Received: 24 May 2021 | Revised: 5 July 2021 | Accepted: 7 July 2021

DOI: 10.1111/cge.14028

LETTER TO THE EDITOR

CLINICAL GENETICS WILEY

A 24-generation-old founder mutation impairs splicing of *RBBP8* in Pakistani families affected with Jawad syndrome

Dear Editor,

Jawad syndrome (JWDS) (MIM #251255) is a multiple congenital anomaly and intellectual disability (ID) syndrome characterized by congenital microcephaly, facial dysmorphism, digital anomalies, onychodysplasia, and white spots on hands and feet present in all adult patients. So far, only two families have been reported, hereafter referred to as F1 and F2.[1,2] Both families originate from Pakistan and carry mutation (NM_002894.3:c.1808-1809delTA;p.[Ile603Lysfs*7]) in *RBBP8*.[2]

We recruited two new families (F3 and F4) afflicted with JWDS from distant areas of Pakistan. After getting informed consents from parent(s)/guardians, clinical details were documented. Approval of this study was obtained by the Ethical Review Board of the National Institute for Biotechnology and Genetic Engineering (NIBGE), Pakistan. In F3, phenotypic variability with respect to height, digital anomalies, white spots, and nail dysplasia was observed (Figure 1(A)). F4 showed variable expression of onychodysplasia and white spots (Figure 1(A)).

Exome sequencing of a patient from F3 revealed above-mentioned *RBBP8* variant. Using Sanger sequencing, we found it also in F4 (Figure 1(B)).

To analyze mutational effects on splicing, we amplified mutation spanning region, which showed an expected band size of ~390 bps in all three samples. However, the patients showed an additional band of ~260 bps (Figure 1(C)). Sequencing of ~390 bps amplicons showed a frameshift and a premature termination codon (p.[Ile603Lysfs*7]) whereas ~260 bps revealed an in-frame deletion as the consequence of the skipping of exon 12, which entails a loss of 43 amino acids, p.(Ile603_Gln646delinsLys) (Figure 1(D,E)).

To assess the possibility of a founder mutation, all four families were genotyped. Haplotype analyses revealed a block of concordant SNP alleles of 1.06 Mb in the region of *RBBP8* that turned out to be shared by all four Pakistani JWDS families (Figure 1(F)). Based on a calculation described earlier,[3] we estimate the minimum age of the mutation to be 24 generations, which corresponds to about 600 years assuming a generation time of 25 years (Figure 1(F)).

The identified mutation in *RBBP8* is, obviously, loss-of-function; however, depletion of *Rbbp8* in mice cause embryonic lethality.[4] We propose that the transcript with the 2-bp deletion is degraded by nonsense-mediated decay (Figure 1(G)) which may encode RBBP8 with residual function. This seems to explain the alleviated phenotype, since the missing amino acids are far from functionally important domains (Figure 1(E)).

Our findings add important details to the clinical spectrum of this mutation and highlight it as a founder mutation.

ACKNOWLEDGMENTS

Funding was awarded by Koeln Fortune Program, University of Cologne (Muhammad Sajid Hussain) (381/2020) and the Center for Molecular Medicine Cologne (Muhammad Sajid Hussain and Peter Nürnberg) (2635/8029/01, 2635/8326/01).

FUNDING INFORMATION

Center for Molecular Medicine Cologne (CMMC), University of Cologne, 2635/8029/01, , 2635/8326/01; Koeln Fortune Program, Faculty of Medicine, University of Cologne, 381/2020

CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.14028.

DATA AVAILABILITY STATEMENT

Available on request to corresponding author.

Emrah Kaygusuz,[1,2,3] Arwa Ishaq A. Khayyat,[2,4] Uzma Abdullah,[5] Birgit Susanne Budde,[1] Maria Asif,[1,2] Ilyas Ahmed,[6] Ehtisham Ul Haq Makhdoom,[1,2,7,8] Ilknur Sur-Erdem,[9] Jamshaid Mahmood Baig,[10] Muhammad Mohsin Ali Khan,[7] Mohammad Reza Toliat,[1] Christian Becker,[1] Haseeb Anwar,[8] Maria Iqbal,[1,2,7] Sarah Fischer,[2] Muhammad Jameel,[7] Muhammad Sher,[7] Muhammad Tariq,[7] Naveed Altaf Malik,[7] Angelika A. Noegel,[2] Muhammad Jawad Hassan,[11] Holger Thiele,[1] Sigrid Tinschert,[12] Ludwig Eichinger,[2] Stefan Höning,[2] Shahid Mahmood Baig,[7] Peter Nürnberg,[1] and Muhammad Sajid Hussain,[1,2]

[1]*Cologne Center for Genomics (CCG) and Center for Molecular Medicine Cologne (CMMC), University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany*

[2]*Center for Biochemistry, Medical Faculty, University of Cologne, Cologne, Germany*

[3]*Molecular Biology and Genetics, Bilecik Şeyh Edebali University, Bilecik, Turkey*

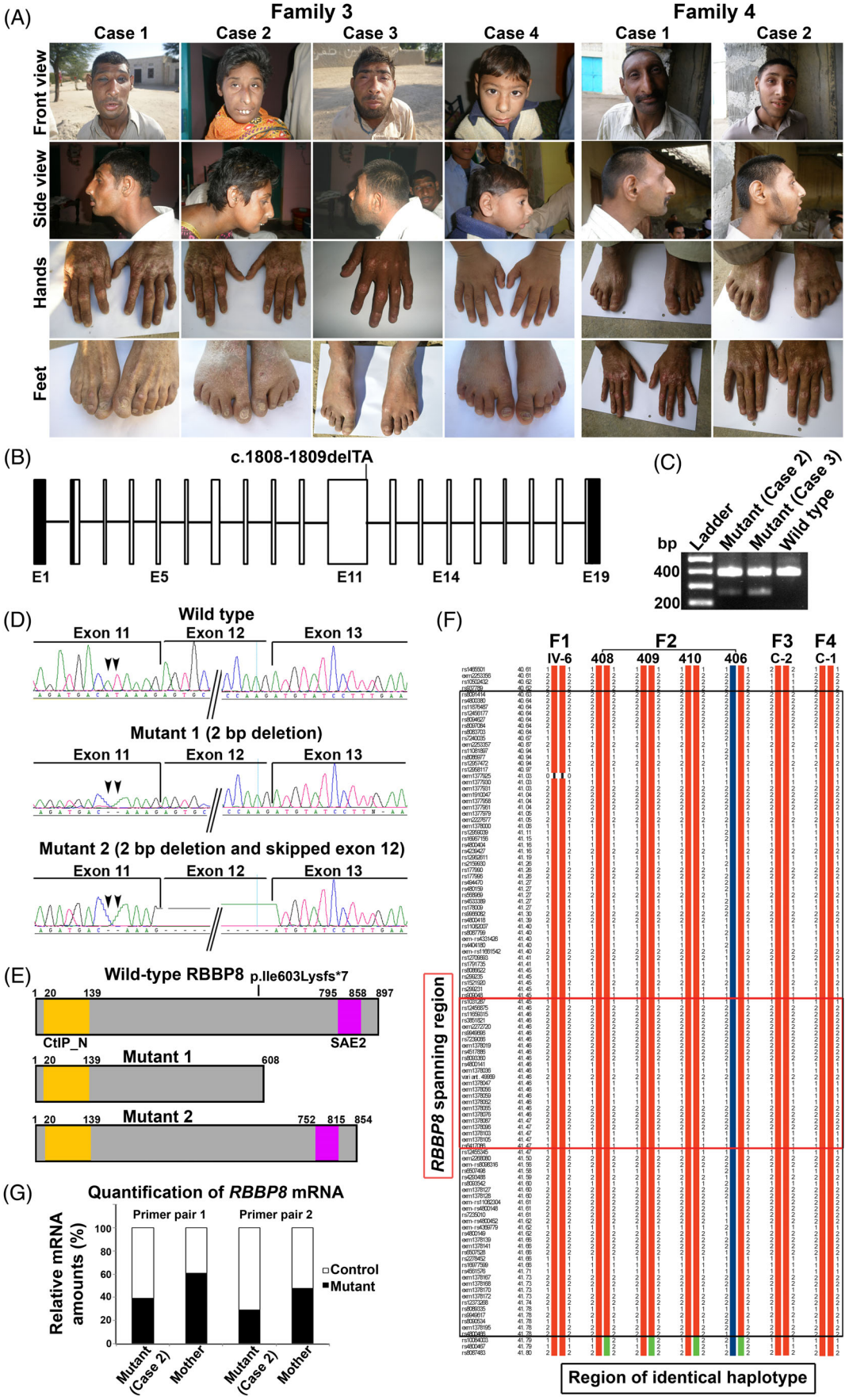

FIGURE 1 (A) Clinical features of F3 and F4. (B) Graphical representation of *RBBP8*. (C) Electropherogram showing transcript amplicons obtained from cDNA of two patients and a control. (D) Sanger traces corresponding to transcript amplicons of 387 bps (Mutant 1) and 260 bps (Mutant 2) from a patient in comparison to control. (E) Graphical illustration and domain structure of the RBBP8 protein. (F) Shared haplotype of four patients from four different families. (G) Quantitative real-time PCR of *RBBP8* mRNA [Colour figure can be viewed at wileyonlinelibrary.com]

[4]Biochemistry Department, King Saud University, Riyadh, Saudi Arabia
[5]University Institute of Biochemistry and Biotechnology, PMAS-Arid Agriculture University, Rawalpindi, Pakistan
[6]Institute for Cardiogenetics, University of Luebeck, Luebeck, Germany
[7]Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE) College, PIEAS, Faisalabad, Pakistan
[8]Department of Physiology, Government College University, Faisalabad, Pakistan
[9]Department of Molecular Biology, Koc University School of Medicine, Istanbul, Turkey
[10]Department of Bioinformatics and Biotechnology, International Islamic University, Islamabad, Pakistan
[11]Department of Biological Sciences, National University of Medical Sciences, Rawalpindi, Pakistan
[12]Zentrum Medizinische Genetik, Medizinische Universität, Innsbruck, Austria

Correspondence
Muhammad Sajid Hussain, Cologne Center for Genomics (CCG) and Center for Molecular Medicine Cologne, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany.
Email: mhussain@uni-koeln.de

Emrah Kaygusuz and Arwa Ishaq A. Khayyat contributed equally to this study.

ORCID

Peter Nürnberg https://orcid.org/0000-0002-7228-428X
Muhammad Sajid Hussain https://orcid.org/0000-0002-1353-8809

REFERENCES

1. Hassan MJ, Chishti MS, Jamal SM, Tariq M, Ahmad W. A syndromic form of autosomal recessive congenital microcephaly (Jawad syndrome) maps to chromosome 18p11.22-q11.2. *Hum Genet*. 2008;123(1):77-82. https://doi.org/10.1007/s00439-007-0452-x
2. Mumtaz S, Yildiz E, Jabeen S, Khan A, Tolun A, Malik S. RBBP8 syndrome with microcephaly, intellectual disability, short stature and brachydactyly. *Am J Med Genet A*. 2015;167(12):3148-3152. https://doi.org/10.1002/ajmg.a.37299
3. Budde BS, Namavar Y, Barth PG, et al. tRNA splicing endonuclease mutations cause pontocerebellar hypoplasia. *Nat Genet*. 2008;40(9):1113-1118. https://doi.org/10.1038/ng.204
4. Chen PL, Liu F, Cai SN, et al. Inactivation of CtIP leads to early embryonic lethality mediated by G(1) restraint and to tumorigenesis haploid insufficiency. *Mol Cell Biol*. 2005;25(9):3535-3542. https://doi.org/10.1128/Mcb.25.9.3535-3542.2005