Expanding the genotypic spectrum of TXNL4A variants in Burn-McKeown syndrome

Katherine A. Wood1,2 | Jamie M. Ellingford1,2 | Huw B. Thomas1 | Genomics UK Research Consortium | Sofia Douzgou1,2,3 | Glenda M. Beaman1,2 | Emma Hobson4 | Katrina Prescott4 | Raymond T. O’Keefe1 | William G. Newman1,2

1Division of Evolution, Infection and Genomics, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK
2Manchester Centre for Genomic Medicine, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK
3Department of Medical Genetics, Haukeland University Hospital, Bergen, Norway
4Yorkshire Regional Genetics Service, Chapel Allerton Hospital, Leeds Teaching Hospitals NHS Trust, Leeds, UK

Correspondence
William G. Newman, Manchester Centre for Genomic Medicine, Manchester University NHS Foundation Trust, Manchester M13 9WL, UK.
Email: william.newman@manchester.ac.uk

Present address
Katherine A. Wood, MRC Weatherall Institute of Molecular Medicine, The University of Oxford, Oxford, UK

Funding information
Biotechnology and Biological Sciences Research Council, Grant/Award Number: BB/N000358/1; Health Education England Genomics Education Programme; Medical Research Council, Grant/Award Number: 1916606; National Institute for Health Manchester Biomedical Research Centre, Grant/Award Number: IS-BRC-1215-20007

Abstract
The developmental disorder Burn-McKeown Syndrome (BMKS) is characterised by choanal atresia and specific craniofacial features. BMKS is caused by biallelic variants in the pre-messenger RNA splicing factor TXNL4A. Most patients have a loss-of-function variant in trans with a 34-base pair (bp) deletion (type 1 Δ34) in the promoter region. Here, we identified two patients with BMKS. One individual has a TXNL4A c.93_94delCC, p.His32Argfs *21 variant combined with a type 1 Δ34 promoter deletion. The other has an intronic TXNL4A splice site variant (c.258-3C>G) and a type 1 Δ34 promoter deletion. We show the c.258-3C>G variant and a previously reported c.258-2A>G variant, cause skipping of the final exon of TXNL4A in a minigene splicing assay. Furthermore, we identify putative transcription factor binding sites within the 56 bp of the TXNL4A promoter affected by the type 1 and type 2 Δ34 and use dual luciferase assays to identify a 22 bp repeated motif essential for TXNL4A expression within this promoter region. We propose that additional variants affecting critical transcription factor binding nucleotides within the 22 bp repeated motif could be relevant to BMKS aetiology. Finally, our data emphasises the need to analyse the non-coding sequence in individuals where a single likely pathogenic coding variant is identified in an autosomal recessive disorder consistent with the clinical presentation.

KEYWORDS
Burn-McKeown syndrome, choanal atresia, craniofacial abnormalities, DIM1, non-coding variant, promoter, RNA splicing, spliceosome, TXNL4A

1 | INTRODUCTION

Burn-McKeown Syndrome (BMKS, MIM 608572) is an autosomal recessive developmental craniofacial disorder with fewer than 20 families being described in the literature. Although there is clinical overlap with other craniofacial disorders including Treacher Collins syndrome, the recessive mode of inheritance and characteristic constellation of features differentiate BMKS from other craniofacial...
disorders. Affected individuals present with choanal atresia/stenosis, short palpebral fissures, lower eyelid coloboma, prominent nasal bridge, cleft lip and/or palate and large protruding ears.\(^1\)–\(^7\) Choanal atresia/stenosis has been reported in all affected individuals to date. Extra-craniofacial phenotypes of conductive and sensorineural hearing loss, congenital heart defects, inguinal hernias and short stature are observed in some patients. One BMKS individual has been reported with intellectual disability and developmental delay.\(^8\)

Wieczorek et al. identified biallelic variants in \textit{TXNL4A} as causative in BMKS.\(^4\) Most affected individuals carry a 34-base pair (bp) deletion (chr18: g.77748581_77,748614del [GRCh37, hg19]), known as the type 1 \(\Delta 34\)) in the \textit{TXNL4A} promoter of one allele combined with a loss-of-function variant on the other allele. Loss-of-function variants include microdeletions, splice site, nonsense and frameshift variants.\(^4,6\) Alternatively, some affected individuals are homozygous for a different 34 bp deletion, (chr18: g.77748604_77,748637 [GRCh37, hg19], known as the type 2 \(\Delta 34\)) in the \textit{TXNL4A} promoter.\(^4,6,7\) It is proposed that reduced \textit{TXNL4A} expression causes BMKS, with complete loss-of-function likely embryonically lethal.

\textit{TXNL4A}/DIM1 is a spliceosomal U5 small nuclear ribonucleoprotein particle (snRNP) component, responsible for all precursor mRNA (pre-mRNA) splicing.\(^9\)–\(^11\) It is postulated that decreased \textit{TXNL4A} expression reduces tri-snRNP assembly disrupting splicing of a specific subset of pre-mRNAs required for craniofacial development.\(^4,12,13\) Mis-splicing of pre-mRNAs relevant to craniofacial development would result in the tissue-specific and restricted phenotype of BMKS patients.

A difficulty hindering the diagnosis of BMKS is the identification of the 34 bp \textit{TXNL4A} promoter deletions from sequencing data. Promoter deletions may not be identified by whole-exome sequencing (WES), while bioinformatics pipelines for whole-genome sequencing (WGS) frequently do not cover non-coding sequences encompassing promoter and deep intronic regions.\(^14\) Here, we identify two unreported individuals with BMKS with novel \textit{TXNL4A} genotypes. We show that a novel \textit{TXNL4A} c.258-3C>G splice acceptor variant in one patient, as well as a previously reported c.258-2A>G variant affecting the adjacent nucleotide, cause skipping of the final exon of \textit{TXNL4A}. Furthermore, we identify potential transcription factor binding sites within the \textit{TXNL4A} type 1 and type 2 \(\Delta 34\) promoter deletions and use a dual luciferase assay to identify a 22 bp repeated motif which is crucial for \textit{TXNL4A} promoter activity. These findings expand the genetic spectrum of \textit{TXNL4A} variants underlying BMKS and identify why \textit{TXNL4A} \(\Delta 34\) promoter deletions influence \textit{TXNL4A} expression.

\section{MATERIALS AND METHODS}

See Data S1.

\section{RESULTS AND DISCUSSION}

\subsection{Identification of novel patients with BMKS from WGS data}

We sought to identify undiagnosed patients with BMKS using WGS data from the 100 000 (100 K) Genomes Project. Using available sequence variant data from the standard variant filtering pipeline, we identified heterozygous loss-of-function variants in \textit{TXNL4A} in two individuals with phenotypes consistent with BMKS. Sequence variant filtering identified only \textit{TXNL4A} mono-allelic coding variants meaning the potential diagnoses of BMKS had not been made. We then used manual bioinformatics analysis of WGS data to screen for \textit{TXNL4A} promoter deletions in affected individuals. Both patients were found

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Confirmation of biallelic variants in \textit{TXNL4A} from patients with BMKS identified from whole-genome sequencing data. (A) i. heterozygous type 1 34 bp deletion and ii. heterozygous \textit{TXNL4A} c.93_94delCC variants in family 1 proband. Red line indicates position of 2 bp deletion, after which a double sequencing trace indicates heterozygous frameshift. (B) i. heterozygous type 1 34 bp deletion and ii. heterozygous \textit{TXNL4A} c.258-3C>G variant in family 2 proband. Red box indicates single nucleotide variant, with double peak indicating variant heterozygosity [Colour figure can be viewed at wileyonlinelibrary.com]}
\end{figure}
to have a heterozygous type 1 Δ34 promoter deletion, which was confirmed by Sanger sequencing (Figure 1).

3.2 | Proband phenotyping

The family 1 proband is a white British female only child born to unrelated parents who presented in the genetic clinic in adulthood with mixed conductive sensorineural hearing loss and jaw ankylosis (Figure 2A). She had previous treatment for bilateral choanal atresia and displayed dysmorphic craniofacial features including lower eyelid coloboma, malar flattening, a high palate and micrognathia, right-sided microtia and protruding ears (Figure 2B, Table S1). Sequencing revealed a heterozygous chr18:77748298TGG>T (GRCh37), TXNL4A c.93_94delCC (NM_006701), p.His32Argfs*21 (NP_006692) variant with a heterozygous type 1 Δ34 promoter deletion (Table S1). The frameshift variant is not present in gnomAD and has not been previously associated with BMKS. Parental genotyping revealed that the type 1 Δ34 promoter was maternally inherited, while the c.93_94delCC was paternally inherited (Figure 2A, Table S1). The mother is clinically unaffected. The father died at 44 years of oesophageal carcinoma. He possibly had choanal atresia as, at 11–12 years, he had an operation to drill one side out of his nose as his nasal passages had not fully developed. He also possibly had a flat malar region (Table S1). It is possible that the father may be mildly clinically affected based on his reported phenotype. Sanger sequencing of the whole TXNL4A coding and promoter sequence for the father did not reveal any additional variants which could account for his craniofacial features. As he was deceased, the father was not recruited to the 100 K Genomes Project. Therefore, WGS was not available. It is unlikely that the oesophageal carcinoma is related to his TXNL4A genotype as this association has not been described in other carriers of TXNL4A variants. While somatic mutations in some core spliceosome components have been associated with cancer, there are no reports to date of TXNL4A mutations in tumours.15,16

The family 2 proband is a white British male and only child of healthy, non-related, parents with phenotypic features including choanal atresia, conductive hearing impairment, a cleft upper lip and distinctive craniofacial features including downslanted palpebral fissures, malar flattening and dysplastic ears (Figure 2A). The left ear was atretic with closure of the external auditory ear canal (microtia) (Figure 2C, Table S1). Sequencing revealed a heterozygous chr18:77733859G>C (GRCh37), TXNL4A c.258-3C>G (NM_006701) splice acceptor variant and a heterozygous type 1 Δ34 promoter deletion (Table S1). The splice acceptor variant is not observed in the gnomAD population database and has not been previously described in a BMKS patient. However, a variant in the adjacent nucleotide, TXNL4A c.258-2A>G (NM_006701) has been described in an individual with BMKS.4 In silico prediction of variant pathogenicity suggested both splice site variants are disease-causing by disrupting the splice acceptor site (Table S1). We conducted minigene splicing assays for the c.258-2A>G and c.258-3C>G variants; both led to complete skipping of the TXNL4A final exon (Data S1; Figure S1). Deletion of TXNL4A exon 3 in trans to a type 1 Δ34 has been reported in another BMKS patient.4 The heterozygous c.258-3C>G splice acceptor variant was maternally inherited while the heterozygous type 1 Δ34 was paternally inherited (Figure 2A, Table S1). Comparison of the clinical features observed in patients here and previously reported patients is provided in Table S1.

3.3 | Identifying putative transcription factor binding sites within the human TXNL4A promoter type 1 Δ34

Wieczorek et al. found that TXNL4A type 1 and type 2 Δ34 deletions reduced promoter activity by 59% and 72%, respectively.4 This promoter region consists of two repeated 22 bp motifs separated by a 12 bp spacer, with each Δ34 deletion containing one of the 22 bp repeated motifs with the spacer region overlapping the type 1 and type 2 Δ34 (Figure 3A). These 34 bp regions were proposed to contain binding sites for transcription factors which promote TXNL4A expression, the loss of which cause decreased promoter activity in patients and carriers of the deletions.4 We predicted potential binding sites for four transcription factors (XBP-1, c-JUN, AhR/ARNT and ATF3) in the type 1 Δ34 (Figure 3A). All but three nucleotides in these binding sites were within the
repeated 22 bp motif rather than the 12 bp spacer, meaning of the four predicted transcription factors, only XBP-1 is not predicted to also bind to the type 2 Δ34. Interestingly, only twelve heterozygous and one homozygous variant in the 56 bp region of the TXNL4A promoter were found in the gnomAD database, indicating an important and sequence-specific role in promoter activity (Data S1; Table S2).

3.4 In vitro analysis of putative transcription factor binding sites on promoter function

To test whether the identified putative transcription factor binding sites are important in TXNL4A promoter function, we cloned a 601 bp TXNL4A promoter fragment into a luciferase reporter vector and performed dual luciferase assays. Constructs contained the wild type promoter region, the type 1 Δ34 or several smaller deletions (Figure 3A). Similar to Wieczorek et al., we found type 1 Δ34 reduced promoter activity to 46% (Figure 3B). Smaller deletions (deletions 1 and 2) reduced promoter activity to 59% and 63%, respectively, while deletion 3 (12 bp spacer) only reduced promoter activity by 7% (Figure 3B). Deletion 4 (spanning deletions 1 and 2) reduced activity to 47% (Figure 3B). Scrambling deletion 4 reduced activity to 70%, suggesting sequence specificity of this region (Figure 3B). We then deleted the 22 bp repeated motif within the type 1 Δ34 (repeated region 2, RR2) or type 2 Δ34 (repeated region 2, RR2) (Figure 3A). RR1 reduced promoter activity to 54%, while RR2 reduced activity to 45%, the same as the full type 1 Δ34 (Figure 3B). Deleting or scrambling both RR1 and RR2 together reduced promoter activity to 10% (Figure 3B). These findings suggest

FIGURE 3 Analysis of the human TXNL4A promoter. (A) Structure of the TXNL4A promoter region affected by type 1 (orange) and type 2 (red) 34 bp deletions in BMKS patients; 12 bp spacer region (yellow) and 22 bp repeated regions (pink). Putative transcription factor binding sites identified using ALGGEN PROMO indicated in grey. Hypothetical deletions 1–4 in luciferase reporter gene constructs are highlighted in purple. B) Effects of TXNL4A promoter deletions on luciferase expression. Relative firefly luciferase expression for each construct, normalised to renilla luciferase expression, is indicated as a percentage of the wild type promoter region expression. n = 4. **p-value <0.01, ****p-value <0.0001 [Colour figure can be viewed at wileyonlinelibrary.com]
that RR1 and RR2 contain the critical nucleotides for TXNL4A promoter activity and act independently and cumulatively to promote TXNL4A expression.

This study has reiterated the power of WGS in diagnosing patients with rare disorders and emphasises the need to consider non-coding regions when analysing WGS data, especially when a single pathogenic coding variant is identified in a disease-associated gene known to cause a recessive condition consistent with the clinical presentation. We have also developed an analysis approach for screening existing and novel promoter variants in a gene of interest. This approach may prove useful for disorders associated with promoter variants where few patients have been identified and where it is unclear whether a single pathogenic variant or spectrum of different promoter variants underlie the phenotype.

ACKNOWLEDGEMENTS
The authors thank the participants for their involvement in this study and Hannah Musgrave and Jacqueline Hodgkinson for coordinating the study.

We acknowledge support from: Medical Research Council 1916606 (KAW); Biotechnology and Biological Sciences Research Council (BB/N000358/1 (RTO, WGN, HBT)). WGN is supported by the National Institute for Health Manchester Biomedical Research Centre funding IS-BRC:1215-2007. JME is supported by the Health Education England Genomics Education Programme.

This research was made possible through access to the data and findings generated by the 100,000 Genomes Project. The 100,000 Genomes Project is managed by Genomics England Limited (a wholly owned company of the Department of Health and Social Care). The 100,000 Genomes Project is funded by the National Institute for Health Research and NHS England. The Welcome Trust, Cancer Research UK and the Medical Research Council have also funded research infrastructure. The 100,000 Genomes Project uses data provided by patients and collected by the National Health Service as part of their care and support.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1111/cge.14082.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES
1. Burns J, McKeown C, Wagget J, Bray R, Goodship J. New dysmorphic syndrome with choanal atresia in siblings. Clin Dysmorphol. 1992;1(3):137-144. doi:10.1097/00019605-199207000-00003
2. Toriello HV, Higgins JV. A boy with choanal atresia and cardiac defect. Clin Dysmorphol. 1999;5(2):143-146. doi:10.1097/00019605-199904000-00011
3. Wieczorek D, Deber OA, Lohmann D, Gillessen-Kaebbach G. Two brothers with Burn-McKeown syndrome. Clin Dysmorphol. 2003;12(3):171-174. doi:10.1097/01.mcd.0000072163.33788.c4
4. Wieczorek D, Newman WG, Wieland T, et al. Compound heterozygosity of low-frequency promoter deletions and rare loss-of-function mutations in TXNL4A causes Burn-McKeown syndrome. Am J Hum Genet. 2014;95(6):698-707. doi:10.1016/j.ajhg.2014.10.014
5. Lehalle D, Wieczorek D, Zechi-Ceide RM, et al. A review of craniofacial disorders caused by spliceosomals defects. Clin Genet. 2015;88(5):405-415. doi:10.1111/cge.12596
6. Goos JAC, Swagemakers SMA, Twigg SRF, et al. Identification of causative variants in TXNL4A in Burn-McKeown syndrome and isolated choanal atresia. Eur J Hum Genet. 2017;25(10):1126-1133. doi:10.1038/ejhg.2017.107
7. Narayanan DL, Purushothama G, Bhavani GS, Shukla A. Burn-McKeown syndrome with biallelic promoter type 2 deletion in TXNL4A in two siblings. Am J Med Genet A. 2020;182(6):1313-1315. doi:10.1002/ajmg.a.61554
8. Strang-Karlsson S, Urquhart J, Newman WG, Douzou S. Severe intellectual disability in a patient with Burn-McKeown syndrome. Clin Dysmorphol. 2017;26(3):193-194. doi:10.1097/MCD.0000000000000175
9. Liu S, Rauhut R, Vornlocher H-P, Lührmann R. The network of protein–protein interactions within the human U4/U6.U5 tri-snRNP. RNA. 2006;12(7):1418-1430. doi:10.1261/ma.55406
10. Nguyen THD, Galej WP, Bai X-C, et al. Cryo-EM structure of the yeast U4/U6.U5 tri-snRNP at 3.7 Å resolution. Nature. 2016;530(7590):298-302. doi:10.1038/nature16940
11. Will CL, Lührmann R. Spliceosome structure and function. Cold Spring Harb Perspect Biol. 2011;3(7):a003707. doi:10.1101/cshperspect.a003707
12. Beauchamp M-C, Alam SS, Kumar S, Jerome-Majewska LA. Spliceosomopathies and neurocristopathies: two sides of the same coin? Dev Dyn. 2020;249(8):924-945. doi:10.1002/dvdy.183
13. Wood KA, Rowlands CF, Thomas HB, et al. Modelling the developmental spliceosomal craniofacial disorder Burn-McKeown syndrome using induced pluripotent stem cells. PLoS ONE. 2020;15(7):e0233582. doi:10.1371/journal.pone.0233582
14. Martin AR, Williams E, Foulger RE, et al. PanelApp crowdsources expert knowledge to establish consensus diagnostic gene panels. Nat Genet. 2019;51(11):1560-1565. doi:10.1038/s41588-019-0528-2
15. Knorr LE-HK, Abdel-Wahab O. Aberrant RNA splicing in cancer. Published online 2019:21.
16. Wood KA, Eadsforth MA, Newman WG, O’Keefe RT. The role of the US snRNP in genetic disorders and cancer. Front Genet. 2021;12:20. doi:10.3389/fgene.2021.636620

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

How to cite this article: Wood KA, Ellingford JM, Thomas HB, et al. Expanding the genotypic spectrum of TXNL4A variants in Burn-McKeown syndrome. Clinical Genetics. 2022;101(2):255-259. doi:10.1111/cge.14082

ORCID
Sofia Douzou https://orcid.org/0000-0001-8890-7544
Raymond T. O’Keefe https://orcid.org/0000-0001-8764-1289
William G. Newman https://orcid.org/0000-0002-6382-4678