Hu et al., 2014). Recently, the role of membrane trafficking during brain development and maturation has been highlighted with a central contribution played by the Golgi apparatus and its impact on key processes : neurogenesis, neuronal migration, myelination and maturation of postmitotic neurons ((Passemar et al., 2017; Rasika et al., 2019). In particular, the Golgi-associated retrograde protein (GARP) acts in promoting the retrograde fusion of endosome-derived carriers with the *trans*-Golgi network (TGN) (Bonifacino and Hierro, 2011; Conibear and Stevens, 2000; Perez-Victoria et al., 2010). GARP is a multisubunit complex made of four distinct proteins (VPS51, VPS52, VPS53 and VPS54). GARP differs only in one subunit with another tethering complex, known as the endosome-associated recycling protein (EARP) complex, in which the VPS54 subunit is substituted by syndetin (VPS50). EARP ensures the fusion of endosome-derived carriers with recycling endosomes and therefore promotes recycling of endocytic membranes back to the plasma membrane (Masschaele et al., 2017; Schindler et al., 2015). Among the five subunits of the GARP/EARP complexes, VPS53 was the first to be associated with a human disease. Loss-of-function mutations in *VPS53* have been associated with two forms of degenerative NDDs, autosomal recessive pontocerebellar hypoplasia type 2E (PCH2E, MIM #615851), characterized by a severe early-onset neurodegeneration with profound intellectual disability (ID), progressive microcephaly, spasticity, and early-onset epilepsy (Feinstein et al., 2014), and progressive encephalopathy with edema, hypsarrhythmia and optic atrophy (PEHO) characterized by a severe developmental delay, limb and facial edema, intractable epilepsy, optic atrophy and dysmorphic features (Hady-Cohen et al., 2018). Recently, a 6-year-old patient with severe global developmental delay, pontocerebellar abnormalities, microcephaly, hypotonia, epilepsy and several systemic and peripheral dysfunctions has been reported (Gershlick et al., 2019). This girl was found to carry compound heterozygous variants in *VPS51* affecting both GARP and EARP complexes. Here, we report two Rwandan sisters with a homozygous three base pairs intragenic deletion in *VPS51* associated with developmental delay, absent speech, severe ID and postnatal microcephaly.

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183 The present study confirms the key role of the GARP and EARP complexes in normal brain  
184 development and homeostasis.  
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## 189 Patients and Methods

### 190 Patient 1

191 Patient 1, seen in the Centre of Medical Genetics of Rwanda University, in Kigali, was born to first-  
192 cousin once removed parents of Rwandese origin (f=1/32), for severe psychomotor developmental  
193 delay. This girl was born by normal delivery from an uneventful pregnancy (birth weight: 3600 g).  
194 Head circumference was not recorded. At 1 month of age, she had a poorly described episode of fever  
195 with loss of consciousness treated by an unknown medication. Further development was severely  
196 delayed. She was able to sit unsupported at age 3. At the first academic evaluation, at age 5, she was  
197 still unable to walk and had no language. Clinical examination revealed microcephaly (head  
198 circumference of 46,5 cm: -3.23 SD), a weight of 15 kg (-1.36 SD) and a height of 118 cm (+ 1.8 SD).  
199 She had hypertelorism, upturned nasal tip, short philtrum, thick vermilion of the upper lip, large teeth  
200 (Fig. 1 A), high arched palate, multiple dental carries, left internal strabismus, and bilateral pes  
201 planus. Brain CT-scan showed Dandy Walker variant anomaly with a cyst of the posterior fossa,  
202 communicating with the 4th ventricle, and mild ventriculomegaly. At the age of 9, she had ataxic gait.  
203 The language was still absent. Brain MRI showed mild vermis atrophy, a thin corpus callosum,  
204 dilated lateral ventricles and a mega cisterna magna (Fig. 2 A,B). Serum transferrin isoelectrofocusing  
205 excluded congenital disorder of glycosylation (CDG).  
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### 221 Patient 2

222 Patient 1's sister was 30-month-old at examination (Fig 1 B). She was born at term following an  
223 uneventful pregnancy and delivery (birth weight 3200 g). At 12 months, she developed an episode of  
224 fever, diarrhoea and vomiting and became hypotonic. At 30 months, she was not able to walk and had  
225 no speech. Feeding difficulties at home lead to severe hypotrophy. She had upturned nasal tip and left  
226 internal strabismus (Fig 1 B). Her head circumference was 43 cm (- 4.56 SD), her weight was 7 kg (<  
227 -3 SD) and her height was 83 cm (- 2.3 SD). Brain MRI showed no malformation of the brain but a  
228 reduced white matter volume and a thin corpus callosum with a normal vermis (Fig. 2 C, D).  
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## Methods

Genomic DNA was extracted from peripheral blood leukocytes using the standard phenol/chloroform method. Whole-genome SNP array was performed in patients, their parents and one unaffected sibling using HumanOmni2.5 BeadChip (Illumina, San Diego, CA). Fifty nanograms of DNA were processed according to Illumina's Infinium HD Assay Super protocol. Arrays were scanned with an iScan System and data were extracted by the Genome Studio software (Illumina, San Diego, CA) and analysed using the PLINK software. Exome sequencing (WES) was performed in both patients and their parents. Library preparation, sequencing, alignment and variant calling were performed in the Unit of Human Genetics, GIGA, at the University of Liege (Belgium). Exons were captured using the Agilent SureSelect Human All Exon V5+UTR (Agilent Technologies, Santa Clara, CA, USA), and sequence was generated on a HiSeq2000 instrument (Illumina San Diego, CA, USA). Base calling was performed using the Illumina CASAVA software. All the raw reads were aligned to the reference human genome (GRCh37 / hg19) using the Burrows–Wheeler aligner (BWA). Optical and PCR duplicates were marked and removed with Picard tools (<http://picard.sourceforge.net>). Single nucleotide variants and small insertions/deletions were then called using the GATK Unified Genotyper v2.7. Variant annotation was performed using ANNOVAR. Variant screening was restricted to genes included in homozygous loci revealed by homozygosity mapping. Variants with at least a minor allele frequency (MAF) of 0,01% in dbSNP138, 1000 genomes, ExAC and ESP6500 databases were excluded. Missense variants were tested for mutational effect by prediction algorithm with Polyphen-2, Sift, Provean, mutation taster, and CADD score. The analysis process was based on the latest release of GnomAD, dbSNP, ExAC databases and the literature review. The variant identified by WES was confirmed by Sanger sequencing in the patients and their parents (primers sequence available on request). The study was approved by the Rwandan National Ethics Committee (N°394/RNEC/2013) and informed consents were obtained from the participants.

## Results

Homozygosity mapping revealed a large homozygous locus on chromosome 11 (chr11:44,081,150\_74,821,392) of about 30 Mb (11p11.2-q13.4) in both sisters. WES revealed a

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303 variant in the *VPS51* gene (reference sequence : NM\_013265.3) consisting in an in-frame deletion of  
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305 a CTT codon in exon 5 (c.1419\_1421del / p.(Phe474del) ) detected at the homozygous state in both  
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307 sisters and at the heterozygous state in both parents and in the unaffected sister. Phe474 is a highly  
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309 conserved residue. This mutation was present in only 2 individuals in the GnomAD database  
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311 (MAF:0.00083%) and was never reported at the homozygous state.  
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## 313 314 Discussion

315 In this study, WES was performed in two patients from a Rwandan consanguineous family with  
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317 NDD. Our patients were selected from a cohort of a previously analysed series of 50 Rwandan  
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319 patients presenting ID and global development delay with or without dysmorphic features (Uwineza  
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321 et al., 2014). The phenotype of our patients includes minor dysmorphic features, a severe global  
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323 developmental delay, a microcephaly, and a reduced volume of the subcortical white matter  
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325 associated with vermis atrophy in the eldest sister.  
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327 Although the deletion identified in the present study does not induce a frameshift in the coding  
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329 sequence of *VPS51*, the loss of the highly conserved Phe474 is predicted to impact the function  
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331 and/or stability of the subunit, possibly by altering a site of interaction with other partners.  
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333 Furthermore, this mutation has never been reported in patients of African origin suggesting that this  
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335 mutation is not a rare polymorphism associated with an ethnic group.  
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337 A single 6-year-old patient has recently been reported with compound heterozygous mutations  
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339 (c.1468C>T/p.Asp745Thrfs\*93 and c.2232delC/p.Arg490Cys) in *VPS51* (Gershlick et al., 2019).  
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341 The patient had failure to thrive, severe ID, microcephaly, thin corpus callosum, pontocerebellar  
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343 abnormalities (small vermis and thin brainstem) and a Dandy-Walker variant. EEG showed electrical  
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345 status epilepticus in sleep (ESES) requiring anticonvulsant therapy. She further had cortical blindness,  
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347 chronic cholestasis requiring ursodiol treatment, edema of the lower limbs reminiscent of PEHO  
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349 syndrome, and some dysmorphic features (including epicanthal folds, long eyelashes, upturned nasal  
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351 tip, a thin upper lip, full cheeks, increased hair on the upper back. Isoelectric focusing showed a  
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353 hypoglycosylated profile of serum transferrin Authors showed that the c.2232delC produces an  
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355 unstable frameshifted protein 54 aminoacids longer than the wild type and that the c.1468C>T  
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363 mutation produces a stable protein that assembles less efficiently with the other GARP/EARP  
364 subunits. In skin fibroblasts from the patient, the levels of fully-assembled GARP and EARP  
365 complexes were lowered. The distribution of the cation-independent mannose 6-phosphate receptor  
366 was altered and swelling of lysosomes was noted. Our patients appear thus to show a milder  
367 phenotype lacking epilepsy, the PEHO-like features (edema, optic impairment), the liver involvement  
368 and abnormal glycosylation profile of serum transferin.  
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372 Clinical features of our patients also have similarities with those of patients reported with mutation in  
373 *VPS53*, such as microcephaly and developmental delay in patients presenting PCH2E (Feinstein et al.,  
374 2014). However, our patients do not have epilepsy and their developmental delay is less severe than  
375 those with PCH2E. Interestingly, mutations in both *VPS53* and *SEPSECS*, the gene for PCH2D  
376 (OMIM #613811), can result in PEHO-like phenotype or PCCA, suggesting that these conditions  
377 belong to a common clinical spectrum (Hady-Cohen et al., 2018). In agreement with these  
378 observations, defective Golgi proteins are increasingly linked to a wide spectrum of  
379 neurodevelopmental defects that have been recently classified as "Golgipathies" (Rasika et al., 2019)  
380 and that usually associate progressive microcephaly and ID with white matter defects.  
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384 The observation of Gershlick and colleagues (Gershlick et al., 2019) that not only the proteasomal  
385 degradation of VPS51 but also a single aminoacid substitution in its sequence can result in a  
386 decreased stability and/or efficiency of the whole GARP and EARP complexes indicates that VPS51  
387 integrity is essential to GARP/EARP function. Of interest, the substitution reported in this paper is  
388 16-aminoacid close to the Phenylalanine deletion observed in the present study. On the other hand, a  
389 residual activity of GARP/EARP complexes is most likely necessary to avoid embryonic lethality and  
390 may explain why these NDDs, although very severe, are viable. Further studies are obviously required  
391 to validate the consequence of this mutation on both complexes.  
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395 Among the four subunits of the complex, VPS51 is required for the proper recruitment of GARP  
396 complex at the Golgi by promoting its interaction with the SNARE machinery (Conibear et al., 2003).  
397 In EARP, VPS51 likely plays a similar function in promoting SNARE-mediated membrane fusion  
398 events. VPS51 also assembles with the other three subunits of GARP/EARP complexes in a  
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423 stoichiometric manner which implies that its instability likely compromises that of the whole  
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425 complexes (Perez-Victoria et al., 2010). Therefore, VPS51 lies at the core of GARP/EARP complexes  
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427 and thereby participates to many functions essential for cell homeostasis such as traffic and recycling  
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429 of endosomes, acid hydrolase sorting, lysosome function, endosomal cholesterol traffic and autophagy  
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431 (Perez-Victoria et al., 2010). That the phenotype resulting from the deficiency of these complexes is  
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433 mainly neurodevelopmental is somehow surprising regarding their ubiquitous expression throughout  
434  
435 life and their critical function in maintaining cellular homeostasis. The same issue has been raised and  
436  
437 discussed recently for a number of Golgipathies (Rasika et al., 2019). More studies are now required  
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439 to understand why the nervous system appears more vulnerable than other organs to the deregulation  
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441 of the endomembrane system.  
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483 **Figures**  
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486 **Figure 1.**

487 Patient 1 (left) and 2 (right)  
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490 **Figure 2.** MRI of both siblings in T1 weight images (A,C: sagittal - B, D: axial).

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492 A and B: MRI of the patient 1 at age 9; C & D MRI of the patient 2 aged 30 months. Note the  
493 thin corpus callosum and the reduced volume of white matter for both siblings. Cerebellum  
494 (vermis and lobes) shows atrophy in patient 1, whereas it is preserved in patient 2,  
495 suggesting a progressive atrophy with age.  
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