HHS Public Access

Author manuscript

Prenat Diagn. Author manuscript; available in PMC 2024 April 24.

Published in final edited form as:

Prenat Diagn. 2024 March ; 44(3): 343–351. doi:10.1002/pd.6527.

The expanded spectrum of human disease associated with *GREB1L* likely includes complex congenital heart disease

Emily Zhao[1], **Miles Bomback**[2], **Atlas Khan**[3], **Sarath Krishna Murthy**[3], **David Solowiejczyk**[4], **Neeta L. Vora**[5], **Kelly L. Gilmore**[5], **Jessica L. Giordano**[6], **Ronald J. Wapner**[6], **Simone Sanna-Cherchi**[3], **Alex Lyford**[7], **Angie C. Jelin**[1], **Ali G. Gharavi**[3], **Thomas Hays**[4]

[1]Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[2]Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

[3]Department of Medicine, Columbia University Irving Medical Center, New York, New York, USA

[4]Department of Pediatrics, Columbia University Irving Medical Center, New York, New York, USA

[5]Department of Obstetrics and Gynecology, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, North Carolina, USA

[6]Department of Obstetrics and Gynecology, Columbia University Irving Medical Center, New York, New York, USA

[7]Department of Mathematics and Statistics, Middlebury College, Middlebury, Vermont, USA

Abstract

Objective: *GREB1L* has been linked prenatally to Potter's sequence, as well as less severe anomalies of the kidney, uterus, inner ear, and heart. The full phenotypic spectrum is unknown. The purpose of this study was to characterize known and novel pre- and postnatal phenotypes associated with *GREB1L*.

Methods: We solicited cases from the Fetal Sequencing Consortium, screened a population-based genomic database, and conducted a comprehensive literature search to identify disease cases associated with *GREB1L*. We present a detailed phenotypic spectrum and molecular changes.

Results: One hundred twenty-seven individuals with 51 unique pathogenic or likely pathogenic *GREB1L* variants were identified. 24 (47%) variants were associated with isolated kidney anomalies, 19 (37%) with anomalies of multiple systems, including one case of hypoplastic left heart syndrome, five (10%) with isolated sensorineural hearing loss, two (4%) with isolated uterine agenesis; and one (2%) with isolated tetralogy of Fallot.

Correspondence Thomas Hays, 622 West 168th St, Suite PH 17W-303, New York, NY 10032, USA. th2712@cumc.columbia.ed.
Emily Zhao and Miles Bomback have contributed equally.

CONFLICT OF INTEREST STATEMENT
The authors declare no competing conflicts of interest.

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

Conclusion: *GREB1L* may cause complex congenital heart disease (CHD) in humans. Clinicians should consider *GREB1L* testing in the setting of CHD, and cardiac screening in the setting of *GREB1L* variants.

1 | INTRODUCTION

GREB1L (growth regulation by estrogen in breast cancer 1-like; MIM: 617782) was discovered in two studies as a gene responsible for severe congenital anomalies of the kidneys and urinary tract (CAKUT), including renal agenesis and hypodysplasia (MIM: 617805).[1,2] Prenatal cases in these studies were often characterized by Potter's sequence; postnatal presentations included unilateral renal anomalies, as well as uterine agenesis and dysgenesis, aortic stenosis, and sensorineural hearing loss (MIM: 277000, 619274). Determining the full spectrum of phenotypes associated with pathologic variants of *GREB1L* involves understanding multiple complex genetic factors such as incomplete penetrance and variable expressivity. Human investigations have been limited by fetal demise, and neonatal or childhood death. The full extent of manifestations of *GREB1L*-associated disorders and the molecular mechanism(s) of pathogenesis are unknown.

GREB1L was initially reported in two independent cohorts of individuals with severe forms of CAKUT. De Tomasi, et al. conducted classical linkage analysis in multiple familial CAKUT cases (most families included cases of bilateral renal agenesis), followed by exome sequencing and a collapsing analysis of sporadic cases of CAKUT.[1] Sanna–Cherchi, et al. assembled a cohort of 612 individuals with renal agenesis, severe hypodysplasia, and multicystic dysplastic disease.[2] Both investigations found enrichment of rare, deleterious *GREB1L* variants, and used animal models to functionally validate the gene's role in CAKUT.

Severe forms of CAKUT, such as renal agenesis and hypodysplasia, often cause fetal demise or neonatal death. Consistently, far fewer loss of function (LoF) *GREB1L* variants are found in the gnomAD population database than expected by chance (LOEUF score = 0.07, indicating a gene highly intolerant to inactivation in the extant human population).[3] This intolerance, and perinatal lethality have made it challenging to determine the full phenotypic spectrum related to *GREB1L*, as affected individuals are rare, and often die before extra renal manifestations can be investigated or sequencing can be performed. Paradoxically, cohorts affected by familial CAKUT have included healthy relatives bearing *GREB1L* disease-causing variants without apparent disease.[1,4]

GREB1L has been associated with congenital anomalies of other organ systems. One of the *GREB1L* discovery cohorts included an individual with sensorineural hearing loss.[2] Schrauwen, et al. subsequently demonstrated that *GREB1L* variants were responsible for isolated inner ear malformations and sensorineural hearing loss.[5] This finding was validated in multiple following studies also demonstrating isolated ear anomalies and deafness caused by *GREB1L* variants.[6–8] These cranial abnormalities have been further validated by the *Greb1L* mutant mouse that developed craniofacial abnormalities such as exencephaly and cleft lip.[4]

The *GREB1L* discovery cohorts also included individuals with uterine malformations.[1,2] Subsequent investigation focused on individuals with Mayer-Rokitanksy-Küster-Hauser syndrome (MIM 277000: characterized by uterine-vaginal atresia, variably accompanied by cardiac, skeletal, auditory, and renal anomalies) demonstrated that variants in *GREB1L* also caused this disease presentation.[9–11] These cohorts included individuals with isolated uterine anomalies in the absence of other urogenital findings. Rare, putatively deleterious *GREB1L* variants are more common in cohorts of individuals with scoliosis.[12]

Finally, simple cardiac anomalies in humans have been attributed to *GREB1L* variants. De Tomasi and colleagues identified *GREB1L* variants in cases that included as part of their phenotype the following: left ventricular hypertrophy, aortic stenosis, and retroesophageal subclavian artery.[1] Additionally *Greb1l* homozygous knockout mouse embryos develop crisscross heart, characterized by torsion of the atrioventricular plane, perpendicular crossing inflows to the right and left ventricles, and the displacement of the right ventricle (RV) superiorly and leftward of the LV.[1,13] Bernheim and colleagues demonstrated important mechanistic insights preceding this anomaly in mice. Loss of *GREB1L* resulted in impaired ribosome biogenesis, growth arrest of cardiac precursor cells, and malpositioning of the cardiac outflow tract.[13] However, to our knowledge, complex cardiac anomalies, as observed in this animal model, have not been described in humans with *GREB1L* variants.

The phenotypic spectrum of *GREB1L*-associated disease has been defined by studies focusing on these phenotypic descriptions (renal and uterine anomalies and sensorineural hearing loss). Intriguingly, *Greb1L* knockout mice demonstrated a broader phenotype including major anomalies of multiple other organ systems.[1] Furthermore, *GREB1L* is expressed broadly in human fetal and adult organs beyond the kidney, gonads, and ear.[1] Given this background, and evidence suggesting that anomalies secondary to *GREB1L* variants lead to stillbirth or early death, we sought to characterize phenotypes associated with perinatal disease and congenital anomalies.

2 | METHODS

2.1 | Fetal sequencing consortium

We solicited investigators from the Fetal Sequencing Consortium (FSC) for cases of perinatal disease attributable to variants in *GREB1L*. The FSC is a growing community of clinical and research experts from more than 30 institutions worldwide working to study the genetic contribution to perinatal disorders and improve clinical care.[14] Members meet regularly to collaborate on ongoing cases and to build a novel repository of fetal genomic data. We solicited the FSC for cases in which clinical exome or genome sequencing of fetuses or infants identified pathogenic or likely pathogenic variants in *GREB1L*. From this, we identified two sporadic cases and one affected family. These included two variants which have not been previously described to our knowledge. This research was approved by the institutional review boards of Johns Hopkins University, the University of North Carolina, and Columbia University.

2.2 | Retrospective cases from the institute of genomic medicine

The Columbia Institute for Genomic Medicine (IGM) maintains exome and genome sequence data from 123,992 individuals, which are analyzed using the in-house Analysis Tool for Annotated Variants (ATAV).[15] Individuals were recruited for multiple indications, including the study of congenital anomalies and neurodevelopmental disorders (e.g., epilepsy, autism, CAKUT, cardiovascular disease). Healthy relatives of the research participants were recruited as well. Using ATAV, we screened for *GREB1L* variants meeting the following criteria: gnomAD allele frequency <0.01, minimum read-depth of 10×, minimum Phred genotype score of 20, and minimum variant Phred quality of 30. This returned 294 unique variants in 280 individuals. We then excluded previously published cases. Using American College of Medical Genetics and Genomics (ACMG) criteria, we annotated two variants as pathogenic or likely pathogenic, which have not been previously described to our knowledge. We also used the IGM database to assess the coverage of *GREB1L* by commonly used exome capture kits. We surveyed all available, exome-sequenced cases in the IGM and determined the portion of coding variants with 10× coverage.

2.3 | Literature review

We first conducted a comprehensive literature review of published human cases with disease caused by rare variants in *GREB1L*. This review was conducted by searching for the term "*GREB1L*" in two databases of peer-reviewed manuscripts (PubMed [https://pubmed.ncbi.nlm.nih.gov] and Mastermind[16] [https://genomenon.com/mastermind]) for the term "*GREB1L.*" PubMed returned 41 unique articles and Mastermind returned an additional 372 unique articles. These articles were each manually reviewed to identify unique cases in which fetal or postnatal disease was found to be caused by rare variants in *GREB1L*. Variants were then manually re-annotated using diagnostic criteria of the ACMG.[17] Only individuals with pathogenic or likely pathogenic variants were included, which were present in 23 articles.[1,2,4–6,8–12,18–30] We also characterized the ascertainment criteria with which these studies identified individuals.

2.4 | Combined cohort

Cases from the FSC, IGM, and literature review were combined, and each individual was categorized based on phenotype including isolated anomalies, anomalies of multiple organ systems, and no apparent disease. We then classified the unique variants by phenotype. For variants that were found in more than one case, the individual phenotypes were combined to determine the phenotype associated with each variant. Variants were categorized as protein disrupting (loss of canonical splice site, stop-gain, or frameshift) or as missense. Mean (SD) of rare exome variant ensemble learner (REVEL) scores were determined for missense variants. REVEL is an in silico prediction of the deleteriousness of missense variants, particularly LoF, variants.[31,32]

3 | RESULTS

We identified two sporadic cases, and one affected family from the FSC. The familial case consisted of a maternally inherited variant (c.4881_4882delCA [p.His1627frameshift]),

which resulted in one pregnancy with bilateral renal agenesis and ventricular hypertrophy and systolic dysfunction, another pregnancy with bilateral renal agenesis and aortic stenosis, and a living son with unilateral renal agenesis. Details regarding whether further genitourinary anomalies were present are not available. The mother had a normal renal ultrasound, and no known anomalies. This was previously described in a report not identified in our literature search.[33] Another case consisted of sporadic bilateral renal agenesis presenting in a male fetus found to have a nonsense *GREB1L* variant (c.5016G>A [p. Trp1672Ter]). In this case the inheritance is unknown, and no other anomalies were identified. Additionally, we identified a female infant with prenatally diagnosed complex CHD (hypoplastic left heart syndrome and anomalous pulmonary venous connection; Figure 1), an ectopic, dysplastic kidney, hemivertebrae, and scoliosis found to have a de novo variant (c.4440C>A; p.Phe1480>Leu). A review of the IGM identified two cases, each from the investigation of individuals with congenital kidney anomalies. These included one individual with a unilateral renal agenesis and renal ectopy and a variant with unknown inheritance (c.4777del [p.Leu1593frameshift]), and another individual with unilateral renal agenesis, renal ectopy, renal dysplasia, and vesicoureteral reflux bearing a variant with unknown inheritance (c.4276_4277del [p.Val1426frameshift]). A literature review identified 46 unique variants, including six that were independently identified in multiple studies.

Combining cases from the FSC, IGM, and literature review provided a cohort of 127 individual cases with multiple phenotypes (Table 1). In order of frequency, these consisted of 66 (52.0%) individuals with isolated renal anomalies, 25 (19.7%) individuals with anomalies of multiple organ systems, 21 (16.5%) healthy relatives of probands with no apparent disease, 10 (7.9%) individuals with isolated sensorineural hearing loss, four (3.1%) individuals with isolated uterine anomalies, and one (0.8%) individual with isolated CHD.

In the combined cohort of 127 individuals, 51 unique pathogenic and likely pathogenic variants were identified (Table 2). Forty variants of uncertain significance were excluded. The cohort included multiple families, as well as sporadic cases in which identical variants were identified. Twenty-six variants were found in multiple individuals. And 23 variants were found in individuals with different phenotypes, including 16 variants found in unaffected relatives with no known disease phenotype. The genetic and phenotypic details of each individual are listed in Table S1. Of the 51 unique variants, 24 (47.1%) were associated with cases of isolated kidney anomalies, 19 (37.3%) were associated with anomalies in multiple organ systems, five (9.8%) were associated with isolated sensorineural hearing loss, two (3.9%) were associated with isolated uterine agenesis, and one (2.0%) was associated with isolated CHD (Figure 2). Two cases of CHD were classified as moderate or of great complexity using the Bethesda classification system.[34] These consisted of one case of hypoplastic left heart syndrome accompanied by renal ectopy and one case of isolated tetralogy of Fallot (Table S1).

Our literature review identified 23 peer-reviewed articles describing individuals with *GREB1L*-associated disease, including one study cited by another retrieved from Mastermind (Table 3).[21,35] These included investigations into the genetic basis of congenital anomalies of the kidney, ear, and uterus, as well as in the setting of perinatal disorders. Eight studies of severe CAKUT (renal agenesis and renal hypodysplasia) identified 28

variants in *GREB1L*; three additional kidney disease studies (including less severe forms of CAKUT and related nephropathy) identified five variants. Five studies of perinatal disorders, including congenital anomalies (organs other than the uterus, kidneys, or ears), stillbirth, and critical illness identified five variants. Four studies of uterine anomalies identified nine variants. Three studies of sensorineural hearing loss identified six variants. These studies predominantly employed exome sequencing of proband-parent trios or extended families (Table S2).

Overall, 24 (47.1%) of variants were missense, with a mean (SD) REVEL score of 0.36 (0.21). Protein truncating (frameshift, nonsense, and splice site disruption) comprised the remaining 27 (52.9%) variants. The distribution of protein truncating and missense variants did not differ (p-value = 0.84 by chi-square test) across phenotypic categories (isolated renal, multiple organ systems, isolated sensorineural hearing loss; isolated uterine and cardiac cases were excluded given sample sizes of 2 and 1 respectively). Of the 51 unique variants, 27 were found in multiple individuals, and 17 were found in probands, as well as apparently unaffected relatives.

Finally, we determined the exome sequencing coverage of *GREB1L* within IGM cases to assess for blind spots in previous clinical and research exome sequencing. This database included exome sequencing using commercial capture kits (Agilent V4, Agilent V5, IDTERPv1, IDTERPv2, Roche Nimblegen, and Roche Nimblegen V2). All kits except the IDTERPv1 had >90% mean 10× coverage of *GREB1L*, however the mean (SD) and median (minimum, maximum) 10× coverage with exome capture by IDTERPv1 was 0.24 (0.43) and 0.00 (0.00, 1.00) respectively (Table 4).

4 | DISCUSSION

We assembled a combined cohort of individuals with disease secondary to pathogenic and likely pathogenic *GREB1L* variants. The phenotypic spectrum of these cases demonstrated several important aspects of *GREB1L*-associated disease, including an association with complex CHD in humans. Previously reported disease caused by *GREB1L* variants included congenital anomalies of the kidney, uterus, vagina, and sensorineural hearing loss. However, these descriptions were based on phenotype-driven, forward genetics studies of cohorts with anomalies in these organ systems. We identified two cases of complex CHD using the FSC multisite network of investigators and a broad literature search using Mastermind, which includes genetic data from online Supporting Information S1. This work demonstrates the importance of broad research collaborations, such as the FSC, as well as deep search algorithms when determining the phenotypic spectrum of rare genetic disorders.

Zaidi, et al., described an excess burden of de novo variants in genes expressed in the developing heart in individuals with severe CHD. This included an individual with tetralogy of Fallot and a single left coronary artery found to have a nonsense variant in *GREB1L* (c.4119G>A; p.Trp1373*).[21] CHD in this case was primarily attributed to a nonsense de novo variant in *NAA15*, a gene encoding a ribosomal component that facilitates post-translational acetylation, and which is associated with neurodevelopmental impairment and CHD.[36] Both genes are associated with autosomal dominant, incompletely penetrant

disease. Given the shared roles of NAA15 and *GREB1L* in ribosome function, this case may represent digenic disease, in which heterozygous loss of each gene impaired ribosome biogenesis, disrupted growth of cardiac precursor cells, and led to a malpositioned cardiac outflow tract, as found with homozygous loss of *GREB1L* in mouse.[13]

Using the FSC, we also identified an individual bearing a de novo missense variant in *GREB1L* (c.4440C>A; p.Phe1480>Leu) with hypoplastic left heart syndrome, double-outlet RV, total anomalous pulmonary venous return, hemivertebrae, scoliosis, as well as an ectopic, hypoplastic kidney. No other genetic disorders were identified by exome sequencing in this individual. While human CHD has been described related to *GREB1L*, this has been limited to aortic stenosis and thickening of the LV.[1] Intriguingly, a complex structural cardiac anomaly known as crisscross heart was found in homozygous *Greb1L* knockout mice prior to embryonic lethality.[1,13] This anomaly was preceded by impaired ribosome biogenesis, growth arrest of cardiac precursor cells, and malpositioning of the cardiac outflow tract. This mechanism may mediate human CHD. Three individuals with CHD in our cohort had outflow tract anomalies: aortic stenosis, double-outlet RV, and tetralogy of Fallot. Additionally, left ventricular hypertrophy may represent fetal cardio renal syndrome caused by increased systemic vascular resistance in the setting of bilateral renal agenesis.[37] Interestingly, the *GREB1L* variants associated with complex CHD lie in exons 24 and 26, near previously described variants associated with aortic stenosis and left ventricular hypertrophy in exons 26 and 27.[1,12] This region in *GREB1L* is highly homologous to a region in *GREB1*, which includes a putative glycosyltransferase domain.[38] The significance of the clustering of variants associated with CHD is unclear given that three of the four variants are protein disrupting (two stop-gain and one splicing disruption).

Our combined cohort included four types of CHD found in five individuals: (1) tetralogy of Fallot, (2) hypoplastic left heart syndrome with double-outlet RV and total anomalous pulmonary venous return, (3) hypertrophic LV found in two individuals, and (4) aortic stenosis. These cases support two important hypotheses. First, *GREB1L* LoF variants may cause complex CHD in humans. To our knowledge, human CHD attributed to *GREB1L* has been limited to aortic stenosis and hypertrophic left ventricles. The mouse *Greb1L* knockout model demonstrates complex CHD, but this has not yet been reported in humans with *GREB1L* variants to our knowledge. Second, human cases of CHD may arise secondary to cardiac outflow tract anomalies. The mouse *Greb1L* complex CHD is preceded by disrupted development of the outflow tract. Outflow tract anomalies in humans could account for the tetralogy of Fallot, double-outlet RV, and aortic stenosis. Hypertrophy of the LV could be attributable to undetected outflow tract obstructions, or alternatively could represent fetal cardiorenal syndrome related to renal agenesis.[37]

Determining the full spectrum of *GREB1L*-associated disease has been limited by several factors. As demonstrated by our literature review, human studies of *GREB1L* have focused predominantly on anomalies of the kidney, uterus, vagina, and sensorineural hearing loss. Phenotypic evaluations (autopsy, audiography, renal ultrasound, echocardiography, etc.) were not uniform in these studies, and negative results were often not reported. Therefore, the incidence of congenital anomalies may be underestimated. Studies have mostly utilized exome sequencing, which has variable *GREB1L* coverage. *GREB1L* is highly intolerant

to LoF variants at the population level. Consistent with this intolerance, individuals with *GREB1L* variants have been observed to have disease incompatible with survival or reproduction: fetal demise, stillbirth, renal agenesis, Potter's sequence, cyanotic heart disease, and uterine agenesis. Data regarding the timing of diagnosis (pre- or postnatal) were not uniformly provided in literature cases. Prenatal presentations frequently included Potter's sequence and subsequent lethality. Paradoxically, postnatal presentations (including unaffected relatives and those with minor anomalies) have also been found bearing *GREB1L* variants, including protein truncating variants. The variable expressivity and incomplete penetrance of *GREB1L*-associated disease may explain this apparent paradox. Loss of *GREB1L* function may confer a risk of severe early presentations of pre- and perinatal disease, mediated by unknown second hit(s). Population-level LoF variants in *GREB1L* would be expected to be rare, as alleles would be unlikely to persist through multiple generations. As we demonstrated, the description of *GREB1L*-associated disease may also have been limited by poor coverage in exome capture.

We could not identify a pattern between molecular changes in *GREB1L* and the observed phenotypes. We found highly variable phenotypes associated with *GREB1L* variants. Of the variants found in multiple individuals, most (23 of 26) resulted in different phenotypic presentations, and 16 variants were found in individuals with no apparent disease. Disruptive protein truncating variants were found across phenotypes, and missense variants varied widely in the predicted deleteriousness as assessed by REVEL scores. Grouping variants associated with phenotypes not compatible with survival or reproduction (bilateral renal agenesis, uterovaginal agenesis, stillbirth, complex CHD) demonstrated a similar distribution of protein truncating variants as well as predicted deleteriousness of missense changes. Scarce data exist regarding the functional domains or molecular role of *GREB1L*. Without these insights, it is as yet unclear how molecular changes in *GREB1L* relate to the variable-observed phenotypes. A bias toward maternal inheritance has been proposed for disease attributable to *GREB1L*.[1,12] We could not clarify patterns of inheritance given heterogeneous, limited data compiled from multiple sources. Additionally, cases identified in the IGM were not confirmed by Sanger sequencing.

These findings have important implications for clinical care and human genetics research. Clinicians should strongly consider genome sequencing or verify *GREB1L* coverage by exome sequencing when testing individuals with presentations consistent with *GREB1L*-associated disease. Clinicians and researchers should consider this when re-analyzing exome sequenced negative cases. Clinicians may consider analysis or re-analysis of *GREB1L* for individuals with CHD, especially anomalies of the outflow tract. Clinicians should consider screening individuals with *GREB1L* variants and their relatives for the full spectrum of associated diseases, including uterine anomalies, sensorineural hearing loss, as well as CHD, especially outflow tract anomalies. Finally, a basic investigation into the molecular function of *GREB1L* is urgently needed. This may illuminate the mechanism of its variable penetrance and incomplete expressivity, as well as novel phenotypes related to *GREB1L*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

The authors wish to acknowledge the members of the FSC for their assistance, as well as the families and individuals described in this work for their participation. We also wish to acknowledge two core laboratories at UNC-CH, the High Throughput Sequencing Facility and the Biospecimen Processing Facility, as well as the core facilities of Columbia University and Johns Hopkins, for helping complete the sequencing work. This work was supported by grants to the following authors. SSC: P20 DK116191 (NIDDK), R01 DK103184 (NIDDK), R01 DK115574(NIDDK). AJ: K23 DK119949 (NIDDK). AG: R01DK080099 (NIDDK). TH: 15705 (Thrasher Research Fund Early Career Award), KL2 TR001874 (NIH/NCATS), K23 HD113827 (NICHD). NV: K23 HD088742 (NICHD).

Funding information

National Institutes of Health, Grant/Award Number: KL2 TR001874; National Institute of Diabetes and Digestive and Kidney Diseases, Grant/Award Numbers: K23 DK119949, P20 DK116191, R01 DK103184, R01 DK115574, R01DK080099; National Institute of Child Health and Human Development, Grant/ Award Numbers: K23 HD088742, K2 HD113827; Thrasher Research Fund, Grant/Award Number: 15705

DATA AVAILABILITY STATEMENT

Descriptions of confirmed genetic variants and phenotype are available in CinVar (https://www.ncbi.nlm.nih.gov/clinvar/). Further data are available on request due to privacy/ethical restrictions. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

REFERENCES

1. De Tomasi L, David P, Humbert C, et al. Mutations in GREB1L cause bilateral kidney agenesis in humans and mice. Am J Hum Genet.2017;101(5):803–814. 10.1016/j.ajhg.2017.09.026 [PubMed: 29100091]
2. Sanna-Cherchi S, Khan K, Westland R, et al. Exome-wide association study identifies GREB1L mutations in congenital kidney malformations. Am J Hum Genet. 2017;101(5):789–802. 10.1016/j.ajhg.2017.11.003 [PubMed: 29100090]
3. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020;581(7809):434–443. 10.1038/s41586-020-2308-7 [PubMed: 32461654]
4. Brophy PD, Rasmussen M, Parida M, et al. A gene implicated in activation of retinoic acid receptor targets is a novel renal agenesis gene in humans. Genetics. 2017;207(1):215–228. 10.1534/genetics.117.1125 [PubMed: 28739660]
5. Schrauwen I, Kari E, Mattox J, et al. De novo variants in GREB1L are associated with non-syndromic inner ear malformations and deafness. Hum Genet. 2018;137(6–7):459–470. 10.1007/s00439-018-1898-8 [PubMed: 29955957]
6. Schrauwen I, Liaqat K, Schatteman I, et al. Autosomal dominantly inherited GREB1L variants in individuals with profound sensorineural hearing impairment. Genes. 2020;11(6):687. 10.3390/genes11060687 [PubMed: 32585897]
7. Adadey SM, Aboagye ET, Esoh K, et al. A novel autosomal dominant GREB1L variant associated with non-syndromic hearing impairment in Ghana. BMC Med Genom. 2022;15(1):237. 10.1186/s12920-022-01391-w
8. Kim BJ, Jeon H, Lee SY, et al. Major contribution of GREB1L alterations to severe inner ear malformation largely in a non-mendelian fashion. Clin Exp Otorhinolaryngol. 2022;15(1):115–118. 10.21053/ceo.2021.01935 [PubMed: 35012281]
9. Herlin MK, Le VQ, Højland AT, et al. Whole-exome sequencing identifies a GREB1L variant in a three-generation family with Müllerian and renal agenesis: a novel candidate gene in Mayer-

Rokitansky-Küster-Hauser (MRKH) syndrome. A case report. Hum Reprod. 2019;34(9):1838–1846. 10.1093/humrep/dez126 [PubMed: 31424080]

10. Jacquinet A, Boujemla B, Fasquelle C, et al. GREB1L variants in familial and sporadic hereditary urogenital adysplasia and Mayer-Rokitansky-Kuster-Hauser syndrome. Clin Genet. 2020;98(2):126–137. 10.1111/cge.13769 [PubMed: 32378186]
11. Buchert R, Schenk E, Hentrich T, et al. Genome sequencing and transcriptome profiling in twins discordant for mayer-rokitansky-küster-hauser syndrome. J Clin Med. 2022;11(19):5598. 10.3390/jcm11195598 [PubMed: 36233463]
12. Jolly A, Du H, Borel C, et al. Rare variant enrichment analysis supports GREB1L as a contributory driver gene in the etiology of Mayer-Rokitansky-Küster-Hauser syndrome. HGG Adv. 2023;4(3):100188. 10.1016/j.xhgg.2023.100188 [PubMed: 37124138]
13. Bernheim S, Borgel A, Le Garrec JF, et al. Identification of Greb1l as a genetic determinant of crisscross heart in mice showing torsion of the heart tube by shortage of progenitor cells. Dev Cell. 2023;58(21):2217–2234.e8. 10.1016/j.devcel.2023.09.006 [PubMed: 37852253]
14. Giordano JL, Wapner RJ. The fetal sequencing consortium: the value of multidisciplinary dialog and collaboration. Prenat Diagn. 2022;42(7):807–810. 10.1002/pd.6190 [PubMed: 35661204]
15. Ren Z, Povysil G, Hostyk JA, Cui H, Bhardwaj N, Goldstein DB. ATAV: a comprehensive platform for population-scale genomic analyses. BMC Bioinf. 2021;22(1):149. 10.1186/s12859-021-04071-1
16. Chunn LM, Nefcy DC, Scouten RW, et al. Mastermind: a comprehensive genomic association search engine for empirical evidence curation and genetic variant interpretation. Front Genet. 2020;11:577152. 10.3389/fgene.2020.577152 [PubMed: 33281875]
17. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. Genet Med. 2015;17(5):405–424. 10.1038/gim.2015.30 [PubMed: 25741868]
18. van der Ven AT, Connaughton DM, Ityel H, et al. Whole-exome sequencing identifies causative mutations in families with congenital anomalies of the kidney and urinary tract. J Am Soc Nephrol. 2018;29(9):2348–2361. 10.1681/asn.2017121265 [PubMed: 30143558]
19. Domingo-Gallego A, Pybus M, Bullich G, et al. Clinical utility of genetic testing in early-onset kidney disease: seven genes are the main players. Nephrol Dial Transpl. 2022;37(4):687–696. 10.1093/ndt/gfab019
20. Seltzsam S, Wang C, Zheng B, et al. Reverse phenotyping facilitates disease allele calling in exome sequencing of patients with CAKUT. Genet Med. 2022;24(2):307–318. 10.1016/j.gim.2021.09.010 [PubMed: 34906515]
21. Zaidi S, Choi M, Wakimoto H, et al. De novo mutations in histone-modifying genes in congenital heart disease. Nature. 2013;498(7453):220–223. 10.1038/nature12141 [PubMed: 23665959]
22. Kingsmore SF, Cakici JA, Clark MM, et al. A randomized, controlled trial of the analytic and diagnostic performance of singleton and trio, rapid genome and exome sequencing in ill infants. Am J Hum Genet. 2019;105(4):719–733. 10.1016/j.ajhg.2019.08.009 [PubMed: 31564432]
23. Zhao C, Chai H, Zhou Q, et al. Exome sequencing analysis on products of conception: a cohort study to evaluate clinical utility and genetic etiology for pregnancy loss. Genet Med. 2021;23(3):435–442. 10.1038/s41436-020-01008-6 [PubMed: 33100332]
24. Marangoni M, Smits G, Ceysens G, et al. Implementation of fetal clinical exome sequencing: comparing prospective and retrospective cohorts. Genet Med. 2022;24(2):344–363. 10.1016/j.gim.2021.09.016 [PubMed: 34906519]
25. Byrne AB, Arts P, Ha TT, et al. Genomic autopsy to identify underlying causes of pregnancy loss and perinatal death. Nat Med. 2023;29(1):180–189. 10.1038/s41591-022-02142-1 [PubMed: 36658419]
26. Rasmussen M, Sunde L, Nielsen ML, et al. Targeted gene sequencing and whole-exome sequencing in autopsied fetuses with prenatally diagnosed kidney anomalies. Clin Genet. 2018;93(4):860–869. 10.1111/cge.13185 [PubMed: 29194579]

27. Boissel S, Fallet-Bianco C, Chitayat D, et al. Genomic study of severe fetal anomalies and discovery of GREB1L mutations in renal agenesis. Genet Med. 2018;20(7):745–753. 10.1038/gim.2017.173 [PubMed: 29261186]

28. Wang A, Ji B, Wu F, Zhao X. Clinical exome sequencing identifies a novel mutation of the GREB1L gene in a Chinese family with renal agenesis. Genet Test Mol Biomarkers. 2020;24(8):520–526. 10.1089/gtmb.2020.0036 [PubMed: 32598191]

29. Mallett AJ, Quinlan C, Patel C, et al. Precision medicine diagnostics for rare kidney disease: twitter as a Tool in clinical genomic translation. Kidney Med. 2019;1(5):315–318. 10.1016/j.xkme.2019.06.006 [PubMed: 32734212]

30. Wu S, Wang X, Dai S, Zhang G, Zhou J, Shen Y. A novel missense mutation in GREB1L identified in a three-generation family with renal hypodysplasia/aplasia-3. Orphanet J Rare Dis. 2022;17(1):413. 10.1186/s13023-022-02553-w [PubMed: 36371238]

31. Hopkins JJ, Wakeling MN, Johnson MB, Flanagan SE, Laver TW. REVEL is better at predicting pathogenicity of loss-of-function than gain-of-function variants. medRxiv. 2023;2023:1–6. 2023.06.06. 23290963. 10.1155/2023/8857940

32. Ioannidis NM, Rothstein JH, Pejaver V, et al. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. Am J Hum Genet. 2016;99(4):877–885. 10.1016/j.ajhg.2016.08.016 [PubMed: 27666373]

33. Vora NL, Gilmore K, Brandt A, et al. An approach to integrating exome sequencing for fetal structural anomalies into clinical practice. Genet Med. 2020;22(5):954–961. 10.1038/s41436-020-0750-4 [PubMed: 31974414]

34. Warnes CA, Liberthson R, Danielson GK, et al. Task force 1: the changing profile of congenital heart disease in adult life. J Am Coll Cardiol. 2001;37(5):1170–1175. 10.1016/s0735-1097(01)01272-4 [PubMed: 11300418]

35. Genovese G, Fromer M, Stahl EA, et al. Increased burden of ultrarare protein-altering variants among 4,877 individuals with schizophrenia. Nat Neurosci. 2016;19(11):1433–1441. 10.1038/nn.4402 [PubMed: 27694994]

36. Sugiura N, Adams SM, Corriveau RA. An evolutionarily conserved N-terminal acetyltransferase complex associated with neuronal development. J Biol Chem. 2003;278(41):40113–40120. 10.1074/jbc.m301218200 [PubMed: 12888564]

37. Merz WM, Kübler K, Fimmers R, Willruth A, Stoffel-Wagner B, Gembruch U. Cardiorenal syndrome is present in human fetuses with severe, isolated urinary tract malformations. PLoS One. 2013;8(5):e63664. 10.1371/journal.pone.0063664 [PubMed: 23717461]

38. Shin EM, Huynh VT, Neja SA, et al. GREB1: an evolutionarily conserved protein with a glycosyltransferase domain links ERα glycosylation and stability to cancer. Sci Adv. 2021;7(12). 10.1126/sciadv.abe2470

Key points

What is Already Known?

- *GREB1L* is associated prenatally with Potter's sequence, and postnatally with less severe anomalies of the kidney, uterus, inner ear, and heart.
- Known cardiac anomalies are aortic stenosis and ventricular hypertrophy.

What does this Study Add?

- We identified complex congenital heart disease (CHD) associated with *GREB1L*.
- Clinicians should consider *GREB1L* testing in the setting of CHD, particularly outflow tract anomalies.
- Clinicians should consider pre- and postnatal screening for cardiac anomalies in the setting of *GREB1L* variants.

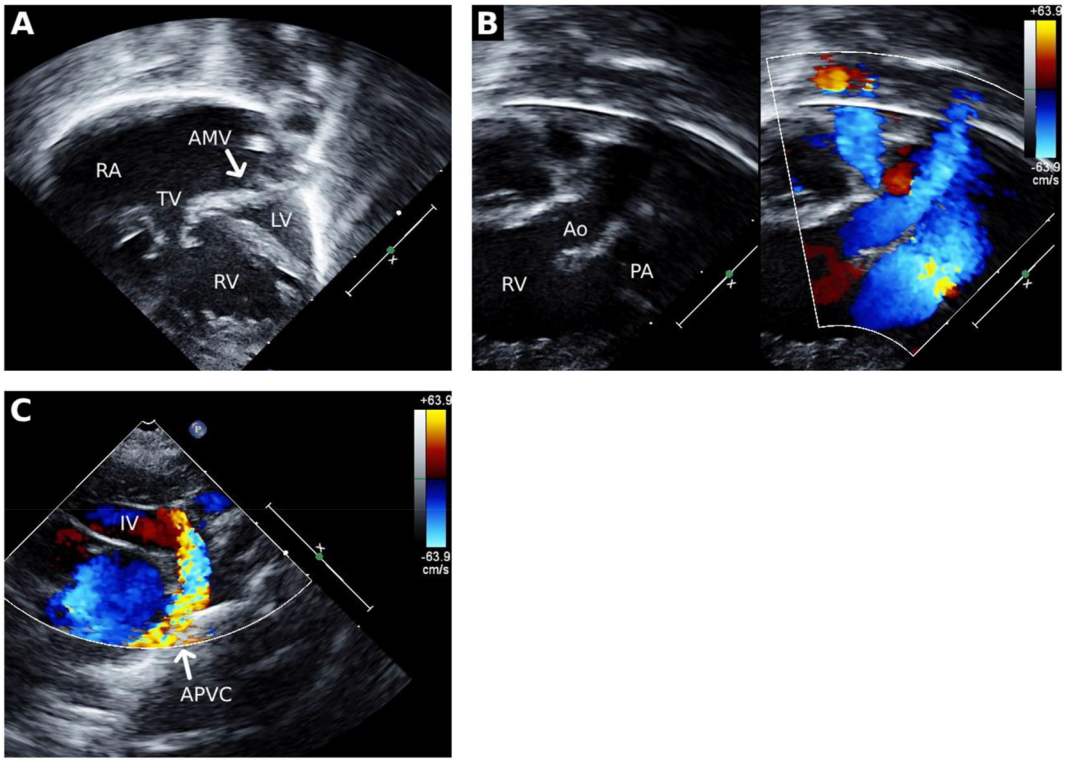

FIGURE 1.

Complex congenital heart disease (CHD) in a female infant with a de novo missense *GREB1L* variant (c.4440C>A; p.Phe1480>Leu). In addition to renal ectopy, renal hypoplasia, hemivertebrae, and scoliosis, the infant had multiple cardiac anomalies. These consisted of hypoplastic left syndrome, double-outlet RV, and total anomalous pulmonary venous return. (A), An echocardiogram apical view demonstrates a hypoplastic LV with atresia of the mitral valve. (B), An apical view with anterior angulation and 2-D and color Doppler image shows both great arteries arising from the RV. (C), A suprasternal view demonstrates a left APVC draining into the IV. Abbreviations: AMV, atretic mitral valve; APVC, anomalous pulmonary venous channel; Ao (aorta), IV, innominate vein; LV, left ventricle; RA, right atrium; RV, right ventricle; TV, tricuspid valve.

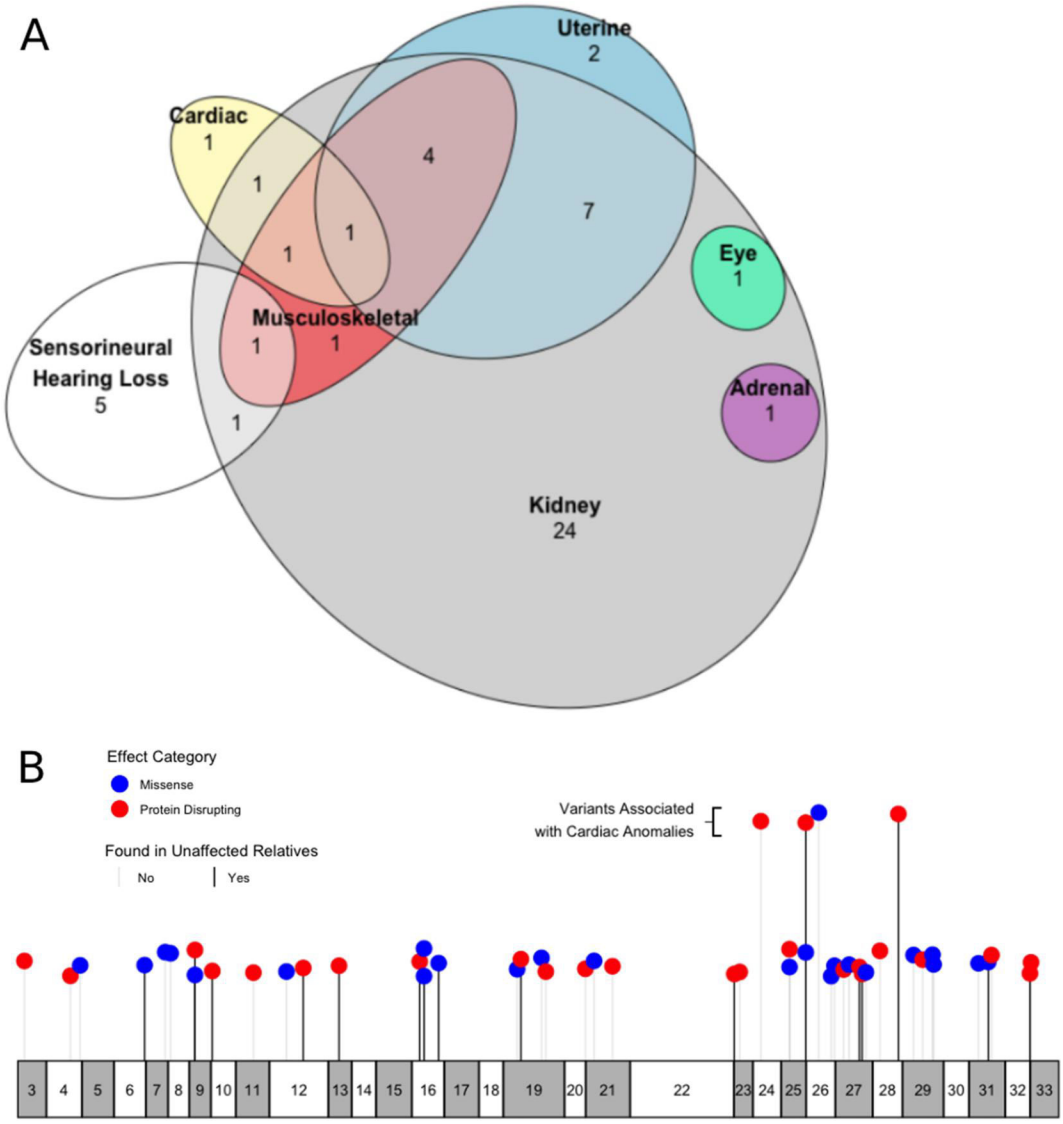

FIGURE 2.

GREB1L phenotypic spectrum and variants. (A), Phenotype classes found in unique pathogenic and likely pathogenic *GREB1L* variants. In the Euler diagram, the number of unique variants is shown for each affected organ system. The area of each ellipse is proportional to the number of variants in each group. Isolated kidney anomalies were associated with 24 unique variants. Isolated sensorineural hearing loss was associated with five unique variants. Isolated uterine anomalies were associated with 2 unique variants. An isolated cardiac anomaly was found in one individual with a unique variant. 19 variants were associated with multiple anomalies, most often kidney anomalies accompanied by uterine and/or musculoskeletal anomalies. (B), Map of disease-causing variants in *GREB1L*. The pathogenic and likely pathogenic variants found in *GREB1L* are shown mapped above the exon structure of *GREB1L*. Missense variants are shown in blue and protein disrupting are shown in red. Variants identified only in individuals with congenital anomalies are shown with gray stems, and variants found in affected individuals as well as in relatives with

no known disease are shown with black stems. Four variants found to be associated with cardiac anomalies are shown with higher stems.

TABLE 1

The prevalence of phenotypes in combined cohorts.

Phenotype category	**Individuals (*n* = 127)**
Isolated renal anomalies	66 (52.0%)
Unilateral or bilateral renal absence	48
Multiple renal anomalies	10
Renal hypo- or dysplasia	4
Urinary tract dilation and related anomalies of the urethra or ureters	3
Renal ectopy or fusion	1
Anomalies of multiple organ systems	25 (19.7%)
No apparent disease (healthy relatives)	21 (16.5%)
Isolated sensorineural hearing loss	10 (7.9%)
Isolated uterine anomaly	4 (3.1%)
Isolated congenital heart disease (tetralogy of fallot)	1 (0.8%)

TABLE 2

Classification of unique variants by phenotype.

	Variant class		
Phenotype	**Protein disrupting**	**Missense**	**REVEL score of missense variants mean (SD)**
Isolated kidney anomalies *n* = 24	11 (45.8%)	13 (54.2%)	0.39 (0.24)
Anomalies of multiple organ systems *n* = 19	11 (57.9%)	8 (42.1%)	0.35 (0.20)
Isolated sensorineural hearing loss *n* = 5	2 (40.0%)	3 (60.0%)	0.26 (0.17)
Isolated uterine anomalies *n* = 2	2(100%)	0	-
Isolated congenital heart disease *n* = 1	1 (100%)	0	-
Total 51	27 (52.9%)	24 (47.1%)	0.36 (0.21)

Abbreviation: REVEL, rare exome variant ensemble learner.

TABLE 3

Published studies of *GREB1L*-associated disease.

Ascertainment criteria of literature cases	Publications (*n* = 23)	Pathogenic/Likely pathogenic variants identified[a] (*n* = 46)
Renal agenesis or renal hypodysplasia	8	27
Perinatal disorders	5	5
Sensorineural hearing loss	3	6
Other renal anomalies or nephropathy	3	5
Uterine anomalies	4	9

[a] 46 unique variants were identified, 6 of which were independently found in multiple studies.

TABLE 4

GREB1L coverage by exome capture kit.

Kit	Mean 10× coverage (SD)	Median 10× coverage (min, max)
Agilent V4	0.97 (0.17)	1.00 (0.00, 1.00)
Agilent V5	0.90 (0.15)	0.99 (0.00, 1.00)
IDTERPv1	0.24 (0.43)	0.00 (0.00, 1.00)
IDTERPv2	1.00 (0.00)	1.00 (0.00, 1.00)
Roche Nimblegen	0.99 (0.03)	1.00 (0.67, 1.00)
Roche Nimblegen V2	0.93 (0.16)	0.97 (0.14, 1.00)