Biallelic TMEM260 variants cause truncus arteriosus, with or without renal defects

Alistair T. Pagnamenta1 | Adam Jackson2,3 | Rahat Perveen2 | Glenda Beaman2 | Gemma Petts4 | Asheeta Gupta5 | Zerin Hyder2 | Brian Hon-Yin Chung6 | Anita Sik-Yau Kan7 | Ka Wang Cheung7 | Wilhelmina S. Kerstjens-Frederikse8 | Kristin M. Abbott8 | Genomics England Research Consortium | Orly Elpeleg9 | Jenny C. Taylor1 | Siddharth Banka2,3 | Asaf Ta-Shma10

1NIHR Biomedical Research Centre, Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK
2Division of Evolution, Infection and Genomics, University of Manchester, Manchester, UK
3Manchester Centre for Genomic Medicine, St. Mary’s Hospital, Manchester University NHS Foundation Trust, Health Innovation Manchester, Manchester, UK
4Department of Paediatric Histopathology, Royal Manchester Children’s Hospital, Manchester, UK
5Birmingham Children’s Hospital, Birmingham, UK
6Department of Paediatrics and Adolescent Medicine, LKS Faculty of Medicine, The University of Hong Kong, Pok Fu Lam, Hong Kong
7Department of Obstetrics and Gynaecology, Queen Mary Hospital, Pok Fu Lam, Hong Kong
8Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands
9Department of Genetics, Hadassah Medical Organization and Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel
10Department of Pediatric Cardiology, Hadassah Medical Organization and Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel

Abstract

Only two families have been reported with biallelic TMEM260 variants segregating with structural heart defects and renal anomalies syndrome (SHDRA). With a combination of genome, exome sequencing and RNA studies, we identified eight individuals from five families with biallelic TMEM260 variants. Variants included one multi-exon deletion, four nonsense/frameshifts, two splicing changes and one missense change. Together with the published cases, analysis of clinical data revealed ventricular septal defects (12/12), mostly secondary to truncus arteriosus (10/12), elevated creatinine levels (6/12), horse-shoe kidneys (1/12) and renal cysts (1/12) in patients. Three pregnancies were terminated on detection of severe congenital anomalies. Six patients died between the ages of 6 weeks and 5 years. Using a range of stringencies, carrier frequency for SHDRA was estimated at 0.0007–0.007 across ancestries. In conclusion, this study confirms the genetic basis of SHDRA, expands its known mutational spectrum and clarifies its clinical features. We demonstrate that SHDRA is a
and renal anomalies syndrome

in foetuses with congenital heart anomalies

tal anomaly scan at 20 weeks gestation (Table 1). The pregnancy was
stenosis and ventricular septal defect (VSD) were detected on antena-
In F1-II-3 (Figure 1A), type I truncus arteriosus (TA) with pulmonary
3.1

| 3.2 |

Identification of additional SHDRA patients

To expand the cohort of patients with SHDRA we interrogated the
100KGP database further and identified a homozygous c.1410C > G:
p.Tyr470Ter TMEM260 variant in Family 2 (Figure 1E). F2-II-2
exhibited a common arterial trunk, tricuspid atresia, VSD, partial
anomalous pulmonary venous connection, bilateral hearing loss, global
developmental delay, protein losing enteropathy and deteriorating
renal function from the age of 15 months (Figure S4). Multi-organ
terminated at 21 weeks. Post-mortem examination confirmed the car-
diac anomalies (Figure S1) and did not reveal any other abnormalities.
In F1-II-4 a large peri-membranous outlet VSD, type I TA with small
pulmonary trunk and small pulmonary artery branches were detected
antenatally and the pregnancy was terminated at 24 weeks gestation.
Post-mortem examination confirmed the cardiac anomalies and rev-
ealed a horseshoe kidney. The placenta was also abnormal with a
two-vessel cord and omphalomesenteric duct remnant.

Trio WGS was performed as part of the 100KGP on F1-II-4 and
both parents. Although the initial analysis focussing on several panels
from PanelApp was negative, a scan for Mendelian inconsistencies
highlighted an apparently homozygous NM_017799.4:c.344G > A:p.
(Arg115Lys) TMEM260 variant in F1-II-4 (Figure 1B). As expected, the
father (F1-I-1) was heterozygous for the c.344G > A TMEM260 vari-
ant, but the mother (F1-I-2) was apparently homozygous for the wild-
type allele. Review of read alignments revealed a maternally inherited
4891 bp deletion (Figure 1B) encompassing exons 2–3. Sanger
sequencing and digital droplet PCR confirmed that F1-II-3 was also
hemizygous for c.344G > A. Neither of the unaffected brothers
(F1-II-1 and F1-II-2) had inherited both TMEM260 variants.

Although the c.344G > A variant was initially annotated as p.
Arg115Lys, it involves the last base of exon 3 and results in a drop in
the predicted splicing efficiency (MaxEntScan: 9.65 → 2.69). We,
therefore, performed RT-PCR on peripheral blood sample from F1-I-1,
which showed the presence of two bands, with only the larger band
seen in controls (Figure 1C). Sanger sequencing confirmed exon 3 skip-
ing (Figure 1D, Figure S2), resulting in a frameshift of exon 4 (p.
Val65AlafsTer32). Similarly, the expected exon 1–4 junction was
detected by RT-PCR in the maternal sample (Figure S3), resulting in p.
(Glu55PhefsTer20). Collectively, the genetic studies, RNA analysis and
similarity of the foetuses’ phenotypes with features reported
previously, can strongly suggest these TMEM260 variants are disease-
causing.

1 | INTRODUCTION

TMEM260 is a 79.5 kDa protein with eight transmembrane spans
(www.uniprot.org/uniprot/Q9NX78) located mainly in the nucleo-
plasm and within focal adhesion sites (www.proteinatlas.org).1
TMEM260 encodes at least four protein-coding transcripts. Of these,
two (ENST00000261556.11 and ENST00000538838.5) are consid-
ered to be the main transcripts. They differ in the utilisation of an
internal exon as well as the final three exons, which in the short iso-
form are non-coding.

Five individuals from two families with biallelic truncating TMEM260
variants and brain, cardiac, renal, and digit abnormalities were reported in
2017.2 The condition is now listed on OMIM as “structural heart defects
and renal anomalies syndrome” (SHDRA; MIM #617478). Notably, the
variants in both families mapped to the long isoform, raising the possibil-
ity of SHDRA being an isoform-specific disorder. Since the original
publication, there have been no further reports in the literature. Knowl-
edge about the variant and the clinical spectrum of this condition is
therefore limited (Supplementary background). In this study, we describe
eight affected individuals from five families, confirming that biallelic
TMEM260 loss of function variants cause SHDRA and helping to define
its clinical spectrum.

2 | MATERIALS AND METHODS

Whole genome sequencing in Families 1 and 2 was performed as part
of the 100 000 genomes project (100KGP; https://doi.org/10.6084/
m9.figshare.4530893.v6, Cambridge South REC: 14/EE/1112). Fami-
lies 3–5 were identified via whole exome sequencing (WES) pipelines
and international collaboration. RNA analysis was performed for Fam-
ilies 1 using PaxGene blood samples. Carrier frequency for SHDRA was
calculated as described previously.3 More details are in Supplemental
methods and Tables S1–S2.

3 | RESULTS

3.1 | Compound-heterozygous TMEM260 variants
in foetuses with congenital heart anomalies

In F1-II-3 (Figure 1A), type I truncus arteriosus (TA) with pulmonary
stenosis and ventricular septal defect (VSD) were detected on antenat-
al anomaly scan at 20 weeks gestation (Table 1). The pregnancy was
severe condition associated with substantial mortality in early childhood and
characterised by congenital cardiac malformations with a variable renal phenotype.

KEYWORDS
exome sequencing, genome sequencing, kidney, phenotypic variability, renal failure, SHDRA,
structural heart defects and renal anomalies syndrome, TMEM260, truncus arteriosus
### TABLE 1 Genetic and clinical information for five newly reported families with rare biallelic variants in TMEM260

<table>
<thead>
<tr>
<th>Family number</th>
<th>Family 1</th>
<th>Family 2</th>
<th>Family 3</th>
<th>Family 4</th>
<th>Family 5</th>
<th>Ta-Shma et al. 2017*</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td>White British</td>
<td>Pakistani</td>
<td>Ashkenazi Jewish</td>
<td>Sudanese</td>
<td>Chinese</td>
<td>Ashkenazi Jewish, Arabic</td>
<td>White British, Pakistani, Arabic, Ashkenazi Jewish (2), Sudanese, Chinese</td>
</tr>
<tr>
<td>Parental consanguinity</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No (no ROH &gt;10 Mb detected)</td>
<td>No</td>
<td>2/2 Families</td>
<td>3/7 Families</td>
</tr>
<tr>
<td>cDNA coordinates</td>
<td>c.(0-44G &gt; A) (NM_017799.4)</td>
<td>c.1410C &gt; G</td>
<td>c.1393C &gt; T (GRCh38)</td>
<td>c.1393C &gt; T (GRCh38)</td>
<td>c.1393C &gt; T (GRCh38)</td>
<td>c.1393C &gt; T (GRCh38)</td>
<td>–</td>
</tr>
<tr>
<td>CADD (splice predictions)</td>
<td>34 (SpliceAI = 0.69, MaxEntScan diff = 6.964), NA</td>
<td>34</td>
<td>41 (SpliceAI = 0.6), 32</td>
<td>NA</td>
<td>34 (SpliceAI = 1.00, MaxEntScan diff = 7.955), 28.3</td>
<td>41 (SpliceAI = 0.06), NA</td>
<td>28.3–41</td>
</tr>
<tr>
<td>Isoforms involved</td>
<td>Short and long</td>
<td>Long</td>
<td>Long</td>
<td>Long</td>
<td>Long</td>
<td>Long</td>
<td>–</td>
</tr>
<tr>
<td>gnomAD AF (v.2.1.1)</td>
<td>2/249706: NA</td>
<td>2/250342</td>
<td>19/273758, 2/251170</td>
<td>Absent</td>
<td>Absent, absent</td>
<td>19/273758, absent</td>
<td>All allele counts of 0–19</td>
</tr>
<tr>
<td>Individual ID</td>
<td>F1-II-3</td>
<td>F1-II-4</td>
<td>F2-II-2</td>
<td>F3-II-2</td>
<td>F3-II-3</td>
<td>F4-II-2</td>
<td>F5-II-1</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Methods</td>
<td>Sanger sequencing</td>
<td>Trio genome sequencing (HiSeqX) as part of 100KGP</td>
<td>Trio genome sequencing (HiSeqX) as part of 100KGP</td>
<td>Exome + Sanger</td>
<td>Exome + Sanger</td>
<td>Exome + Sanger</td>
<td>Exome + Sanger</td>
</tr>
<tr>
<td>Deceased</td>
<td>TOP at 21 weeks</td>
<td>TOP at 24 weeks</td>
<td>Died at 5 years</td>
<td>No (currently 5 years old)</td>
<td>Died at 3 months</td>
<td>No (currently 3 years old)</td>
<td>Died at 4 months</td>
</tr>
<tr>
<td>Cardiac defects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septal defect(s)</td>
<td>VSD</td>
<td>VSD (subvalvular)</td>
<td>VSD</td>
<td>VSD</td>
<td>VSD</td>
<td>VSD and ASD</td>
<td>VSD</td>
</tr>
<tr>
<td>Truncus arteriosus</td>
<td>+ (type I)</td>
<td>+ (type I)</td>
<td>+</td>
<td>+ (type I, complete repair)</td>
<td>+ (type I, complete repair at 1 week of age)</td>
<td>+ (type I, complete repair at 1 week of age)</td>
<td>+ (type I, complete repair at 3.5 months of age)</td>
</tr>
<tr>
<td>Tetralogy of Fallot, pulmonic atresia and major aortopulmonary collateral arteries</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pulmonary stenosis</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Interrupted aortic arch</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Right aortic arch</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

(Continues)
<table>
<thead>
<tr>
<th>TABLE 1 (Continued)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiac defects</strong></td>
</tr>
<tr>
<td>Tricuspid valve atresia</td>
</tr>
<tr>
<td>Partial anomalous pulmonary venous return</td>
</tr>
<tr>
<td>Neurodevelopmental defects</td>
</tr>
<tr>
<td>Agenesis of corpus callosum</td>
</tr>
<tr>
<td>Microcephaly</td>
</tr>
<tr>
<td>Developmental delay</td>
</tr>
<tr>
<td>Renal defects</td>
</tr>
<tr>
<td>Renal failure</td>
</tr>
<tr>
<td>Elevated creatinine levels norm: 0.2–0.9 mg/dL</td>
</tr>
<tr>
<td>Anuria/Oliguria</td>
</tr>
<tr>
<td>Urine protein</td>
</tr>
<tr>
<td>Renal cysts</td>
</tr>
<tr>
<td>Horseshoe kidney</td>
</tr>
<tr>
<td>Limb defects</td>
</tr>
<tr>
<td>Polydactyly</td>
</tr>
<tr>
<td>Overriding toes</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Other features</td>
</tr>
<tr>
<td>Prenatal findings/ polyhydramnios</td>
</tr>
</tbody>
</table>

Abbreviations: ASD, atrial septal defect; NA, not available; N/A, not applicable; ROH, region of homozygosity; VSD, ventricular septal defect.

*Deletion removes exons 2 and 3.

†Annotated as missense but results in exon skipping and c.193_344del:p.(Val65Alafs*32) in a splicing defect.

*Only molecularly confirmed cases from Ta-Shma et al. 2017 (PMID: 28318500) are included.
failure following cardiac surgery led to death at age 5 (Table S1, Supplementary Case Histories).

Through international collaboration, we uncovered three further families ascertained via WES (Figure 1E,F). This included a novel c.1644del:p.Pro549LeufsTer46 allele in trans with the previously described c.1393C > T:p.Gln465Ter in a sib-pair (Family 3). The elder brother presented with type I TA, mild truncal valve insufficiency, a large VSD, with normal renal function (max. Creatinine of 39 $\mu$mol/L) and normal neurodevelopment at the age of 5 years. The younger brother was born with Type I TA, mild to moderate truncal valve insufficiency and a large VSD (Video S1). A few days after cardiac surgery he developed severe chylothorax and progressive renal failure (Figure S5) and died at approaching 4 months from multi-organ failure. In Family 4 we identified homozygous c.1698_1701del:p.Tyr567ThrfsTer27 TMEM260 variant in a 2 year old boy with Type I TA and VSD, and normal renal function (maximum creatinine level of 37 $\mu$mol/L) and normal neurodevelopment. In Family 5 we identified novel compound-heterozygous TMEM260 variants: c.193-2A > G and c.1744G > C:p.Glu582Gln in a girl who died at the age of 4 months. The missense variant is predicted damaging by SIFT/PolyPhen2. The girl was born with TA type I, hypoplastic right ventricle, small main and branch pulmonary arteries, VSD and atrial septal defect. During her neonatal period, she developed necrotizing enterocolitis with perforation of transverse colon, renal impairment (Figure S6) and died at 4 months. Type I TA and VSD were detected antenatally in the proband’s sibling (Figure S7) and the pregnancy was terminated at 22 weeks. The same compound-heterozygous TMEM260 variants were also identified in the foetus.

3.3 SHDRA carrier frequency is 0.7–7 per 1000 individuals

Next, we estimated the carrier frequency of SHDRA using a range of stringency thresholds (Figure S8A). The most stringent criteria included only variants that would be predicted to result in loss-of-
function (without low-confidence flags) and known ClinVar pathogenic or likely pathogenic variants. The least stringent criteria included all variants with a CADD score over 30, a spliceAI score over 0.8 as well as loss-of-function alleles and ClinVar pathogenic and likely pathogenic alleles. This analysis showed that per-ancestry gene carrier rate (GCR) for TMEM260 ranges between 0.001 and 0.007 for least stringent parameters to 0.0007–0.005 for the most stringent. Only 16/94 predicted deleterious variants using the lowest stringency threshold are missense variants (Figure S8B). The GCR was found to be higher in individuals with African/African-American ancestry and lowest in Finnish ancestry. The higher GCR in the African/African-American population is due to a possible founder variant (p.Lys696ThrfsTer7, rs568247949) which has “Likely pathogenic” status in ClinVar with a single submission (SCV00092576.2).

4 | DISCUSSION

We present eight individuals, from five independent families, with biallelic TMEM260 variants (Figure 1). In combination with clinical data published previously, our results suggest congenital cardiac malformations to be the most consistent phenotype of SHDRA. All 12 patients are reported to have VSD and 10/12 had TA (Table 1). In most of these patients, VSD is likely to be secondary to TA. Notably, TA is one of the rarest congenital cardiac anomalies with few known genetic associations in NKX2-5, NKX2-6, GATA6 as well as TBX1. Interestingly, TMEM260, is predicted to be one of 1442 target genes for GATA6 predicted using known transcription factor binding site motifs from the TRANSFAC database. The JASPAR database of transcription factor binding sites predicts a GATA6 binding site within intron 5 of TMEM260 although the functionality of this motif is unknown.

Our results show that the renal phenotype of SHDRA is highly variable. Horseshoe kidney and cysts were noted in one patient each. The renal failure seen in three individuals could be pre-renal injury and acute tubular necrosis secondary to cardiac failure and systemic illness. However, the decline in glomerular filtration prior to the onset of cardiac failure in F2-II-2 suggests the possibility of underlying renal dysfunction. Further studies should address whether the variable renal involvement is secondary to cardiac complications or a primary component of the condition. The intra-familial variability in renal phenotype indicates that this may not be solely due to the precise TMEM260 variant(s) that are involved. A more likely hypothesis is that there is a congenital predisposition to renal failure, leaving the individual vulnerable to a rapid deterioration that can be precipitated by clinical (e.g., cardiac/intestinal) insults.

The combination of congenital heart disease, especially conotruncal defects with renal abnormalities is unusual. Conotruncal abnormalities are seen in 22q11.21 deletion syndrome, in which renal abnormalities, such as hypoplasia or agenesis of the kidney, multicystic dysplasia and vesicoureteral reflux, are thought to occur in over 30% of patients. Another dominant disorder with some phenotypic overlap, including TA and hypoplastic kidneys, is Townes-Brocks syndrome (MIM #107480) due to heterozygous mutations in SALL1. The association of cardiac, cerebral and renal malformations is also reminiscent of ciliopathies, although generally the cardiac features linked to these group of disorders do not include TA.

Antenatal detection of severe congenital malformations led to termination of pregnancy in three cases described here. Out of nine live born pregnancies, six patients died within the age ranges of 6 weeks to 5 years. One of the two individuals whom survived to 5-years old (F2-II-2) had developmental delay and hearing loss. However, due to insufficient numbers it is difficult to confidently associate these features with SHDRA. We note that two other individuals in the present study who survived beyond their first year, were cognitively normal. Although facial dysmorphism was reported in 1/4 of the original cohort, that feature was not replicated here.

This study substantially expands the known mutation spectrum of SHDRA. Including the patients presented here, a total of eight different TMEM260 variants in 12 individuals from seven families have now been identified (Figure 1F). Of these, two variants are stop-gains, two are frameshifts, one is a multi-exon deletion, two disrupt splicing and one is missense. All variants are supported by in silico tools, including CADD scores, which are 28.3–41 (Table 1). The distribution of the variants confirms that variants affecting only the longer isoform are sufficient to cause SHDRA.

We show that the carrier frequency for SHDRA could be up to 1 in 140 in certain populations (Figure S8). This analysis also identified a potential founder variant in the African/African-American population that requires further functional studies to validate its “Likely Pathogenic” status in ClinVar. The c.1698_1701del seen in Family 4 and in an Arabic family described previously may also represent a founder mutation.

In conclusion, our description of five families with biallelic TMEM260 variants confirms the genetic basis of SHDRA and helps delineate the mutational/phenotypic spectrum of the condition. The strong association with TA has important implications for genetic counselling, prenatal diagnostics as well as postnatal targeted genetic testing.

ACKNOWLEDGEMENTS

We thank Katherine Bull for critical comments. Support was provided by the NIHR Oxford Biomedical Research Centre, the Wellcome Trust (203141/Z/16/Z), the Society for the Relief of Disabled Children, Hong Kong (BHC) and Solve-RD (SB, AJ). The Solve-RD project received funding from the European Union’s Horizon 2020 research and innovation program (grant 779257).

CONFLICT OF INTEREST

No conflicting interests are declared.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.14071.

DATA AVAILABILITY STATEMENT

Researchers can apply to access 100KGP data at www.genomicsengland.co.uk/join-a-gecip-domain. Other data can be made available upon request.
REFERENCES


SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.


APPENDIX A


John C. Ambrose1; Prabhj Arumugam1; Roel Bevers1; Marta Bleda1; Freya Boardman-Pretty1,2; Christopher R. Boustred1; Helen Brittain1; Mark J. Caulfield1,2; Georgia C. Chan1; Greg Elgar1,2; Tom Fowler1; Adam Giess1; Angela Hamblin1; Shirley Henderson1,2; Tim J. P Hubbard1; Rob Jackson1; Louise J. Jones1,2; Dalia Kasperavicute1,2; Melis Kayikci1; Athanasios Kousathanas1; Lea Lahnstein1; Sarah E. A. Leigh1; Ionne U. S. Leong1; Javier F. Lopez1; Fiona Maleady-Crowe1; Meriel McEntagart1; Federico Minneci1; Loukas Moutsianas1,2; Michael Mueller1,2; Ninupa Murugaesu1; Anna C. Need1,2; Peter O’Donovan1; Chris A. Odhams1; Christine Patch1,2; Mariana Buongermino Pereira1; Daniel Perez-Gil1; John Pullinger1; Tahrima Rahim1; Augusto Rendon1; Tim Rogers1; Kevin Savage1; Kushmita Sawant1; Richard H. Scott1; Afshan Siddiq1; Alexander Sieghart1; Samuel C. Smith1; Alona Sosinsky1,2; Alexander Stuckey1; Melanie Tanguy1; Ana Lisa Taylor Tavares1; Ellen R. A. Thomas1,2; Simon R. Thompson1; Arianna Tucci1,2; Matthew J. Welland1; Eleanor Williams1; Katarzyna Witkowska1,2; Suzanne M. Wood1,2.

Acknowledgements for the GERC are listed in the supporting information.

1 Genomics England, London, UK
2William Harvey Research Institute, Queen Mary University of London, London, EC1M 6BQ, UK