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BRIEF REPORT

Inactivation of *DRG1*, encoding a translation factor GTPase, causes a recessive neurodevelopmental disorder

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ABSTRACT

Purpose: Developmentally regulated Guanosine-5'-triphosphate-binding protein 1 (DRG1) is a highly conserved member of a class of GTPases implicated in translation. Although the expression of mammalian *DRG1* is elevated in the central nervous system during development, and its function has been implicated in fundamental cellular processes, no pathogenic germline variants have yet been identified. Here, we characterize the clinical and biochemical consequences of *DRG1* variants.
Methods: We collate clinical information of 4 individuals with germline *DRG1* variants and use in silico, in vitro, and cell-based studies to study the pathogenicity of these alleles.
Results: We identified private germline *DRG1* variants, including 3 stop-gained p.Gly54*, p.Arg140*, p.Lys263*, and a p.Asn248Phe missense variant. These alleles are recessively inherited in 4 affected individuals from 3 distinct families and cause a neurodevelopmental disorder with global developmental delay, primary microcephaly, short stature, and craniofacial anomalies. We show that these loss-of-function variants (1) severely disrupt DRG1 messenger RNA/protein stability in patient-derived fibroblasts, (2) impair its GTPase activity, and (3) compromise its binding to partner protein ZC3H15. Consistent with the importance of DRG1 in humans, targeted inactivation of mouse *Drg1* resulted in preweaning lethality.
Conclusion: Our work defines a new Mendelian disorder of DRG1 deficiency. This study highlights DRG1's importance for normal mammalian development and underscores the significance of translation factor GTPases in human physiology and homeostasis.

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Introduction

GTPases are a large enzyme superfamily with critical roles in fundamental cellular processes.[1] Central to their function is the ability to bind and hydrolyze Guanosine-5'-triphosphate (GTP),[1] which confers the ability to act as molecular "switches".

The GTPase family consists of 2 subgroups referred to as "SRP, MinD, and BioD" (SIMIBI) and "Translation Factor" (TRAFAC).[2] The TRAFAC group was named after members that function as translation factors, but also includes RAS and heterotrimeric GTPases.[2] Less well-characterized TRAFAC GTPases include OBG- (spoOB-associated GTP-binding protein) and HflX (high frequency of lysogenization protein X)-like GTPases. OBG/HflX GTPases are an ancient enzyme class with some members present in all domains of life.[2] OBG/HflX GTPases have roles in ribosome regulation/biogenesis, translation, or RNA binding.[3]

The developmentally regulated GTP-binding (DRG) proteins, DRG1 (Q9Y295 / NP_004138.1, an ortholog of the yeast Rbg1) and DRG2 (P55039), are highly conserved OBG/HflX GTPases[2,4] that interact with RNA and ribosomes, consistent with a proposed translational role (Westrip et al[4] and references therein). Indeed, structural analysis places DRG1 within the large ribosomal subunit where it relieves ribosomal pausing.[5] DRGs have also been studied in other contexts.[4] *DRG1* expression is elevated during development of the central nervous system.[4] It is also required for cell proliferation and has been implicated in cancer-associated processes.[4] However, the importance of these functions and their potential roles in physiology and disease remain unclear.

Although mutation of genes encoding small GTPases such as RAS has been widely studied in the context of cancer[6] and, more recently, neurodevelopmental disorders,[7] the role of the wider TRAFAC family is much less well understood. To date, there have been no pathogenic germline variants identified in genes of the OBG/HflX subfamily, including the DRG GTPases. Because gene expression control at the level of translation is now recognized as an increasingly important area of deregulation in inherited disease,[8] further studies of these enigmatic GTPases are warranted.

Here, we identify inherited loss-of-function variants in *DRG1* in 3 pedigrees that present with a novel developmental disorder associated with global developmental delay, failure to thrive, microcephaly, and craniofacial dysmorphism. We show that the disease variants severely damage DRG1 protein level, interactions, and GTPase activity.

Materials and Methods

Isolation of human fibroblasts

Primary human cutaneous fibroblasts from the proband of family 2 and one unaffected parental control were isolated from fresh skin biopsies. Briefly, biopsies were incubated in trypsin overnight at 4 °C to enable the peeling of the epidermis from the dermal compartment. Dermis was chopped up and stuck to a 10-cm plastic dish, allowing the fibroblasts to migrate out of the dermal fragments.

See Supplemental Information for additional Methods.

Results

We report 4 individuals from 3 independently identified families with biallelic deleterious *DRG1* variants resulting in a neurodevelopmental syndrome (Figure 1A and B). With the exception of family 2 (Singaporean ancestry), the other families were of Middle Eastern background and consanguineous (family 1 and family 3). The phenotypes in these 4 individuals consist of a global developmental delay, failure to thrive, microcephaly, intellectual deficit, and craniofacial anomalies. All 4 patients presented with intrauterine growth restriction at birth, and they continued to show significant growth delay. They showed a delay in attaining developmental milestones but, in general, were able to walk and interact with their surroundings. They all had variable speech delay. Detailed clinical descriptions and facial dysmorphism information is presented in Figure 1 and Table 1.

According to gnomAD, no homozygous damaging variants have been reported for *DRG1*. None of the 4 germline variants (p.Gly54*, p.Asn248Phe, p.Lys263*, and p.Arg140*) are present in public databases (gnomAD, BRAVO/TOPmed, ExAC, and 1000G) or in combined in-house databases consisting of >50,000 exomes/genomes. The Regeneron database of >170,000 genomes did not contain the p.Gly54*, p.Asn248Phe, or p.Lys263* variants but did include the p.Arg140* variant with an allele frequency of 0.000012. The truncating variants are predicted to be deleterious, with Combined Annotation Dependent Depletion (CADD) scores >35 (p.Gly54*; CADD = 38, p.Arg140*; CADD = 37, and p.Lys263*; CADD = 41; Figure 1C). The missense p.Asn248Phe variant identified in proband II:1 of family 2 is located in a highly conserved region (Figure 1D) and thus annotated as a possible loss-of-function allele with a CADD score of 26.4.

The *DRG1* gene has a residual variation intolerance score[10] of −0.19 (placing it in the top 40% of human genes most intolerant to genetic variation) and a pLoF observed/expected score of 0.23 (gnomAD). This suggests that *DRG1* is a target of strong negative selection, which may be consistent with an essential function. Consistent with this, *Drg1* is ubiquitously expressed in embryonic day (E) 14.5 (Figure 1F) mouse embryos and is essential for proper murine development: homozygous *Drg1* knockout leads to a significantly lower survival rate at weaning age, with less than 2% $Drg1^{-/-}$ pups obtained from heterozygous crosses (χ^2_2 [N = 105] = 34, $P = 3.8 \times 10^{-8}$; Figure 1G and H).

Overall, these findings suggest that homozygosity for pLOF variants is exceedingly rare in the general population

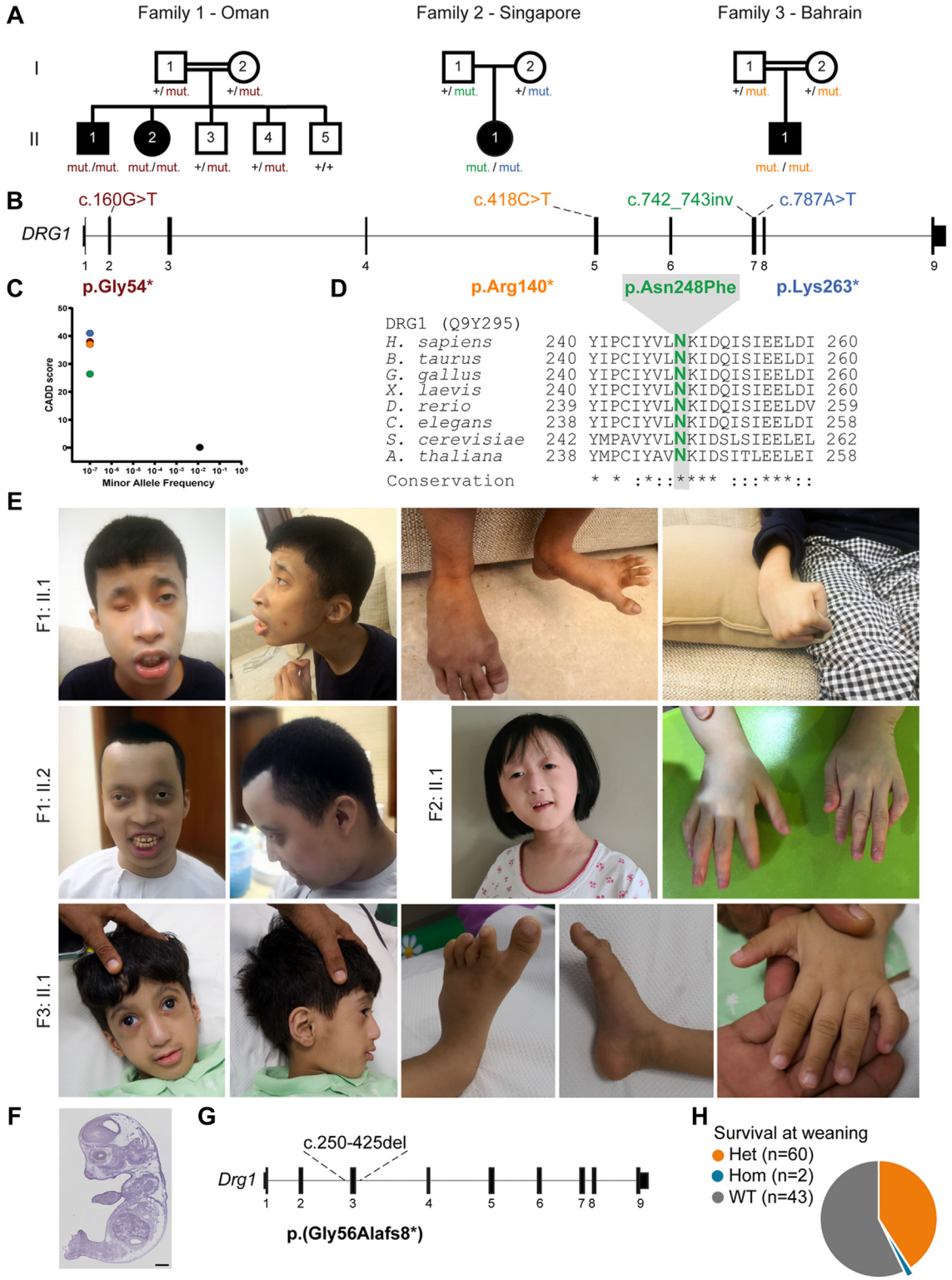

Figure 1 Three families with recessive *DRG1* loss-of-function variants. A. Pedigrees of 3 families in which affected children inherited recessive *DRG1* pLoF variants. B. Structure of the *DRG1* transcript indicating the location of the genomic variants (above) and their corresponding change in amino acid sequence (below). Variants are color-coded according to panel A. C. Minor allele frequency and Combined Annotation Dependent Depletion score of homozygous DRG1 coding variants found in gnomAD v.2.1.1 (black dots) and those found in each family (color-coded dots). DRG1 is intolerant of genetic variation. D. p.Asn248Phe is located in a highly conserved region. Functionally conservative amino acid changes are indicated (* and :). E. Photographs of 4 affected children showing facial dysmorphism and camptodactyly, clubbed feet, and eczema for selected patients. F. Ubiquitous *Drg1* expression in E14.5 mouse embryos by RNA in situ hybridization. Taken from the EMAGE gene expression database[9] (http://www.emouseatlas.org/emage/); EMAGE:31607 June 2022. Scale bar 1

and that the *DRG1* variants observed are probably deleterious, most likely revealing the genetic etiology for this heretofore unknown syndrome.

Next, we experimentally investigated the impact of the *DRG1* variants described above. The p.Asn248Phe variant is located in the highly conserved GTPase domain (Figure 2A and B, Supplemental Figure 1). Analysis of the primary sequence (Figure 1D) and tertiary structure (Figure 2B) indicates that Asn248 is completely conserved and is part of the G4 motif (NKID), which is required for binding to the GTP guanine base.[1] Mutation of the G4 motif is known to inhibit GTP binding and hydrolysis.[12] Furthermore, substitution of asparagine for the larger and more hydrophobic phenylalanine at position 248 could also disrupt structural conformation beyond the GTPase domain (Figure 2B, Supplemental Figure 2).

Three of the alleles are nonsense variants, which likely trigger nonsense-mediated messenger RNA (mRNA) decay. Structural analysis indicates that any residual mRNA would produce a severely truncated protein (p.Gly54*) that lacks essential functional domains,[11] including TGS, S5D2L, and GTPase domains (Figure 2A). The p.Arg140* variant would lack the TGS and S5D2L domains and half of the GTPase domain (Figure 2A, Supplemental Figure 3A). A p.Lys263* DRG1 protein would lack part of the GTPase domain and the entire TGS (Figure 2A, Supplemental Figure 3B). Importantly, DRG1 requires the TGS domain to bind to ZC3H15 (also known as DFRP1, MIM619704) and for recruitment of DRG1/ZC3H15 complexes to polysomes.[11] ZC3H15 binding is also critical for DRG1 stability and GTPase activity.[4,11,13] Overall, the structural analyses strongly suggest that all 4 DRG1 variants are likely to impair GTPase activity and ZC3H15 binding, consistent with a likely loss of function.

To explore the functional consequences of the variants, we first expressed epitope-tagged DRG1 vectors in HeLa cells. Although HA-DRG1^{Gly54*} and HA-DRG1^{Arg140*} proteins were not expressed, the HA-DRG1^{Lys263*} and HA-DRG1^{Asn248Phe} proteins were detected, albeit with significantly reduced abundance (Supplemental Figure 3C). These data indicate that all 4 variants are likely deleterious to normal DRG1 levels. To validate this at the endogenous level, we cultured primary dermal fibroblasts from patient II-6 (p.Lys263*/p.Asn248Phe). Consistent with nonsense-mediated mRNA decay of the endogenous DRG1^{Lys263*} transcript, we observed significantly reduced *DRG1* mRNA (Figure 2C). Sequencing of *DRG1* complementary DNA only detected the p.Asn248Phe variant and no Lys263* (Supplemental Figure 3D), suggesting that the residual transcript is the p.Asn248Phe variant. In line with the mRNA analysis, full-length endogenous DRG1 protein was dramatically reduced in the patient-derived cutaneous fibroblasts (Figure 2D and E), and we were unable to detect a species consistent with p.Lys263* (see Supplemental Figure 3C for antibody validation). Overall, these data indicate that the p.Asn248Phe and p.Lys263* variants seriously impair DRG1 abundance. Consistent with reciprocal regulation of DRG1 and ZC3H15 level,[14] we observed a modest reduction in endogenous ZC3H15 protein in the patient-derived fibroblasts (Figure 2D, Supplemental Figure 3E). These effects on DRG1/ZC3H15 were specific because we did not observe reduced levels of DRG2 or its binding partner RWDD1 (DFRP2) (Figure 2D, Supplemental Figure 3F and G).

We next sought to better understand the impact of the p.Asn248Phe variant on DRG1 protein stability. Because we observed reduced protein expression from a heterologous promoter (Supplemental Figure 3C), we postulated that this variant negatively regulates protein stability. Therefore, we performed cycloheximide-based turnover assays in transfected HeLa cells: the half-life of HA-tagged DRG1 was reduced from about 5 hours in the wild-type to about 1 hour for the HA-DRG1^{Asn248Phe} variant (Supplemental Figure 3H). We observed an even more dramatic effect on the stability of the endogenous protein in patient-derived fibroblasts (Figure 2F, Supplemental Figure 3I). Overall, these results confirm that the p.Asn248Phe variant causes enhanced protein turnover and thus reduced DRG1 protein levels.

Because residual DRG1^{Asn248Phe} protein is expressed in the patient-derived fibroblasts (Figure 2D), we next tested the impact on its enzymatic function. Therefore, we purified HA-DRG1, HA-DRG1^{Asn248Phe}, or HA-DRG1^{Asp117Ala} (a known inactivating mutation) overexpressed in HEK293T cells, before analyzing GTPase activity (Figure 2G). Notably, GTP hydrolysis catalyzed by the DRG^{Asn248Phe} variant was undetectable. For completeness, we also tested the p.Lys263* variant because it retains the bulk of the GTPase domain (Figure 2A). Partially purified DRG1^{Lys263*} also showed a loss of GTPase activity (Supplemental Figure 4). Taken together, these data suggest that these private germline variants are deleterious and therefore pathogenic in the proband from family 2 in which they are inherited in *trans*. Although the p.Asn248Phe allele behaves as a loss-of-function variant in our assays, it could retain some hypomorphic activity toward untested, or hitherto unknown, functions of DRG1.

Interestingly, visual inspection of purified DRG1 variants (Figure 2G, Supplemental Figure 4) suggested reduced ZC3H15 binding. To test this, we immunoprecipitated wild-type or variant HA-DRG1 from transfected HeLa cells

mm. G. Structure of the mouse *Drg1* transcript indicating the site of deletion in *Drg1* KO mice. This deletion leads to a frameshift and premature stop codon within exon 4. H. Survival of *Drg1* KO mice upon weaning. This is significantly different from the expected litter distribution of 25% WT, 50% Het, 25% KO (χ^2_2 test [$N = 105$] = 34, $P = 3.8 \times 10^{-8}$). DRG1, developmentally regulated GTP-binding protein 1; Het, heterozygous; Hom, homozygous; KO, knockout; WT, wild-type.

Table 1 Clinical characteristics of 4 patients with Tan-Almurshedi syndrome caused by biallelic *DRG1* loss-of-function variants

Clinical Synopsis and Genetics	HPO Terms	Family 1		Family 2	Family 3	Total
Country of origin		Oman		Singapore	Bahrain	
Propositus number (refer to pedigrees)		II:1	II:2	II:6	II:7	
Gender		Male	Female	Female	Male	2F:2M
Intrauterine growth restriction (birth weight in kg)	HP:0001511	+ (n.d.)	+ (2.3)	+ (2.12)	+ (1.67)	✓
Failure to thrive	HP:0001508	+	+	+	+	✓
Gene (MIM 603952)		DRG1		DRG1	DRG1	✓
Autosomal-recessive inheritance	HP:0000007	+		+ (compound heterozygous)	+	✓
Genomic change (GRCh38/hg38) (ENSG00000185721.13)		chr22:g.31400737G>T		Maternal: chr22:g.31426688A>T Paternal: chr22:g.31426643_31426644inv	chr22:g.31420261C>T	
Complementary DNA change (NM_004147.3)		c.160G>T		Maternal: c.787A>T Paternal: c.742_743inv	c.418C>T	
Expected protein change (Q9Y295 / NP_004138.1)		p.(Gly54*)		Maternal: p.(Lys263*) Paternal: p.(Asn248Phe)	p.(Arg140*)	
Observed protein change (Q9Y295 / NP_004138.1)		n.d.		Maternal: p.0 Paternal: p.Asn248Phe	n.d.	LoF mutations
Variant classification (ACMG guidelines)		Pathogenic (PVS1)		Maternal: pathogenic (PVS1) Paternal: likely pathogenic (PS3)	Pathogenic (PVS1)	LoF mutations
Craniofacial dysmorphisms						
Facial dysmorphism	HP:0001999	+	+	+	+	✓
Microcephaly	HP:0000252	+	+ (−2.4 SD)	+ (−6.7 SD)	+ (−3.1 SD)	✓
Short palpebral fissure	HP:0012745	+	+	+	+	✓
Brachycephaly	HP:0000248	+	+	+	+	✓
Prominent forehead	HP:0011220	+	+	+	+	✓
Hypoplastic supraorbital ridges	HP:0009891	+	+	+	+	✓
Short eyelashes	HP:0010764	+	+	+	−	3/4
Broad nasal bridge	HP:0000431	+	+	+	+	✓
Low-set ears	HP:0000369	+	+	+	+	✓
Posteriorly rotated ears	HP:0000358	+	+	−	+	3/4
Everted prominent lower lip	HP:0000232	+	+	−	+	3/4
Widely spaced teeth	HP:0000687	+	+	+	+	✓
Bone/skeletal abnormalities						
Proportionate short stature	HP:0003508	+	+	+	+	✓
Camptodactyly	HP:0012385	+	+	+	+	✓
Clubbing of feet	HP:0001762	+	+	−	+	3/4
Immune defects						
Infantile eczema	HP:0000964	+	+	+	+	✓
Repeated infections	HP:0002719	n.d.	n.d.	+	−	1/2
Hypergammaglobulinemia	HP:0010702	n.d.	n.d.	+	−	1/2
Autoimmune hemolytic anemia	HP:0001890	n.d.	n.d.	+	−	1/2
Behavioral/neurological traits						
Neurodevelopmental delay	HP:0012758	+	+	+	+	✓
Spasticity	HP:0001257	+	+	−	−	2/4
Seizure	HP:0001250	+	−	−	−	2/4
Skin findings and its appendages						
Dry skin	HP:0000958	+	+	+	−	3/4
Other clinical manifestations						
Myelomeningocele	HP:0002475	−	−	−	+	1/4

The plus and minus symbols refer to affirmative and negative, respectively.

ACMG, American College of Medical Genetics and Genomics; *HPO*, Human Phenotype Ontology; *n.d.*, not determined.

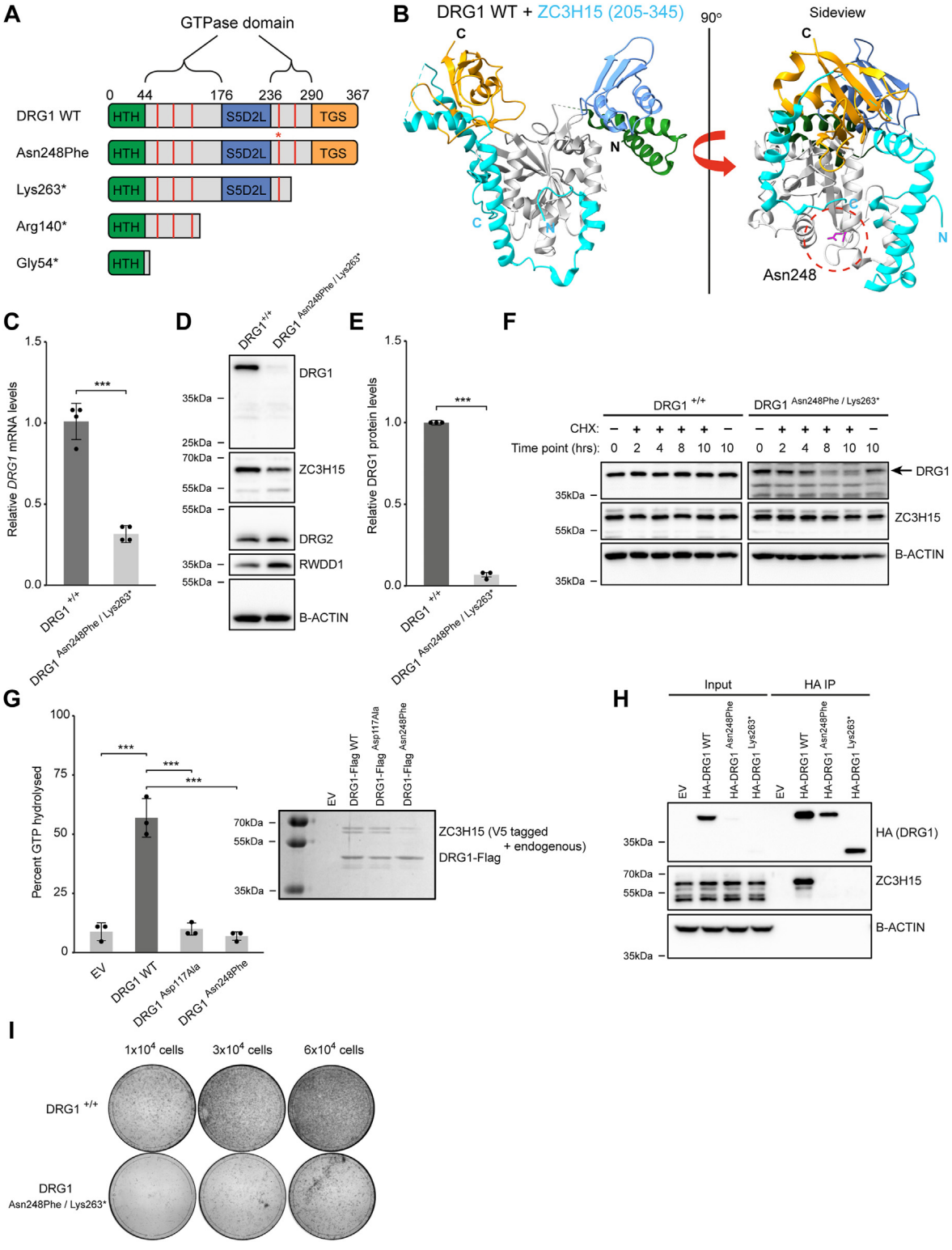

Figure 2 DRG1 variants reduce abundance, GTPase activity, and ZC3H15 binding. A. Domain organization for WT (top) and mutant DRG1 proteins. Red bars indicate the 5 G motifs conserved in DRG GTPases. The location of the Asn248Phe variants is indicated with an asterisk. B. Structure of Rbg1 (yeast DRG1) showing the location of Asn248 and its proximity to the ZC3H15 binding interface. GTPase domain: gray, HTH: green, S5D2L: blue, TGS: orange. The C-terminal fragment of yeast ZC3H15 (Tma46) is also shown in cyan. Structure file PDB: 4A9A.[11] C. Quantification of *DRG1* mRNA levels in WT and *DRG1* (Asn248Phe / Lys263*) mutated fibroblasts using RT-qPCR. Results are normalized to a GAPDH control. The data represent the mean with error bars showing the standard deviation of 4 biological repeats (data points shown). Statistical significance was estimated using a two-sample *t* test. D. Western blots using protein extracts from WT and DRG1 Asn248Phe / Lys263* fibroblasts. E. Quantification of DRG1 protein levels relative to B-actin. Data represent mean with standard

before western blotting for endogenous ZC3H15 (Figure 2H). Importantly, both p.Asn248Phe and p.Lys263* variants were unable to bind ZC3H15. Considering the importance of ZC3H15 binding for DRG1 activity and stability,[11,13,14] the effect of these variants on the complex may partly explain the loss of function observed. Overall, our combined functional analyses demonstrate that these novel patient variants severely affect the level, GTPase activity, and ZC3H15 binding of DRG1. Consistent with this, and the essentiality of DRG1, we find that patient-derived cells show a survival deficit in colony formation assays (Figure 2I).

Discussion

Here, we describe 3 families with recessive loss-of-function variants in the DRG1 translation factor. Detailed biochemical and functional analyses confirmed the pathogenicity of the variants in this novel Mendelian disease, which we propose to refer to as Tan-Almurshedi syndrome. Consistent with these variants driving the pathogenicity of the associated syndrome, we also document that *Drg1* is an essential gene in mice, in which targeted inactivation causes preweaning lethality.

Considering the obligate nature of ZC3H15 for DRG1 function, and the loss of ZC3H15 binding to the DRG1 variants described here, one might predict the existence of a related neurodevelopmental disorder driven by pathogenic variants in this gene. Indeed, ZC3H15 has a similar pattern of tissue distribution to DRG1, including in the developing central nervous system,[13] and the *ZC3H15* gene is located within a chromosomal region altered in 2q32 deletion syndrome (MIM612345).[15] Furthermore, the gene encoding the JMJD7 Jumonji-C oxygenase, which targets DRGs for lysyl hydroxylation,[16] was identified as a candidate gene for autism and intellectual disability.[17,18] Further work is required to fully understand the role of the JMJD7-DRG1/ZC3H15 pathway in human disease.[4]

Although the functions of the DRG1/ZC3H15 GTPase complex are still under debate, there is growing evidence supporting a fundamental role in translation (reviewed in Westrip et al[4]), specifically the elongation step. Cryo-electron microscopy analyses of the yeast orthologs (Rbg1/Tma46) demonstrate associations with the ribosomal A-site transfer RNA, GTPase association center, and 40S subunit (Supplemental Figure 5).[5] Precedence for the importance of translational elongation in neurodevelopment is underlined by other disorders driven by pathogenic variants in elongation factor pathways. Pathogenic variants in the eEF1 complex have been implicated in developmental disorders associated with failure to thrive, developmental delay, intellectual disability, microcephaly, and facial dysmorphism (reviewed in[19]). Furthermore, pathogenic variants in the elongation factor EIF5A (MIM619376; Faundes-Banka syndrome)[20] or an enzyme (deoxyhypusine synthase, MIM600944)[21] involved in its unique and essential modification, hypusination, have also recently been identified in neurodevelopmental disorders with clinical presentations that overlap with those described here. Aside from EEF1A2 (MIM602959),[20] DRG1 represents the only other gene encoding a GTPase component of a translation elongation factor complex to have been identified thus far as the basis of a neurodevelopmental disorder. To our knowledge, our work also represents the first case of a disorder associated with the OBG/HflX GTPase family.

Data Availability

The data that support the findings of this study are available from the corresponding authors upon request.

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deviation. Statistical significance was estimated using a two-sample *t* test. F. Western blots of CHX stability assay with the DRG1 WT and DRG1 Asn248Phe / Lys263* fibroblasts. Cells were treated with 50 μg/mL CHX and then harvested at the indicated time points. A 10-hour dimethyl sulfoxide control was also included. G. GTPase assay using C-terminally flag-tagged DRG1 WT, Asp117Ala (positive control predicted to have no GTPase activity), and Asn248Phe that were co-transfected with C-terminally V5-tagged ZC3H15 in HEK293T cells and purified using antiflag pulldown. Coomassie stained gel of purified DRG1/ZC3H15 complexes shown in inset. The data represent the mean with error bars showing the standard deviation of $n = 3$ biological repeats (data points shown). Statistical significance was confirmed using a one-way analysis of variance with Tukey HSD to estimate *P* values. H. N-terminal HA-tagged DRG1 WT, Asn248Phe, and Lys263* variants were transiently expressed in HeLa cells. Cell lysates were used in an anti-HA pulldown experiment followed by western blotting for the indicated proteins using input and pulldown samples. I. Colony-forming assay using DRG1 WT and Asn248Phe / Lys263* patient fibroblasts. Cells were seeded on 10-cm plates and then stained with crystal violet after 10 days. We note that these primary cutaneous fibroblasts do not form compact colonies with clearly defined borders. EV, Empty Vector; HTH, Helix Turn Helix; S5D2L, Ribosomal protein S5 domain 2-like domain; TGS, ThrRS, GTPase, and SpoT domain; WT, wild-type.

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Ethics Declaration

This study was approved by the Medical Research Ethical Committee of Sultan Qaboos University (family 1), KK Women's and Children's Hospital (family 2), and Centogene (Germany) (family 3). Parents provided written informed consent to participate and to publish their family pedigrees and clinical data. A valid Health Insurance Portability and Accountability Act authorization for participation/publication from every individual whose protected health information and photograph is included has been received and archived. Clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. The study protocol was approved by A*STAR institutional review board (IRB 2019-087). Genetic analyses were performed in accordance with bioethics rules of national laws. Further approvals were obtained from the West Midlands, Coventry, and Warwickshire Research Ethics Committee (REC: Stewart/20/WM/0098)

Conflict of Interest

Maryam Najafi and Salem Alawbathani are employees of Centogene. All other authors declare no conflicts of interest.

Web Resources

1000 Genomes Project Database, http://browser.1000genomes.org/index.html

CRISPRScan, https://www.crisprscan.org

Exome Aggregation Consortium (ExAC), http://exac.broadinstitute.org

Exome Variant Server from NHLBI Exome Sequencing Project (ESP), https://evs.gs.washington.edu/EVS/

https://cadd.gs.washington.edu/score

Genome Aggregation Database (GnomAD), http://gnomad.broadinstitute.org/

Greater Middle East (GME) Variome web, http://igm.ucsd.edu/gme/index.php

NCBI dbSNP, https://www.ncbi.nlm.nih.gov/SNP/

Online Mendelian Inheritance in Man (OMIM), https://www.omim.org

Additional Information

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References

1. Wittinghofer A, Vetter IR. Structure-function relationships of the G domain, a canonical switch motif. *Annu Rev Biochem*. 2011;80:943-971. http://doi.org/10.1146/annurev-biochem-062708-134043
2. Leipe DD, Wolf YI, Koonin EV, Aravind L. Classification and evolution of P-loop GTPases and related ATPases1. *J Mol Biol*. 2002;317(1):41-72. http://doi.org/10.1006/jmbi.2001.5378
3. Verstraeten N, Fauvart M, Versées W, Michiels J. The universally conserved prokaryotic GTPases. *Microbiol Mol Biol Rev*. 2011;75(3):507-542. http://doi.org/10.1128/MMBR.00009-11
4. Westrip CAE, Zhuang Q, Hall C, Eaton CD, Coleman ML. Developmentally regulated GTPases: structure, function and roles in disease. *Cell Mol Life Sci*. 2021;78(23):7219-7235. http://doi.org/10.1007/s00018-021-03961-0
5. Zeng F, Li X, Pires-Alves M, Chen X, Hawk CW, Jin H. Conserved heterodimeric GTPase Rbg1/Tma46 promotes efficient translation in eukaryotic cells. *Cell Rep*. 2021;37(4):109877. http://doi.org/10.1016/j.celrep.2021.109877
6. Sahai E, Marshall CJ. RHO–GTPases and cancer. *Nat Rev Cancer*. 2002;2(2):133-142. http://doi.org/10.1038/nrc725

7. Shieh JTC. Emerging RAS superfamily conditions involving GTPase function. *PLoS Genet*. 2019;15(2):e1007870. http://doi.org/10.1371/journal.pgen.1007870
8. Scheper GC, van der Knaap MS, Proud CG. Translation matters: protein synthesis defects in inherited disease. *Nat Rev Genet*. 2007;8(9):711-723. http://doi.org/10.1038/nrg2142
9. Richardson L, Venkataraman S, Stevenson P, et al. EMAGE mouse embryo spatial gene expression database: 2014 update. *Nucleic Acids Res*. 2014;42(database issue):D835-D844. http://doi.org/10.1093/nar/gkt1155
10. Petrovski S, Wang Q, Heinzen EL, Allen AS, Goldstein DB. Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet*. 2013;9(8):e1003709. http://doi.org/10.1371/journal.pgen.1003709
11. Francis SM, Gas ME, Daugeron MC, Bravo J, Séraphin B. Rbg1-Tma46 dimer structure reveals new functional domains and their role in polysome recruitment. *Nucleic Acids Res*. 2012;40(21):11100-11114. http://doi.org/10.1093/nar/gks867
12. Walter M, Clark SG, Levinson AD. The oncogenic activation of human p21ras by a novel mechanism. *Science*. 1986;233(4764):649-652. http://doi.org/10.1126/science.3487832
13. Ishikawa K, Azuma S, Ikawa S, Semba K, Inoue JI. Identification of DRG family regulatory proteins (DFRPs): specific regulation of DRG1 and DRG2. *Genes Cells*. 2005;10(2):139-150. http://doi.org/10.1111/j.1365-2443.2005.00825.x
14. Ishikawa K, Ito K, Inoue JI, Semba K. Cell growth control by stable Rbg2/Gir2 complex formation under amino acid starvation. *Genes Cells*. 2013;18(10):859-872. http://doi.org/10.1111/gtc.12082
15. Van Buggenhout G, Van Ravenswaaij-Arts C, Mc Maas N, et al. The del(2)(q32.2q33) deletion syndrome defined by clinical and molecular characterization of four patients. *Eur J Med Genet*. 2005;48(3):276-289. http://doi.org/10.1016/j.ejmg.2005.05.005
16. Markolovic S, Zhuang Q, Wilkins SE, et al. The Jumonji-C oxygenase JMJD7 catalyzes (3S)-lysyl hydroxylation of TRAFAC GTPases. *Nat Chem Biol*. 2018;14(7):688-695. http://doi.org/10.1038/s41589-018-0071-y
17. Matsunami N, Hensel CH, Baird L, et al. Identification of rare DNA sequence variants in high-risk autism families and their prevalence in a large case/control population. *Mol Autism*. 2014;5(1):5. http://doi.org/10.1186/2040-2392-5-5
18. de Ligt J, Willemsen MH, van Bon BWM, et al. Diagnostic exome sequencing in persons with severe intellectual disability. *N Engl J Med*. 2012;367(20):1921-1929. http://doi.org/10.1056/NEJMoa1206524
19. McLachlan F, Sires AM, Abbott CM. The role of translation elongation factor eEF1 subunits in neurodevelopmental disorders. *Hum Mutat*. 2019;40(2):131-141. http://doi.org/10.1002/humu.23677
20. Faundes V, Jennings MD, Crilly S, et al. Impaired eIF5A function causes a Mendelian disorder that is partially rescued in model systems by spermidine. *Nat Commun*. 2021;12(1):833. http://doi.org/10.1038/s41467-021-21053-2
21. Ganapathi M, Padgett LR, Yamada K, et al. Recessive rare variants in deoxyhypusine synthase, an enzyme involved in the synthesis of hypusine, are associated with a neurodevelopmental disorder. *Am J Hum Genet*. 2019;104(2):287-298. http://doi.org/10.1016/j.ajhg.2018.12.017