A novel homozygous mutation in TRAPPC9 gene causing autosomal recessive non-syndromic intellectual disability

Mutaz Amin1,2, Cedric Vignal3, Esraa Eltaraifee3, Inaam N. Mohammed4, Ahlam A. A. Hamed4, Maha A. Elseed4, Arwa Babai4, Iman Elbadi4, Doua Mustafa4, Rayan Abubaker4, Mohamed Mustafa4, Severine Drunat2,3, Liena E. O. Elsayed6, Ammar E. Ahmed4, Odile Boespflug-Tanguy2,7 and Imen Dorboz2,7*

Abstract
Background: The etiology of intellectual disabilities is diverse and includes both genetic and environmental factors. The genetic causes of intellectual disabilities range from chromosomal aberrations to single gene disorders. The TRAPPC9 gene has been reported to cause autosomal recessive forms of intellectual disabilities in 56 patients from consanguineous and non-consanguineous families around the world.

Methods: We analyzed two siblings with intellectual disability, microcephaly and delayed motor and speech development from a consanguineous Sudanese family. Genomic DNA was screened for mutations using NGS panel (NextSeq500 Illumina) testing 173 microcephaly associated genes in the Molecular Genetics service in Robert Debre hospital in Paris, France.

Results: A novel homozygous mutation (NM_031466.7 (TRAPPC9):c.2288dup, p. (Val764Glyfs*7) in exon 14 of TRAPPC9 gene was found in the two patients. The mutation was predicted to cause nonsense mediated decay (NSMD) using SIFT prediction tool. The variant has not been found in either gnomAD or Exac databases. Both parents were heterozygous (carriers) to the mutation.

Conclusion: This is the first study to report patients with TRAPPC9-related disorder from Sub-Saharan Africa.

Keywords: Autosomal recessive, Intellectual disability, TRAPPC9, Novel, Sudan

Background
Intellectual disabilities (ID) are a heterogeneous group of disorders that present with variable severity of cognitive impairment which can be associated with other behavioural, syndromic or dysmorphic features [1]. There are currently more than 700 known rare genetic diseases that can present with various forms of intellectual disabilities and can be inherited as autosomal recessive, autosomal dominant, X-linked or mitochondrial [2]. Autosomal recessive forms of intellectual disabilities are relatively rare and account for less than 12% of cases of intellectual disabilities [3]. However, they are particularly more common in consanguineous communities as in the Middle East [4–12].

Intellectual disability-obesity-brain malformations-facial dysmorphism syndrome (ORPHA: 352,530) is a very rare form of autosomal recessive intellectual disability characterized by moderate to severe intellectual impairment, epilepsy, microcephaly, variable dysmorphic features and obesity [13]. The disease is caused by loss of function mutations in TRAPPC9 gene which is located...
in chromosome 8q24.3 and has 23 exons [6]. It encodes a protein that has important roles in brain development and functions as an activator of NF-kappa-B through increased phosphorylation of the IκB kinase (IKK) complex [5]. The clinical spectrum related to TRAPPC9 mutations also include non-syndromic intellectual disability [14] autism [15] and severe developmental delay [16].

So far, mutations in TRAPPC9 gene have been reported in very few families around the world [4–12, 14, 15, 17–25] but none from Sub-Saharan Africa. In this study, we reported a novel homozygous mutation in TRAPPC9 gene causing autosomal recessive intellectual disability in a consanguineous family from Sudan.

Methods
Two affected siblings born to consanguineous parents were studied. Patient one (aged 6 years) and patient two (aged 4 years) were both males and outcomes of normal uncomplicated vaginal delivery. Both patients presented with severe delay in motor development and total lack of speech up to the time of assessment (6 and 4 years respectively) (patient 1 started to walk at age 5 years and patient 2 at 2.5 years). Obesity was noted in the first few months of life followed by acquired microcephaly noted after 1 year of age. Both patients had severe learning difficulty (they were unable to attend school) and cognitive impairment. In addition, they had behavioral abnormalities (hyperactivity, irritability, biting and frequent nocturnal awakening). Patient two also had epilepsy. On examination, they had microcephaly (<3 SD), hypotonia and hyporeflexia (Table 1).

Brain MRIs of both patients revealed cortical and cerebellar atrophy, periventricular white matter changes, thin corpus callosum, dilatation of ventricles, and hyperintensity of posterior limb of internal capsule (Fig. 1).

Targeted gene panel testing
Two ml of saliva was collected from patients and other healthy family members using DNA Oragene Saliva kits (DNA Genotek Inc., Ottawa, ON, Canada). DNA extraction was done according to the prepIT.L2P manual protocol provided by the manufacturer. Genomic DNA was screened for mutations using NGS panel (NextSeq500 Illumina) testing 173 microcephaly associated genes in the Molecular Genetics service in Robert Debre hospital in Paris, France. Library was prepared using Custom SureSelectQXT (GC_V3) Agilent. Burrows-Wheeler Aligner algorithm (BWA) was used to align genomic DNA sequence to human reference genome GRCh37 (hg19) with >99.5% coverage and minimum depth of 20X. Variants were classified using Bench lab NGS Cartagenia v5.0.1.

Results
A novel homozygous mutation (NM_031466.7 (TRAPPC9):c.2288dup, p. (Val764Glyfs*7) in exon 14 of TRAPPC9 gene was found in the two patients. The mutation was a frameshift mutation leading to premature stop codon and was predicted to be deleterious using ENTPRISE-X [26] causing nonsense mediated decay (NSMD) as predicted by the SIFT tool [27]. The variant has not been found in either gnomAD or Exac databases. Parents were screened for the mutation using the same panel NGS analysis described in Methods and both were heterozygous (carriers) to the mutation. No other potentially deleterious variants were found in genes involved in neurodevelopmental disorders.

Discussion
The development of the human brain is very complex and involves a cascade of reactions controlled by significant number of genes [28]. In this study, we reported a novel homozygous mutation in TRAPPC9 gene causing autosomal recessive intellectual disability in a Sudanese family. The mutation reported in our study is a one base pair duplication in exon 14 that results in a premature stop codon and predicted to cause nonsense mediated decay in TRAPPC9-mRNA. All TRAPPC9 mutations reported so far are loss of function mutations (nonsense, frameshift splice site or inframe insertions/deletions) (Fig. 2.) and there was no genotype–phenotype correlation [4–7, 9–12, 18].

Table 1  Clinical features of patients with mutations in TRAPPC9 gene

| No | Reported patients | This study |
|----|-------------------|------------|
|    | 56                | Patient 1  | Patient 2  |
| Consanguinity | 15/20 (75%) | + | + |
| Age in years (mean) | 12.2 | 6 | 4 |
| Sex (M/F) | 19/27 | + | + |
| Intellectual disability | 53/53 (100%) | + | + |
| Microcephaly | 42/47 (89%) | + | + |
| Dysmorphism | 24/39 (61%) | – | – |
| Delayed motor and speech development | 54/54 (100%) | + | + |
| Autistic features | 7/25 (28%) | – | – |
| Epilepsy | 8/39 (18%) | – | + |
| Obesity | 13/28 (46%) | + | + |
| Behavioral abnormalities | 15/18 (83%) | + | + |
| MRI findings | | | |
| Brain atrophy | 11/14 (78%) | + | + |
| White matter changes | 19/22 (86%) | + | + |
| Thin corpus callosum | 24/26 (92%) | + | + |
TRAPPC9-related intellectual disability is an autosomal recessive disease with particular overrepresentation in consanguineous communities [4–12]. To our knowledge, only 56 patients were reported to have mutations in TRAPPC9 gene [4–12, 14, 15, 17–25]. The majority of these patients were from Middle Eastern consanguineous families (Table 1). Sudan has the highest percentage of consanguineous marriages in the Middle East [29]; therefore autosomal recessive forms of intellectual disabilities are expected to be prevalent compared to other modes of inheritance. The most consistent clinical presentations of patients with TRAPPC9-related autosomal recessive intellectual disability were cognitive impairment and delayed speech development. Some patients presented with other behavioral and metabolic abnormalities such as autism and obesity. Nonspecific dysmorphic features were reported in some but not all patients including our study. The clinical features of the two siblings in our study are aligned with those reported with TRAPPC9 mutations. More recently, missense mutations in TRAPPC9 gene have been reported in three patients with intellectual disability and biochemical abnormalities consistent with Congenital disorder of glycosylation [17]. This underlies the clinical heterogeneity of TRAPPC9-related disorder.

The TRAPPC9 gene is imprinted with predominance of the maternal allele [8]. It is highly expressed in postmitotic neurons and plays important roles in neuronal cells differentiation through regulating axon and/or dendrite outgrowth [30]. It is also involved in memory processing and regulation of food intake [8]. Trappc9−/− knockout mice were obese and had reduced brain size [8]. This pleiotropy
can explain the heterogeneous clinical features of patients with TRAPPC9-related disorder.

Conclusion
This is the first study to report patients with TRAPPC9-related disorder from Sub-Saharan Africa.

Abbreviations
TRAPPC9: Trafficking protein particle complex subunit 9; IKK: IκB kinase; NSMD: Nonsense mediated decay; SD: Standard deviation; DNA: Deoxyribonucleic acid; BWA: Burrows-Wheeler Aligner; NGS: Next generation sequencing.

Acknowledgements
Not applicable.

Author contributions
MA, ID and OBT made substantial contributions to the conception and the design of the study. AAAH, INM and MAE recruited the patients, did clinical interpretation and substantially revised the work including data analysis. CV and SD did the laboratory work. Field work, data collection, data analysis and data interpretation was done by AB, IE, RA, EE, DM and MM. MA drafted the manuscript. MAE, LE and AEA critically revised it. ID gave final approval of the version to be published; all authors read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
The raw data generated in this study has been deposited in the European Variation Archive (EVA)[31] at EMBL-EBI under accession number PRJEB55655 (https://www.ebi.ac.uk/eva/?eva-study=PRJEB55655) and the variant has been submitted to Clinvar under accession number: VCV001683498.1.

Declarations
Ethics approval and consent to participate
This study was approved by the Ethical committee of the Faculty of Medicine, University of Khartoum (Sudan) and the LEUKOFANCE research program for undetermined leukodystrophies (authorization CPP AU78; CHill. 1406552; AFSSAPS B90298-60) (France). Written informed consent was obtained from each family member (or parents/legal guardians in case of minors) before participation in the study.

Consent for publication
Written informed consent for publication of clinical details and/or clinical images was obtained from the all of the participants (or parents/legal guardians in case of minors).

Competing interests
The authors declare no conflict of interest.

Author details
1 Faculty of Medicine, Al-Neelain University, Khartoum, Sudan. 2 INSERM UMR 1141 PROTECT, Université Paris Diderot- Sorbonne Paris Cité, Paris, France. 3 Unité de Génétique Moleculaire, Departement de Genetique Médicale, APHP, Hopital Robert-Debré, 75019 Paris, France. 4 Department of Basic Sciences, College of Medicine, Princess Nourah Bint Abdulrahman University, PO. Box 84428, Riyadh 11671, Saudi Arabia. 5 Department of Medicine, University of Khartoum, Khartoum, Sudan. 7 Neuropediatrics and Metabolic Disorders Department, Reference Center for Leukodystrophies and Rare Leukoencephalopathies (LEUKOFANCE), CHU APHP Robert-Debré, Imen DORBOZ: INSERM U1141, Hopital Robert Debre, 48 boulevard Serurier, 75019 Paris, France.

Received: 25 February 2022   Accepted: 9 September 2022
Published online: 08 November 2022

References
1. Vasudevan P, Suri M. A clinical approach to developmental delay and intellectual disability. Clin Med (Northfield Il). 2017;17:558. https://doi.org/10.7861/CLINMEDICINE.17-6-558.
2. Orphanet: Rare genetic intellectual disability. https://www.orpha.net/consor/cgi-bin/Disease_Search.php?lng=EN&data_id=18320& Disease_Disease_Search_diseaseGroup=Rare-intellectual-disability &Disease_Disease_Search_diseaseType=Pat&Disease(s)/group of
1. Abou Jamra R, Wohlfart S, Zweier M, Uebe S, Priebe L, Ekici A, et al.
2. Abbasi AA, Blaesius K, Hu H, Latif Z, Picker-Minh S, Khan MN, et al.
3. Kakar N, Goebel I, Daud S, Nürnberg G, Agha N, Ahmad A, et al.
4. Orphanet: Intellectual disability obesity brain malformations facial dysmorphism
5. Mochida GH, Mahajnah M, Hill AD, Basel-Vanagaite L, Gleason D, Hill RS, et al.
6. Mir A, Kaufman L, Noor A, Motazacker MM, Jamil T, Azam M, et al.
7. Mochida GH, Mahajnah M, Hill AD, Basel-Vanagaite L, Gleason D, Hill RS, et al.
8. Kakar N, Goebel I, Daud S, Nürnberg G, Agha N, Ahmad A, et al.
9. Orphanet: Intellectual disability obesity brain malformations facial dysmorphism
10. Hoonual A, Graidist P, Kritsaneepaiboon S, Limprasert P. Novel concept examples. Am J Med Genet A. 2016;170:1772–9. https://doi.org/10.1002/ajmg.a.37649.
11. Najmabadi H, Motazacker MM, Garshasbi M, Kahrizi K, Tzsach A, Chen W, et al. Homozygosity mapping in consanguineous families reveals extreme heterogeneity of non-syndromic autosomal recessive mental retardation and identifies 8 novel gene loci. Hum Genet. 2007;121:43–8. https://doi.org/10.1007/s00439-006-0292-0.
12. Alvarez-Mora MI, Corominas J, Gilissen C, Sanchez A, Magriñal I, Rodriguez-Reyenga L. Novel compound heterozygous mutation in TRAPPC9 gene: the relevance of whole genome sequencing. Genes (Basel). 2021. https://doi.org/10.3390/GENES12040557.
13. Wilton KM, Gundersen LB, Hasadni L, Wood CP, Schimmenti LA. Profound intellectual disability caused by homozygous TRAPPC9 pathogenic variant in a man from Malta. Mol Genet Genom. 2020. https://doi.org/10.1007/MGG3.1211.
14. Bié Z, Kong X. Diagnosis of a case with mental retardation due to novel compound heterozygous variants of TRAPPC9 gene. Zhonghua Yi Xue Yichu Xue Za Zhi. 2019;36(115–9. https://doi.org/10.3769/CMAJ.JISSN.1003-9406.2019.11.015.
15. Zhou H, Gao M, Skolnick J. ENTPRISE-X: predicting disease-associated frameshift and nonsense mutations. PLoS One. 2018. https://doi.org/10.1371/journal.pone.0171729.
16. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003;31:3812–4. https://doi.org/10.1093/nar/gkg509.
17. Bae-B, Jayaraman D, Walsh CA. Genetic changes shaping the human brain. Dev Cell. 2015;32:423–34. https://doi.org/10.1016/j.devcel.2015.01.035.
18. Tadmouri GO, Nair P, Obied T, Al Ali MT, Al Khaja N, Hamamry HA. Conjugative and reproductive health among Arabs. Reprod Health. 2009. https://doi.org/10.1186/1742-4755-6-17.
19. Zhang Y, Bitner D, Pontes Filho AA, Li F, Liu S, Wang H, et al. Expression and function of NIBP and IKKβ binding protein, in nonsyndromic autosomal-recessive mental retardation. Am J Hum Genet. 2009;85:897–902. https://doi.org/10.1002/ajmg.b.32602.
20. Alphonet: Intellectual disability obesity brain malformations facial dysmorphism syndrome. https://www.orpha.net/orcon/cgi-bin/Disease_Search.php?lng=E&dattit_22075&Disease_Disease_Search&diseaseGroup=intellectual-disability-obesity-brain-malformations-facial-dysmorphism-syndrome&diseaseType=Pat&Disease=0/group of diseases=intellectual-disability-obesity-brain-malformations-facial-dysmorphism-syndrome&title=Intelectual disability-obesity-brain-malformations-facial-dysmorphism syndrome#Search_Simple. Accessed 10 Feb 2022.
21. Kramer J, Beer M, Bodie H, Winter B. Two novel compound heterozygous mutations in the TRAPPC9 gene reveal a connection of non-syndromic intellectual disability and autism spectrum disorder. Front Genet. 2021. https://doi.org/10.3389/FGene.2020.00072.
22. Hinooua A, Gradist P, Kritsaneepaiboon S, Limprasert P. Novel compound heterozygous mutations in the TRAPPC9 gene in two siblings with autism and intellectual disability. Front Genet. 2019. https://doi.org/10.3389/FGene.2019.00611.FULL.
23. Aslanger AD, Goncu B, Duzenli OF, Yuscan E, Sengenc E, Yesil G. Biallelic loss of TRAPPC9 function links vesicle trafficking pathway to autosomal recessive intellectual disability. J Hum Genet. 2022. https://doi.org/10.1038/s41388-021-01007-8.
24. Radenkovic S, Martinelli D, Zhang Y, Preston GI, Maorana A, Terracciano A, et al. TRAPPC9:CDG: A novel congenital disorder of glycosylation with dysmorphic features and intellectual disability. Genet Med. 2022. https://doi.org/10.1016/J.GIM.2021.12.012.