Update of genetic variants in CEP120 and CC2D2A—With an emphasis on genotype-phenotype correlations, tissue specific transcripts and exploring mutation specific exon skipping therapies

Miguel Barroso-Gil1 | Eric Olinger1 | Simon A. Ramsbottom1 | Elisa Molinari1 | Colin G. Miles1 | John A. Sayer1,2,3

1Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle Upon Tyne, UK
2Renal Services, The Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle Upon Tyne, UK
3NIHR Newcastle Biomedical Research Centre, Newcastle University, Newcastle Upon Tyne, UK

Correspondence
John A. Sayer, Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Central Parkway, Newcastle Upon Tyne, NE1 3BZ, UK.
Email: john.sayer@ncl.ac.uk

Funding information
Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: P2ZHP3_195181; Kidney Research UK, Grant/Award Number: Paed_ RP_001_20180925, RP_006_20180227 and ST_001_20171120; Northern Counties Kidney Research Fund, Grant/Award Number: 01/19

Abstract

Background: Mutations in ciliary genes cause a spectrum of both overlapping and distinct clinical syndromes (ciliopathies). CEP120 and CC2D2A are paradigmatic examples for this genetic heterogeneity and pleiotropy as mutations in both cause Joubert syndrome but are also associated with skeletal ciliopathies and Meckel syndrome, respectively. The molecular basis for this phenotypical variability is not understood but basal exon skipping likely contributes to tolerance for deleterious mutations via tissue-specific preservation of the amount of expressed functional protein.

Methods: We systematically reviewed and annotated genetic variants and clinical presentations reported in CEP120- and CC2D2A-associated disease and we combined in silico and ex vivo approaches to study tissue-specific transcripts and identify molecular targets for exon skipping.

Results: We confirmed more severe clinical presentations associated with truncating CC2D2A mutations. We identified and confirmed basal exon skipping in the kidney, with possible relevance for organ-specific disease manifestations. Finally, we proposed a multimodal approach to classify exons amenable to exon skipping. By mapping reported variants, 14 truncating mutations in 7 CC2D2A exons were identified as potentially rescuable by targeted exon skipping, an approach that is already in clinical use for other inherited human diseases.

Conclusion: Genotype-phenotype correlations for CC2D2A support the deleteriousness of null alleles and CC2D2A, but not CEP120, offers potential for therapeutic exon skipping approaches.

KEYWORDS
antisense oligonucleotide, CC2D2A, CEP120, ciliopathy, exon skipping, Joubert syndrome, Meckel syndrome, precision medicine

Miguel Barroso-Gil and Eric Olinger contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. Molecular Genetics & Genomic Medicine published by Wiley Periodicals LLC
1 INTRODUCTION

Human disorders arising from the dysfunction of motile and/or primary cilia are collectively referred to as ciliopathies and there are more than a dozen distinguishable ciliopathy syndromes. The spectrum of disease arising from defects in the primary cilium (primary ciliopathies) includes neurological diseases (e.g., Joubert syndrome (JBTS, MIM PS213300), Meckel syndrome (MKS, MIM PS249000)) and skeletal ciliopathies (e.g., Jeune asphyxiating thoracic dysplasia (JATD), MIM PS208500), which are often accompanied by multisystem involvement including the kidney (e.g., nephronophthisis (NPHP), MIM PS256100) and retina. Other ciliopathies affect primarily kidneys and liver such as autosomal dominant and recessive polycystic kidney disease (ADPKD & ARPKD, MIM PS173900) or lead to isolated retinal disease such as Leber congenital amaurosis (LCA, MIM PS120970) (Novarino et al., 2011; Reiter & Leroux, 2017; Shaheen et al., 2016). Collectively, ciliopathies affect approximately 1 in every 2000 individuals with ADPKD being by far the most common (Kagan et al., 2017). Besides the high level of phenotypic complexity and overlap of clinical phenotypes, mutations within the same gene can give rise to distinct ciliopathy syndromes, known as genetic pleiotropy (Coppieters et al., 2010; Roosing et al., 2016; Shaheen et al., 2016; Shamseldin et al., 2020). In addition, mutations in different genes can cause the same ciliopathy syndrome (genetic heterogeneity) such as it is the case of JBTS, with more than 40 genes associated (Figure S1) (Bachmann-Gagescu et al., 2015, 2020; Braun & Hildebrandt, 2017; Mitchison & Valente, 2017; Parisi, 2019). In addition, we have recently shown that differences in phenotypic presentation in patients with the same mutations are in part due to the presence of genetic modifiers (Ramsbottom et al., 2020). JBTS is characterized by a cerebellar and brainstem malformation, known as the “molar tooth sign” (MTS) (Parisi, 2019; Radha Rama Devi et al., 2020; Romani et al., 2013). MKS is a lethal multiorgans ciliopathy, generally associated with more pronounced central nervous system (CNS) malformations, such as occipital encephalocele, and severe extra-CNS manifestations including cystic renal dysplasia and hepatic abnormalities including ductal plate malformation and hepatic fibrosis (Alexiev et al., 2006; Hartill et al., 2017). As an example for genetic pleiotropy and heterogeneity, variants in both CEP120 (MIM 613446) and CC2D2A (MIM 612013) have been reported to cause JBTS, with CEP120 being associated with skeletal ciliopathies and CC2D2A giving rise to the whole spectrum of neurological disorders (Bachmann-Gacescu et al., 2012; Mougu-Zerelli et al., 2009; Roosing et al., 2016; Shaheen et al., 2015).

Centrosomal protein of 120 kDa (encoded by CEP120) is expressed ubiquitously in embryonic mice tissues with a subcellular expression enriched in the daughter centriole (Mahjoub et al., 2010; Xie et al., 2007). Several studies have investigated the role of CEP120 in centriole biogenesis and ciliogenesis and revealed its requirement for centriole duplication, assembly, elongation and maturation (Tables S1 and S2) (Comartin et al., 2013; Mahjoub et al., 2010). Originally, biallelic genetic variants in CEP120 were detected in four families with JATD but genetic variants have also been linked to JBTS (Roosing et al., 2016; Shaheen et al., 2015). Coiled-coil and C2 domain containing 2A, encoded by CC2D2A, is expressed in multiple human adult tissues, particularly in brain, prostate, pancreas, kidney, lung, liver and retina (Noor et al., 2008). CC2D2A localizes and functions at the transition zone (TZ) (Gorden et al., 2008) where it has a role in cilia assembly and interacts with MKS-JBTS associated proteins (Tables S1 and S2) (Bachmann-Gagetscu et al., 2015; Garcia-Gonzalo et al., 2011; Lewis et al., 2019; Ojeda Naharros et al., 2017; Tallila et al., 2008; Veleri et al., 2014). Mutations in CC2D2A cause a spectrum of clinical phenotypes, ranging from isolated rod-cone dystrophy (RCD) (Mejecase et al., 2019) to JBTS (Bachmann-Gagetscu et al., 2012; Gorden et al., 2008; Noor et al., 2008) and MKS (Mougou-Zerelli et al., 2009; Szymanska et al., 2012; Tallila et al., 2008, 2009). How mutations in these two genes, encoding proteins with different ciliary localization and function, lead to this wide spectrum of distinct clinical presentations with partially overlapping phenotypes is not fully understood.

In 2015, Drivas et al. suggested that basal levels of alternative splicing (AS) with exon skipping may be responsible for some of the genetic pleiotropy observed in CEP290- and CC2D2A-associated disease (Drivas et al., 2015). AS is a mechanism by which a precursor messenger RNA (pre-mRNA) is processed into multiple isoforms (Nilsen & Graveley, 2010; Tabrez et al., 2017) and is thought to occur in around 95% of multieaxon genes (Pan et al., 2008). Basal levels of noncanonical splicing has indeed been shown to occur in patient dermal fibroblasts with CEP290 (MIM 610142) mutations but also in control samples. Deleterious mutations in CEP290 and CC2D2A falling into exons that preserve the reading frame (exons whose length is an exact multiple of 3) were associated with a higher level of residual near-full length protein, as they escape nonsense-mediated mRNA decay (NMD), and correspond with a milder clinical phenotype (Drivas et al., 2015). Nonsense-associated altered splicing (NAS), an endogenous mechanism increasing the level of alternatively spliced transcripts in response to truncating variants, might contribute to this rescue, although no evidence for a selective mechanism was found in this study (Drivas et al., 2015). It is currently unclear whether tissue-specific exon splicing events could underlie differential organ involvement in ciliopathies.

The potential of therapies exploiting this natural mechanism and based on the specific removal of dispensable exons by exon-skipping antisense oligonucleotides (ASOs) has
We screened PubMed® for reports of patients harbouring specific exons within the disease-causing gene, DMD (MIM 300377), leads to exon skipping, and a subsequent restoration of reading frame and a partially functional dystrophin protein (Aartsma-Rus & van Ommen, 2007; Kole & Krieg, 2015; Komaki et al., 2018; Lee et al., 2018; Servais et al., 2015). The same strategy has recently been applied to nonsense mutations within CEP290 (Barny et al., 2019; Garanto et al., 2016; Molinari et al., 2019; Ramsbottom et al., 2018) building on the fundamental finding that exon skipping in CEP290 is tolerated and leads to functional transcripts (Drivas et al., 2015). ASO-mediated exon skipping rescued the ciliary phenotype and CEP290 protein levels in a humanized murine model of LCA (Garanto et al., 2016) and intravitreal injections of ASOs improved visual acuity in LCA patients (Cideciyan et al., 2019). Systemic administration of ASOs via intravenous injections was shown to induce skipping of a gene trap in a JBTS mouse model restoring CEP290 protein levels and rescuing renal ciliary phenotype and the cystic burden in the kidneys (Ramsbottom et al., 2018). As a proof of principle, ex-vivo ASO-mediated skipping restored the ciliary phenotype in human urine-derived renal epithelial cells (hURECs) and fibroblasts derived from a JBTS patient carrying a CEP290 homozygous truncating mutation (Molinari et al., 2019; Ramsbottom et al., 2018).

In this study, we systematically review and curate genetic variants and phenotypes associated with CEP120 and CC2D2A, two genes paradigmatic for the concepts of genetic heterogeneity and pleiotropy, and investigate genotype-phenotype correlations. Extending the concept proposed by Drivas et al. (Drivas et al., 2015; Molinari et al., 2019; Rozet & Gerard, 2015), we detect and validate tissue-specific splicing events and, using these two genes, propose a multimodal approach to identify target exons for future exon skipping therapy approaches.

2 | RESULTS

2.1 | Allelic and clinical spectra of ciliopathies caused by biallelic variants in CEP120 and CC2D2A

We screened PubMed® for reports of patients harbouring biallelic genetic variants in CEP120 or CC2D2A, accessed the Human Gene Mutation Database (HGMD®) for additional entries and manually curated the genetic and phenotypic data available in order to create a comprehensive database for CEP120- and CC2D2A-associated disease compliant with Human Genome Variation Society (HGVS) recommendations.

To date, only nine index patients harbouring homozygous or compound heterozygous genetic variants in CEP120 have been reported. 4/9 presented with IBTS, 3/9 with Jeune asphyxiating thoracic dystrophy (JATD), 1/9 with a MKS/oro-facial-digital syndrome (OFD) overlapping phenotype and 1/9 with tectocerebellar dysraphia with occipital encephalocoele (TCDOE) (Table 1 and Figure S2a). In these patients, 14 missense alleles, 3 frameshift alleles and 1 nonsense allele are reported, in different combinations (Tables 1, S4 and Figure S2b). Variant p.(Ala199Pro) alone is found in homozygosis in 3 index patients and in compound heterozygote state in another patient (representing 7/14 missense alleles) (Roosing et al., 2016; Shaheen et al., 2015). The CEP120 variant p.(Leu712Phe) is reported in compound heterozygote state in one patient (Roosing et al., 2016) (MTI-143, Table 1) but population data indicate an allelic frequency of ~0.004 with 2 homozygous individuals in the normal population (https://gnomad.broadinstitute.org/) (Table S4). Although in vitro experiments indicated that this variant impairs the recruitment of Talpid3 to the centrioles, its pathogenicity is questionable (Tsai et al., 2019).

111 patients from 97 families suffering from CC2D2A-related disease have been reported to date (Tables 2 and S3). Roughly half of the patients suffered from IBTS (59/111), with slightly less than half displaying an MKS (40/111) or Meckel syndrome-like (ML) presentation (3/111). Rare cases were described with RCD (4/111), Cogan-type congenital oculomotor apraxia (1/111) or autism-spectrum disease (1/111). In three reported cases, the phenotype was not unequivocally described (Figure 1a and Table S3). From the total pool of 195 pathogenic alleles reported in all 97 families, 90 alleles corresponded to missense changes, 60 were frameshift variants, 20 were splice-affecting variants, 18 were nonsense alleles, 3 were single amino acid deletions and 4 were large insertions/deletions, including one reported case of retrotransposon insertion (Figure 1b and Table S3). Several alleles are shared between families with their overall allelic counts in family index patients between 1 and 22 (c.1762C>T) (Table S5). Altogether 84 different genetic variants have been identified in the 97 reported families (Table S5). Of note, Srour et al. reported a family with three affected individuals, one of them compound heterozygote for p.(Glu1126Lys) and p.(Asp1556Val) while the other two individuals were compound heterozygote for p.(Glu1126Lys) and p.(Asn1520Ser) (i.e., two different compound heterozygote combinations) (Srour et al., 2012). CC2D2A variants p.(Glu229del) and p.(Pro721Ser) have each been detected in one patient in compound heterozygosis or homozygosis, respectively (Table S3) (Mougou-Zerelli et al., 2009; Otto et al., 2011). The allelic frequencies in gnomAD are 0.062 for p.(Glu1126Lys) (including 3 homozygous individuals) and 0.002 for p.(Pro721Ser) (including three homozygous carriers) (Table S5), suggesting that these are hypomorphic alleles.
rather than fully pathogenic variants (Bachmann-Gagescu et al., 2012).

### 2.2 Genotype-phenotype correlations in disease caused by mutations in CC2D2A

Having systematically assessed the variants in CC2D2A and the associated phenotypes for all 111 reported patients, we wondered whether truncating variants (nonsense or frameshift) were associated with a more severe phenotype than missense variants, as suggested before (Mougou-Zerelli et al., 2009). Considering the patients presenting any combination of truncating (nonsense or frameshift) and/or missense variants, biallelic truncating variants were found in 66% (21/32) of cases presenting with MKS or ML, contrasting with only 2% (1/45) of cases presenting with JBTS (Fisher’s exact test: \( p < .0001 \)). Conversely, biallelic missense variants were detected in only 22% (7/32) of MKS/ML cases versus 58% (26/45) of cases with JBTS (Fisher’s exact test: \( p = .0023 \)) (Figures 1c,d). This systematic analysis of all reported cases to date shows a robust correlation between the type of CC2D2A mutation and the severity of the disease. In addition, we assessed systematically the cases reported in literature with specific mention of either the presence or absence of kidney disease and we show that biallelic truncating variants were more frequently found in presence of kidney disease (50%, 20/40) than in cases without kidney involvement (3%, 1/34) (Fisher’s exact test: \( p < .0001 \)), in line with the notion that missense changes are more frequently associated with a pure JBTS presentation without extra-CNS manifestations (Figure 1e,f). Of note, we found an overlap of biallelic variants that were present in JBTS and MKS/ML as well as shared between patients with kidney disease and without kidney disease (e.g., p.(Pro1122Ser)) suggesting the presence of additional modifying factors. Similar associations were not seen for CEP120, but a meaningful analysis was precluded by the low patient numbers (Figure S2c,d).

### 2.3 In silico analysis of gene expression and tissue-specific basal exon skipping

Based on these results suggesting that truncating variants are associated with a more severe clinical picture, we were interested to assess the applicability of exon skipping therapies to rescue truncating variants (Ramsbottom et al., 2018). Exon skipping events occurring in basal conditions are informative...
| Family ID (1) | Patient ID | Phenotype | Kidney phenotype (2) | Allele 1 (Ex,Int) | Allele 2 (Ex,Int) | Reference |
|--------------|------------|-----------|----------------------|-------------------|-------------------|-----------|
| 15           | UW41-IV:1  | JBTS      | no                   | c.2848C>T; p.(Arg950Ter) (Ex23) | c.2848C>T; p.(Arg950Ter) (Ex23) | Gorden et al. (2008) |
| 16           | UW47-II:1  | JBTS      | no                   | c.3055C>T; p.(Arg1019Ter) (Ex25) | c.3288G>C; p.(Gln1096His) (Ex26) | Gorden et al. (2008) |
| 20           | UMI10      | MKS       | n/a                  | c.3084del; p.(Lys1029ArgfsTer3) (Ex25) | c.4179+1del (Int33) | Tallila et al. (2009) |
| 26           | MKS-54     | MKS       | yes                  | c.517C>T; p.(Arg173Ter) (Ex8) | c.517C>T; p.(Arg173Ter) (Ex8) | Mougu-Zerelli et al. (2009) |
| 29           | MKS-977    | MKS       | yes                  | c.3084del; p.(Lys1029ArgfsTer3) (Ex25) | c.3084del; p.(Lys1029ArgfsTer3) (Ex25) | Mougu-Zerelli et al. (2009) |
| 33           | MKS-365    | MKS       | yes                  | c.2773C>T; p.(Arg925Ter) (Ex22) (3) | c.2486+1G>C (Int20) | Mougu-Zerelli et al. (2009) |
| 34           | UW67       | JBTS      | yes                  | c.3347C>T; p.(Thr1116Met) (Ex27) | c.3145C>T; p.(Arg1049Ter) (Ex25) | Doherty et al. (2010) |
| 35           | F434-21    | JBTS      | no                   | c.517C>T; p.(Arg173Ter) (Ex8) | c.1676T>C; p.(Leu559Pro) (Ex16) | Otto et al. (2011) |
| 36           | A2421-21   | MKS       | yes                  | c.3544T>C; p.(Trp1182Arg) (Ex29) | c.3774dup; p.(Glu1259Ter) (Ex31) | Otto et al. (2011) |
| 38           | M506       | MKS       | n/a                  | c.3084del; p.(Lys1029ArgfsTer3) (Ex25) | c.3084del; p.(Lys1029ArgfsTer3) (Ex25) | Hopp et al. (2011) |
| 40           | UW75-3     | JBTS      | no                   | c.1676T>C; p.(Leu559Pro) (Ex16) | c.3892_3893del; p.(Val1298PhefsTer17) (Ex31) | Bachmann-Gagescu et al. (2012) |
| 42           | UW78-3     | JBTS      | n/a                  | c.3055C>T; p.(Arg1019Ter) (Ex25) | c.4667A>T; p.(Asp1556Val) (Ex37) | Bachmann-Gagescu et al. (2012) |
| 43           | UW79-3     | JBTS      | no                   | c.1263_1264insGGCATGTGTGGGC; p.(Ser422GlyfsTer19) (Ex13) (4) | c.3452T>C; p.(Val1151Ala) (Ex28) | Bachmann-Gagescu et al. (2012) |
| 43           | UW79-4     | JBTS      | no                   | c.1263_1264insGGCATGTGTGGGC; p.(Ser422GlyfsTer19) (Ex13) (4) | c.3452T>C; p.(Val1151Ala) (Ex28) | Bachmann-Gagescu et al. (2012) |
| 51           | 128        | MKS       | n/a                  | c.3544T>C; p.(Trp1182Arg) (Ex29) | c.3774dup; p.(Glu1259Ter) (Ex31) | Szymanska et al. (2012) |
| 61           | MKS        | yes       | c.3774dup; p.(Glu1259Ter) (Ex31) | c.4550C>G; p.(Thr1517Ser) (Ex37) | Jones et al. (2014) |
| 62           | MTI-127    | JBTS (5)  | n/a                  | c.4583G>A; p.(Arg1528His) (Ex37) (6) | c.3082del; p.(Arg1028GlyfsTer4) (Ex25) (7) | Ben-Salem et al. (2014) |
### TABLE 2 (Continued)

| Family ID (1) | Patient ID | Phenotype | Kidney phenotype (2) | Allele 1 (Ex,Int) | Allele 2 (Ex,Int) | Reference |
|---------------|------------|-----------|----------------------|-------------------|-------------------|-----------|
| 72            | 3          | JBTS/MKS (8) | n/a                  | c.2803C>T; p.(Arg935Ter) (Ex22) | c.3774dup; p.(Glu1259Ter) (Ex31) | Watson et al. (2016) |
| 73            | 4          | JBTS/MKS (8) | n/a                  | c.2875del; p.(Glu959AsnfsTer3) (Ex23) | c.2875del; p.(Glu959AsnfsTer3) (Ex23) | Watson et al. (2016) |
| 74            | FT-1       | MKS        | yes                  | c.3084del; p.(Lys1029ArgfsTer3) (Ex25) | c.3084del; p.(Lys1029ArgfsTer3) (Ex25) | Al-Hamed et al. (2016) |
| 78            | FT-15      | MKS        | yes                  | c.3084del; p.(Lys1029ArgfsTer3) (Ex25) | c.3084del; p.(Lys1029ArgfsTer3) (Ex25) | Al-Hamed et al. (2016) |
| 79            | FT-21      | MKS        | yes                  | c.3084del; p.(Lys1029ArgfsTer3) (Ex25) | c.3084del; p.(Lys1029ArgfsTer3) (Ex25) | Al-Hamed et al. (2016) |
| 81            | F850-21    | Cogan      | yes                  | c.1267C>T; p.(Arg423Ter) (Ex13) | c.4667A>T; p.(Asp1556Val) (Ex37) | Schueler et al. (2016) |
| 84            |            | JBTS       | yes                  | c.2581G>A; p.(Asp861Asn) (Ex21) | c.2848C>T; p.(Arg950Ter) (Ex23) | Xiao et al. (2017) |
| 89            | 44:36      | JBTS       | no                   | c.3744_3747dup; p.(Pro1250GlyfsTer11) (Ex30) (9) | c.3989G>A; p.(Arg1330Gln) (Ex32) | Vilboux et al. (2017) |
| 89            | 45:36      | JBTS       | no                   | c.3744_3747dup; p.(Pro1250GlyfsTer11) (Ex30) (9) | c.3989G>A; p.(Arg1330Gln) (Ex32) | Vilboux et al. (2017) |

**Note:** CC2D2A transcript: NM_001080522.2. Cogan, Cogan-type congenital oculomotor apraxia; JBTS, Joubert syndrome; MKS, Meckel syndrome; ML, Meckel-like syndrome. (1) Relates to family ID of complete database in Table S3. (2) Designated as n/a, unless renal phenotype clearly stated. (3) Variant initially reported as c.2673C>T; p.(Arg925Ter). We assumed c.2773C>T is the correct nucleotide change given that it is predicted to give rise to the reported protein change. (4) Variant initially reported as c.1263_4InsGCGATGTGGGC; c.1268G>A; p.(Ser423Glyfs*19). (5) Study does not state potential extra-CNS manifestations. (6) Variant initially reported as c.2580G>A; p.(Arg861Asn). This variant was corrected as c.2581G>A; p.(Asp861Asn) (Lam et al., 2020). (7) Variant initially reported as c.1412delG; p.(Lys472Argfs*). This variant was corrected as c.3082del; p.(Arg1028Glyfs*4) (Ben-Salem et al., 2015). (8) In this study (Watson et al., 2016), patients were referred with a clinical diagnosis of either JBTS (9 patients) or MKS (17 patients). The genetic diagnosis was confirmed in 14 of the 26 cases, a diagnostic yield of 54%. The exact phenotype is not reported. (9) Variant initially reported as c.3743_3746dup; p.(Pro1250Glyfs*11).
as they indicate that skipping of these particular exons is likely well-tolerated.

CEP120 and CC2D2A are ubiquitously expressed in human tissues, with highest expression levels in the female reproductive system and cerebellum for the former and smooth muscle and female reproductive system for the latter (Figure S3). Expression of both genes has also been reported in the human kidney (Figure S3 and http://www.proteinatlas.org, Uhlén et al., 2015). Cerebellum and kidney phenotypes are classically encountered in primary ciliopathies (Badano et al., 2006; Braun & Hildebrandt, 2017). Using human RNA sequencing data available through the Genotype-Tissue Expression (GTEx) project (https://www.gtexportal.org/home/), we investigated the tissue-specific expression and splicing of CEP120 and CC2D2A in kidney medulla and the cerebellar hemisphere. Abundant expression of ENST00000306481.10 (“transcript 1”) is the main CEP120 transcript detected in the kidney medulla and the cerebellar hemisphere. Abundant expression of ENST00000328236.9 (“transcript 2”) and ENST00000306467.9 (“transcript 3”) were also detected in the cerebellum but nearly absent in the kidney (Figure 2a(i)). These transcript isoforms are generated through alternative splicing events at the pre-mRNA 5′-end, with exon 2 (ref.: ENST00000328236.9 or NM_153223.3) predicted to be skipped in the kidney (Figure 2a(ii)). These changes are reflected by a predicted protein product lacking the first 26 amino acids for transcript 1 (Figure 2a(iii)).

For CC2D2A, the main protein coding transcripts in kidney medulla are ENST00000515124.5 (“transcript 1”) and ENST00000503292.5 (“transcript 2”) and in the cerebellar hemisphere are “transcript 1” and ENST00000389652.9 (“transcript 3”) (Figure 2b(i)). Transcript 1 is short (1,474 bp), lacking functional CC2D2A domains and generated by alternative splicing resulting in an additional exon (including a premature stop codon) after exon 5 (ref.: ENST00000503292.5 or NM_001080522.2). This transcript is supported by the detection in GTEx of the specific junction in nearly all tissues but enriched in the kidney (Figure 2b(ii)). Transcript 3 is detected in the cerebellum but not the kidney and has an incomplete open reading frame with the 5′-end not fully annotated. However, based on GTEx junction expression data, exon 2 appears to be spliced in the kidney but not the cerebellum, while an exon predicted in the cerebellum (between exons 30 and 31) is skipped in the kidney. Of interest, a splice junction leading to skipping of exon 30 is detected at low frequency and almost exclusively in the kidney medulla (Figure 2b(iii)). At the protein level, transcript 1 encodes a 111 amino acid product, sharing the first 82 amino acids with canonical transcript 2 (Figure 2b(iii)).

In summary, human RNAseq data suggest the presence of
tissue-specific transcripts for \textit{CEP120} and \textit{CC2D2A}. Exons that are predicted to undergo organ-specific splicing events, such as exon 30 of \textit{CC2D2A}, represent optimal candidates to apply exon skipping therapeutic strategies. However, isoform expression predicted from RNA sequencing data must be interpreted with caution and specific isoforms should be confirmed by dedicated RT-PCR (Molinari et al., 2018).

2.4 Confirmation of tissue-specific basal exon skipping and possible implications for organ disease manifestations

We performed RT-PCR on total RNA from whole blood, kidney and human urine-derived renal epithelial cells (hURECs) using a primer pair targeted to exon 29 and exon 31 from the canonical \textit{CC2D2A} transcript (ENST00000503292.5). Beside the predicted amplification product of 327 bp detected in the kidney and whole blood (at lower levels), a shorter transcript is clearly seen in the kidney but not in whole blood RNA. The observed size (~150 bp) is in line with the expected size of an amplification product lacking exon 30 of \textit{CC2D2A} (150 bp) and strongly suggests basal exon 30 skipping in the kidney (Figure 3a). Furthermore, we were able to detect this basal exon skipping event in hURECs (Figure 3a, right panel), highlighting the utility of this “liquid biopsy” system to study kidney-specific splicing events (Molinari et al., 2018). Given that truncating \textit{CC2D2A} variants are associated with more severe disease and a generally high penetrance for kidney disease (Figure 1) as well as our observation that a small fraction of \textit{CC2D2A} exon 30 undergoes basal exon skipping in the kidney, we postulated that truncating variants in exon 30 are partially rescued and therefore associated with lower prevalence of (and/or milder) kidney...
disease. To assess this hypothesis, we examined the relative prevalence of kidney disease associated with truncating variants in the different CC2D2A exons (Figure 3b). There are only 2 patients known to harbour a truncating variant in exon 30, limiting the strength of any conclusions. However, neither of these patients showed kidney involvement, compared to documented kidney disease in 33.3%–100% of truncating variants in the other exons of CC2D2A (Figure 3b). Considering all patients with either monoallelic or biallelic truncating variants in CC2D2A, 35/48 presented with kidney disease, and only 13 did not present with kidney disease (including the two patients with exon 30 truncating variants). Patient MTI-991 (Table 1) harbours a CEP120 biallelic intronic variant at the exon-intron boundary 3′ of exon 2 which has been shown to lead to intron retention (Roosing et al., 2016). However, GTEx data predict that exon 2 of CEP120 is skipped in the kidney (Figure 2) and we confirmed via RT-PCR that basal CEP120 exon 2 skipping occurs in the human kidney (Figure S4). Assuming that exon 2 is not spliced in the kidney, a variant located at the exon-intron boundary 3′ of exon 2 would be likely “silent” in the kidney and not lead to a disease phenotype. Indeed, no kidney involvement has been reported for this patient (Roosing et al., 2016).

2.5 | Multimodal identification of skippable exons in CEP120 and CC2D2A and mapping of reported truncating mutations

The CEP120 transcript ENST00000328236.9 contains 20 coding exons. Among them, the nucleotide length of 11 exons is a multiple of three and therefore amenable to exon skipping without change in reading frame (Figure 4a). Considering the location of encoded protein domains of functional importance (coiled-coil and C2 domains), only exons 14 and 15 of CEP120 are predicted to be skippable without inducing loss of protein function. As their boundaries fall between codons (phase 0), skipping of exons 14 and 15 will not alter reading frame and not lead to potential amino acid substitutions. Based on the GTEx alternative splicing data of CEP120 presented above, exon 2 (predicted to be skipped in kidney tissue, see transcript ENST0000306481.10) might represent an additional target for tolerated exon skipping with an alternative start codon functional in exon 3. However, none of the reported CEP120 mutations to date fall in these identified exons, suggesting that CEP120, at the current state of knowledge, is not a good candidate gene to apply exon skipping therapies.
The CC2D2A transcript ENST00000503292.5 contains 36 coding exons, 17 of which are potentially amenable to exon skipping without change in reading frame (Figure 4b). Considering the location of protein domains of functional importance (coiled-coil and C2 domains) (Bachmann-Gagescu et al., 2012; Noor et al., 2008), exons 4, 7, 8, 9, 12, 13, 22, 23, 31, 32, 33, 34 and 35 might be skippable without inducing loss of protein function. These exons all contain full complements of codons (exon boundaries fall between codons (phase 0)) and can therefore be skipped without introducing potential amino acid substitutions. Based on the data presented above, exon 30 appears as a possible candidate for exon skipping. Furthermore, exon 30 is predicted to encode only the last 3 amino acids of the C2 domain or to have no overlap with the C2 domain at all (Bachmann-Gagescu et al., 2012; Gorden et al., 2008; Noor et al., 2008; Srour et al., 2012). Prediction tools and available literature provide conflicting data with respect to C2 domain overlap with exon 25, indicating the need for functional studies confirming this potential exon skipping target (http://smart.embl-heidelberg.de/) (Bachmann-Gagescu et al., 2012; Noor et al., 2008; Srour et al., 2012). Because of their clear pathogenic implications, we have focused on truncating variants in CC2D2A as potential targets for exon skipping approaches. Table 2 lists the reported patients harbouring at least one of the predicted skippable exons in CC2D2A (Figure 4b). Four of these truncating variants have been described in homozygosis: c.517C>T, p.(Arg173Ter), exon 8; c.2848C>T, p.(Arg950Ter), exon 23; c.2875del, p.(Glu959AsnfsTer3), exon 23 and c.3084del,
p.(Lys1029ArgfsTer3), exon 25. Skipping of each of the seven identified exons that harbour truncating variants and are potentially tolerant to skipping would lead to a predicted near-full length protein product (Figure S5).

3 | DISCUSSION

CEP120 and CC2D2A both encode ciliary proteins, with different subcellular localization and function. Mutations in these genes are associated with a spectrum of both overlapping and distinct ciliopathies, illustrating the concepts of genetic heterogeneity and pleiotropy, inherent to most ciliopathy genes. To capture the genetic and clinical spectrum of CEP120- and CC2D2A-associated disease, we reviewed the literature, including previous mutation summaries (Bachmann-Gagescu et al., 2012; Lam et al., 2020), and open-access tools to generate a curated, annotated and HGVS compliant database. Furthermore, we used in silico tools to identify tissue-specific basal (endogenous) exon skipping events. Using this database, we establish genotype-phenotype correlations, including possible insights into tissue-specific disease expression, and show that several exons in CC2D2A, but not in CEP120, are good candidates for future exon skipping approaches. In addition to creating an updated and annotated database for CEP120- and CC2D2A-associated disease, we provide a possible roadmap of how open-access tools can be used to identify future targets for splice-altering therapeutic approaches in large multi-exon genes.

To date, only nine patients from nine different families have been described with biallelic genetic variants in CEP120 and 111 patients from 97 families with biallelic variants in CC2D2A. In these patient populations, nine different genetic variants have been described in CEP120 and 84 different variants in CC2D2A. It has been previously shown that mutations in CC2D2A cluster to the C-terminal half of the protein (Bachmann-Gagescu et al., 2012) (Figure 4b). Several variants are shared between unrelated families and might follow geographical clusters. For instance, CC2D2A variant c.1762C>T was detected in 11 unrelated cases with MKS in the Finnish population but not reported outside Scandinavia (Tallila et al., 2008). In contrast, CC2D2A missense variant c.4667A>T is found in 13 unrelated cases, always in compound heterozygous state and without apparent geographical patterns. Finally, by crossing reported disease-causing variants with genomic data from the general population, we detected common variants (CC2D2A: p.(Glu229del) & p.(Pro721Ser); CEP120: p.(Leu712Phe)) that are most likely misclassified, echoing similar concerns for other ciliopathy genes (Barroso-Gil et al., 2020; Pauli et al., 2019; Shaheen et al., 2016).

Mutations in CC2D2A is a common cause of a ciliopathy syndrome accounting for about 10% of both JBTS and MKS patients (Bachmann-Gagescu et al., 2015; Mougou-Zerelli et al., 2009; Vilboux et al., 2017). The relative prevalence of CC2D2A-associated disease enabled more detailed analyses of genotype-phenotype correlations that are important to prioritize genetic testing (if targeted tests are performed), provide better prognostic information but can also give insights into disease mechanisms. Previous studies have shown that subjects with CC2D2A-related JBTS were more likely to have ventriculomegaly and seizures than subjects without CC2D2A mutations (Bachmann-Gagescu et al., 2012). Furthermore, it has been previously noted that patients with at least one missense mutation in CC2D2A are more likely to suffer from JBTS while patients with biallelic truncating variants display more often MKS or ML, in line with a more deleterious effect of null alleles (Bachmann-Gagescu et al., 2012; Mougou-Zerelli et al., 2009). A similar correlation between biallelic truncating variants and more severe phenotypes has been suggested for the ciliopathy genes TME67 and RPGRIP1L (Delous et al., 2007; Iannicelli et al., 2010). In this study, we provide a systematic analysis of all reported patients with CC2D2A mutations and indeed show a strikingly more severe clinical presentation for patients with biallelic null variants. Out of 45 patients with CC2D2A-related JBTS caused by any combination of truncating (nonsense or frameshift) and/or missense variants, only one harboured biallelic truncating variants, whereas the majority of CC2D2A-related MKS/ML was caused by biallelic null alleles. We also show that this association holds true for extra-CNS manifestations as patients with biallelic truncating variants were strikingly more likely to suffer from kidney disease (Figure 1). This observation is compatible with the notion that some of the observed genetic pleiotropy might be explained by the effects of particular mutations on total protein expression. In support of this hypothesis, Drivas et al. showed that basal exon skipping events modulate total protein expression in patients with CEP290 and CC2D2A mutations and that protein expression inversely correlated with disease severity (Drivas et al., 2015). Interestingly, a minority of patients with MKS/ML presented with biallelic missense changes in CC2D2A and a homozygous missense variant p.(Pro1122Ser) in CC2D2A was detected in patients with JBTS and MKS, suggesting additional phenotype modifying factors are at work, such as trans-acting genetic modifiers. Along these lines, a recent study established enrichment for secondary variants beyond the driver locus in cohorts of recessive ciliopathy patients (Bardet–Biedl syndrome) that might potentially contribute to disease expressivity (Kousi et al., 2020). Large-scale human sequencing projects suggest major differences in pre-mRNA splicing and basal exon skipping between different organs for most of our transcriptome. Whether these tissue-specificities contribute to genetic pleiotropy is currently unknown.
Here, we provide ex vivo data showing tissue-specific differences in basal exon skipping and illustrate how these effects might be exploited for a better understanding of different organ involvement in ciliopathies. While our limited data by no means prove this concept, we estimate that this is an exciting field for future studies. As alternative splicing events are conserved in human urine-derived epithelial cells (hURECs), they provide the ideal tool to investigate splicing in the kidney (Figure 3) (Molinari et al., 2018).

Using bioinformatic tools, including sequence data, domain annotations and alternative splicing predictions, we identified potentially skippable exons in CC2D2A and CEP120 and populated them with reported truncating variants to assess the applicability of therapeutic exon skipping for these two ciliopathy genes. Only exons 14 and 15 in CEP120 are skippable without inducing a frameshift or disrupting a functional domain. None of the reported genetic variants to date map into these two exons. Given the low number of mutations reported, it is currently impossible to say whether this is purely down to chance or whether this observation reflects the fact that these particular exons are functionally not important and/or skippable and therefore mutations in these exons are tolerated.

In contrast, we identified 15/38 exons in CC2D2A as potentially skippable and we mapped 14 distinct truncating variants in seven of them. Our analysis highlights exon 30 as a particularly good candidate as this exon undergoes some degree of basal exon skipping in the kidney, potentially modulating the severity of kidney disease associated with truncating mutations therein. Using this example, we show how open-access databases for tissue-specific splicing could be used to identify targets for exon skipping therapy and we suggest exon 30 skipping as a potential therapeutic option for future patients with kidney disease caused by truncating mutations in exon 30. According to GTEx, CC2D2A exon 30 skipping is only observed in the kidney and the female reproductive tract. Assuming that endogenous exon splicing points towards tolerated splicing events that lead to functional transcripts, we hypothesize that ASO-mediated exon 30 splicing might also constitute a therapeutic option in other tissues such as the liver or retina in patients with truncating exon 30 mutations. Indeed, the skippable CC2D2A mutations identified in a total of 26 patients are a starting point for in vitro analysis to determine if a functional rescue using ASO mediated exon skipping is possible and to what extent this rescue can be translated between different tissues. Our group has previously applied ASO-mediated exon skipping to rescue kidney phenotypes in a mouse ciliopathy model (Ramsbottom et al., 2018). Delivery of ASO via systemic administration to the kidney appears effective in contrast to the brain and retinal tissues where blood-brain and blood-retinal barriers, respectively, cause reduction in delivery (Daneman & Prat, 2015; Himawan et al., 2019; Pardridge, 2002; Yu et al., 2007). In rodents, systemic administration of ASOs revealed greatest accumulation in kidney and liver (Geary et al., 2015; Zhao et al., 1998) and abundant proximal tubular uptake (Janssen et al., 2019; Oberbauer et al., 1995). Given the high morbidity associated with kidney disease, the potential for adequate ASO delivery, the tissue-specific splicing events that convey important information about potential target exons and the availability of relevant cell systems (hURECs) for non-invasive validation, ASO-mediated exon skipping offers exciting therapeutic perspectives for nephrology and particularly ciliopathy patients suffering from kidney disease (Molinari et al., 2018, 2019). As this approach was successfully tested in pre-clinical models (Ramsbottom et al., 2018), the next big step is to bring this innovative therapy from bench to bedside, following the path set out by other diseases including Duchenne muscular dystrophy (Kole & Krieg, 2015; Komaki et al., 2018; Lee et al., 2018; Servais et al., 2015).

4  |  MATERIAL AND METHODS

4.1  |  Ethical compliance

The study was conducted with full ethical approval and consent. Ethical approval was obtained from the National Research Ethics Service Committee North East–Newcastle & North Tyneside 1 (08/H0906/21+5).

4.2  |  Web resources

The URLs for data presented herein are as follows:

Ensembl (release 100): https://www.ensembl.org/index.html

Ensembl VEP: https://www.ensembl.org/info/docs/tools/vep/index.html

GnomAD v2.1.1: https://gnomad.broadinstitute.org/

GTEx: https://www.gtexportal.org/home/

The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. The data used for the analyses described in this manuscript were obtained from: the GTEx Portal on 15/02/2020.

HGMD®: http://www.hgmd.cf.ac.uk/ac/index.php

NCBI ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/

NCBI Primer-BLAST: https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi

ProteinPaint: https://pecan.stjude.cloud/proteinpaint

PubMed: https://pubmed.ncbi.nlm.nih.gov/
4.3 | Patient database

We searched PubMed® (using global keywords: “cep120” and “cc2d2a”, with subsequent manual curation for relevant literature) and HGMD® (Stenson et al., 2017) databanks (last query 05/2020) for reported patients with biallelic genetic variants in CEP120 (NG_042125.1) and CC2D2A (NG_013035.1) and detected 33 relevant publications. All genetic variants were manually curated and annotated using Ensembl Variant Effect Predictor (Ensembl release 100) (Yates et al., 2020), NCBI ClinVar and VarSome (Kopanos et al., 2019), matched with allele frequency data from the general population assessed via gnomAD v2.1.1. (Karczewski et al., 2020) and compiled using an identifier following the HGVS identification standard (den Dunnen et al., 2016). Patients reported in multiple publications were only included once in our database (if possible to detect) and patients with incomplete genetic or phenotypic information were not included. For each included patient, available phenotypic data were reviewed and where necessary adapted with following disease categories (Drivas et al., 2015): JBTS: All patients with hypoplasia of the cerebellar vermis and/or brain stem abnormalities, often intellectual disability and with or without extra-CNS manifestations; Meckel-like syndrome (ML): lethality during the first months or years, characterized by cystic kidney disease, CNS malformation (typically Dandy Walker malformation), polydactyly, and hepatic fibrosis; MKS: Similar to ML but uniformly perinatal lethal with occipital encephalocele being the predominant CNS malformation.

4.4 | RNA preparation and RT-PCR

Total RNA from human kidney (ThermoFisher AM7976) was used together with total RNA from whole blood samples and urine-derived renal epithelial cells (hUREC) isolated using RNeasy mini kit (Qiagen) according to the manufacturer’s instructions and quantified using a NanoDrop 2000 spectrophotometer. 0.75 µg RNA was reverse-transcribed using an oligo-dT primer and SuperScript III Reverse Transcriptase (Thermo Fisher Scientific). The resulting cDNA was used for PCR with a GoTaq® DNA Polymerase (Promega). A CC2D2A gene-specific primer pair (5- TGAGAGACACTGGCTGGGAT -3 and 5- AGGCACTGACGATTGGAAAC -3) to identify basal skipping of exon 30 and a CEP120 gene-specific primer pair (amplifying only in the event of exon 2 skipping) (5- TACAGCAGTAGCGCGTGG -3 and 5- GGGAAATGGCCGACCTCAG -3) to identify basal skipping of exon 2 have been used. Primers were designed using NCBI Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi). Amplification of HPRT1 housekeeping gene cDNA was performed alongside. Product electrophoresis was performed on a 2% agarose gel.

ACKNOWLEDGMENTS

Miguel Barroso-Gil is funded by Kidney Research UK (ST_001_20171120) and the Northern Counties Kidney Research Fund. Eric Olinger is supported by an Early Postdoc MobilityStipendium of the Swiss National Science Foundation (P2ZHP3_195181) and Kidney Research UK (Paed_RP_001_20180925). Elisa Molinari is Funded by Kidney Research UK (RP_006_20180227). John Sayer and Colin Miles are funded by Kidney Research UK and the Northern Counties Kidney Research Fund.

AUTHOR CONTRIBUTIONS

MB-G. and EO carried out the experimental work and wrote the manuscript with support from EM, SAR, CGM and JAS. CGM and JAS supervised the project. JAS conceived the original idea. All authors discussed the results and contributed to the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available through The Human Gene Mutation Database at http://www.hgmd.cf.ac.uk/ac/index.php and PubMed® at https://pubmed.ncbi.nlm.nih.gov. Data presented here were derived from the following resources available in the public domain: The Genotype-Tissue Expression (GTEx) Project (https://www.gtexportal.org/home/), The Genome Aggregation Database v2.1.1 (https://gnomad.broadinstitute.org/) and Ensembl (release 100): (https://www.ensembl.org/index.html).
REFERENCES

Aartsma-Rus, A., & van Ommen, G. J. (2007). Antisense-mediated exon skipping: A versatile tool with therapeutic and research applications. *RNA, 13*(10), 1609–1624. https://doi.org/10.1261/rna.653607

Al-Hamed, M. H., Kurdi, W., Alsahan, N., Alabdullah, Z., Abudraz, R., Tulbah, M., & Albaqumi, M. (2016). Genetic spectrum of Saudi Arabian patients with antenatal cystic kidney disease and ciliopathy phenotypes using a targeted renal gene panel. *Journal of Medical Genetics, 53*(5), 338–347. https://doi.org/10.1136/jmedgenet-2015-103469

Alexiev, B. A., Lin, X., Sun, C. C., & Brenner, D. S. (2006). Meckel-Gruber syndrome: Pathologic manifestations, minimal diagnostic criteria, and differential diagnosis. *Archives of Pathology and Laboratory Medicine, 130*(8), 1236–1238. https://doi.org/10.1043/1543-2165(2006)130[1236:MS2.COC2]

Bachmann-Gagescu, R., Dempsey, J. C., Bulgheroni, S., Chen, M. L., D’Arrigo, S., Glass, I. A., & Doherty, D. (2020). Healthcare recommendations for Joubert syndrome. *American Journal of Medical Genetics. Part A, 182*(1), 229–249. https://doi.org/10.1002/ajmg.a.61399

Bachmann-Gagescu, R., Dempsey, J. C., Phelps, I. G., O’Roak, B. J., Knutzen, D. M., Rue, T. C., & Doherty, D. (2015). Joubert syndrome: A model for untangling recessive disorders with extreme genetic heterogeneity. *Journal of Medical Genetics, 52*(8), 514–522. https://doi.org/10.1136/jmedgenet-2015-103087

Bachmann-Gagescu, R., Dona, M., Hetterschijt, L., Tonnaer, E., Peters, T., de Vrieze, E., & van Wijk, E. (2015). The ciliopathy protein CC2D2A associates with NINL and functions in RAB8-MICAL3-dependent vesicle trafficking. *PLoS Genetics, 11*(10), e1005575. https://doi.org/10.1371/journal.pgen.1005575

Bachmann-Gagescu, R., Ishak, G. E., Dempsey, J. C., Adkins, J., O’Day, D., Phelps, I. G., & Doherty, D. (2012). Genotype-phenotype correlation in CC2D2A-related Joubert syndrome reveals an association with ventriculomegaly and seizures. *Journal of Medical Genetics, 49*(2), 126–137. https://doi.org/10.1136/jmedgenet-2011-100552

Badano, J. L., Mitsuma, N., Beales, P. L., & Katsanis, N. (2006). The ciliopathies: An emerging class of human genetic disorders. *Annual Review of Genomics and Human Genetics, 7*, 125–148. https://doi.org/10.1146/annurev.genom.7.080505.115610

Barny, I., Perrault, I., Michel, C., Goudin, N., Defoort-Dhellemmes, S., Ghazi, I., & Gerard, X. (2019). AON-mediated exon skipping to bypass protein truncation in retinal dystrophies due to the recurrent CEP290 c.4723A > T mutation. Fact or fiction? *Genes (Basel), 10*(5), 368. https://doi.org/10.3390/genes10050368

Barroso-Gil, M., Powell, L., & Sayer, J. A. (2020). RE: Clinical and molecular diagnosis of Joubert syndrome and related disorders. *Pediatric Neurology, 112*, 10. https://doi.org/10.1016/j.pediatrneurol.2020.07.010

Ben-Salem, S., Al-Shamsi, A. M., Gleeson, J. G., Ali, B. R., & Al-Gazali, L. (2014). Mutation spectrum of Joubert syndrome and related disorders among Arabs. *Human Genome Variation, 1*, 14020. https://doi.org/10.1038/hgv.2014.20

Ben-Salem, S., Al-Shamsi, A. M., Gleeson, J. G., Ali, B. R., & Al-Gazali, L. (2015). Erratum: Mutation spectrum of Joubert syndrome and related disorders among Arabs. *Human Genome Variation, 2*, 15001. https://doi.org/10.1038/hgv.2015.1

Bennett, C. F., & Swayze, E. E. (2010). RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. *Annual Review of Pharmacology and Toxicology, 50*, 259–293. https://doi.org/10.1146/annurev.pharmtox.010909.105654

Braun, D. A., & Hildebrandt, F. (2017). Ciliopathies. *Cold Spring Harbor Perspectives in Biology, 9*(3), a028191. https://doi.org/10.1101/cshperspect.a028191

Cideciyan, A. V., Jacobson, S. G., Drack, A. V., Ho, A. C., Chang, J., Garafalo, A. V., & Russell, S. R. (2019). Effect of an intravitreal antisense oligonucleotide on vision in Leber congenital amaurosis due to a photoreceptor cilium defect. *Nature Medicine, 25*(2), 225–228. https://doi.org/10.1038/s41591-018-0295-0

Comartin, D., Gupta, G. D., Fussner, E., Coyaud, E., Hasegan, M., Archinti, M., & Pelletier, L. (2013). CEP120 and SPICE1 cooperate with CPAP in centriole elongation. *Current Biology, 23*(14), 1360–1366. https://doi.org/10.1016/j.cub.2013.06.002

Coppieters, F., Lefever, S., Leroy, B. P., & De Baere, E. (2010). CEP290, a gene with many faces: Mutation overview and presentation of CEP290-base. *Human Mutation, 31*(10), 1097–1108. https://doi.org/10.1002/humu.21337

Daneman, R., & Prat, A. (2015). The blood-brain barrier. *Cold Spring Harbor Perspectives in Biology, 7*(1), a020412. https://doi.org/10.1101/cshperspect.a020412

Delous, M., Baala, L., Salomon, R., Laclef, C., Vierkotten, J., Tory, K., & Saunier, S. (2007). The ciliary gene RPGRIP1L is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. *Nature Genetics, 39*(7), 875–881. https://doi.org/10.1038/ng2039

den Dunnen, J. T., Dalgleish, R., Maglott, D. R., Hart, R. K., Greenblatt, M. S., McGowan-Jordan, J., & Taschner, P. E. (2016). HGVS recommendations for the description of sequence variants: 2016 update. *Human Mutation, 37*(6), 564–569. https://doi.org/10.1002/humu.22981

Doherty, D., Parisi, M. A., Finn, L. S., Gunay-Aygun, M., Al-Mateen, M., Bates, D., & Glass, I. A. (2010). Mutations in 3 genes (MKS3, CC2D2A and RPGRIP1L) cause COACH syndrome (Joubert syndrome with congenital hepatic fibrosis). *Human Mutation, 31*(10), 1097–1108. https://doi.org/10.1002/humu.21337

Garanto, A., Chung, D. C., Duijkers, L., Corral-Serrano, J. C., Drivas, T. G., Wojno, A. P., Tucker, B. A., Stone, E. M., & Bennett, J. (2015). Basal exon skipping and genetic pleiotropy: A predictive model of disease pathogenesis. *Science Translational Medicine, 7*(291), 291ra297. https://doi.org/10.1126/scitranslmed.aaa5370

Gazali, L. (2014). Mutation spectrum of Joubert syndrome and related disorders among Arabs. *Human Genome Variation, 1*, 14020. https://doi.org/10.1038/hgv.2014.20

Gazali, L. (2015). Erratum: Mutation spectrum of Joubert syndrome and related disorders among Arabs. *Human Genome Variation, 2*, 15001. https://doi.org/10.1038/hgv.2015.1

Messchaert, M., Xiao, R., & Collin, R. W. (2016). In vitro and in vivo rescue of aberrant splicing in CEP290-associated LCA by antisense oligonucleotide delivery. *Human Molecular Genetics, 25*(12), 2552–2563. https://doi.org/10.1093/hmg/ddw118
Garcia-Gonzalo, F. R., Corbit, K. C., Sirerol-Piquer, M. S., Ramaswami, G., Otto, E. A., Noriega, T. R., & Reiter, J. F. (2011). A transition zone complex regulates mammalian cilogenesis and ciliary membrane composition. *Nature Genetics, 43*(8), 776–784. https://doi.org/10.1038/ng.891

Geary, R. S., Norris, D., Yu, R., & Bennett, C. F. (2015). Pharmacokinetics, biodistribution and cell uptake of antisense oligonucleotides. *Advanced Drug Delivery Reviews, 87*, 46–51. https://doi.org/10.1016/j.addr.2015.01.008

Gorden, N. T., Arts, H. H., Parisi, M. A., Coene, K. L., Letteboer, S. J., van Beersum, S. E., & Doherty, D. (2008). CC2D2A is mutated in Joubert syndrome and interacts with the ciliopathy-associated basal body protein CEP290. *American Journal of Human Genetics, 83*(5), 559–571. https://doi.org/10.1016/j.ajhg.2008.10.002

Hamosh, A., Scott, A. F., Amberger, J., Valle, D., & McKusick, V. A. (2000). Online mendelian inheritance in man (OMIM). *Human Mutation, 15*(1), 57–61. https://doi.org/10.1002/(SICI)1098-1004(20000115):15<1:57::AID-HUMU12>3.0.CO;2-G

Hartill, V., Szymanska, K., Sharif, S. M., Wheway, G., & Johnson, C. A. (2017). Meckel-Gruber syndrome: An update on diagnosis, clinical management, and research advances. *Frontiers in Pediatrics, 5*, 244. https://doi.org/10.3389/fped.2017.00244

Himawan, E., Ekstrom, P., Buzgo, M., Gaillard, P., Stefansson, E., Marigo, V., & Paquet-Durand, F. (2019). Drug delivery to retinal photoreceptors. *Drug Discovery Today, 24*(8), 1637–1643. https://doi.org/10.1016/j.drudis.2019.03.004

Hopp, K., Heyer, C. M., Hommerding, C. J., Henke, S. A., Sundsbak, J. L., Patel, S., & Harris, P. C. (2011). B9D1 is revealed as a novel Meckel syndrome (MKS) gene by targeted exon-enriched next-generation sequencing and deletion analysis. *Human Molecular Genetics, 20*(13), 2524–2534. https://doi.org/10.1093/hmg/ddr151

Iannicelli, M., Brancati, F., Mougou-Zerelli, S., Mazzotta, A., Thomas, S., Elkhartoufi, N., & Valente, E. M. (2010). Novel TMEM67 mutations and genotype-phenotype correlates in meckelin-related ciliopathies. *Human Mutation, 31*(5), E1319–E1331. https://doi.org/10.1002/humu.21239

Janssen, M. J., Nieskens, T. T. G., Steevels, T. A. M., Caetano-Pinto, P., van Beersum, S. E., & Doherty, D. (2008). CC2D2A is mutated in Joubert syndrome and interacts with the ciliopathy-associated basal body protein CEP290. *American Journal of Human Genetics, 83*(5), 559–571. https://doi.org/10.1016/j.ajhg.2008.10.002

Jones, D., Fiozzo, F., Waters, B., McKnight, D., & Brown, S. (2014). First-terminus diagnosis of Meckel-Gruber syndrome by fetal ultrasound with molecular identification of CC2D2A mutations by next-generation sequencing. *Ultrasonad in Obstetrics and Gynecology, 44*(6), 719–721. https://doi.org/10.1002/ugo.13381

Kagan, K. O., Dufke, A., & Gebruhr, U. (2017). Renal cystic disease and associated ciliopathies. *Current Opinion in Obstetrics and Gynecology, 29*(2), 85–94. https://doi.org/10.1097/GCO.000000000000348

Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alfoldi, J., Wang, Q., & MacArthur, D. G. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature, 581*(7809), 434–443. https://doi.org/10.1038/s41586-020-2308-7

Kole, R., & Krieg, A. M. (2015). Exon skipping therapy for Duchenne muscular dystrophy. *Advanced Drug Delivery Reviews, 87*, 104–107. https://doi.org/10.1016/j.addr.2015.05.008

Komaki, H., Nagata, T., Saito, T., Masuda, S., Takeshita, E., Sasaki, M., & Takeda, S. (2018). Systemic administration of the antisense oligonucleotide NS-065/NCNP-01 for skipping of exon 53 in patients with Duchenne muscular dystrophy. *Science Translational Medicine, 10*, 437. https://doi.org/10.1126/scitranslmed.aan0713

Kopanos, C., Tsiolkas, V., Kouri, A., Chapple, C. E., Albarca Aguilera, M., Meyer, R., & Massouras, A. (2019). VarSome: The human genomic variant search engine. *Bioinformatics, 35*(11), 1978–1980. https://doi.org/10.1093/bioinformatics/bty897

Kousi, M., Söylemez, O., Ozanturk, A., Mourti, N., Akle, S., Jungreis, I., & Katsanis, N. (2020). Evidence for secondary-variant genetic burden and non-random distribution across biological modules in a recessive ciliopathy. *Nature Genetics, 52*(11), 1145–1150. https://doi.org/10.1038/s41588-020-0707-1

Lam, Z., Altaba, S., & Balasubramanian, M. (2020). Atypical, milder presentation in a child with CC2D2A and KIDINS220 variants. *Clinical Dysmorphology, 29*(1), 10–16. https://doi.org/10.1097/MCD.000000000000299

Lee, J. J. A., Saito, T., Duddy, W., Takeda, S., & Yokota, T. (2018). Direct reprogramming of human DMD fibroblasts into myotubes for in vitro evaluation of antisense-mediated exon skipping and exons 45–55 skipping accompanied by rescue of dystrophin expression. *Methods in Molecular Biology, 1828*, 141–150. https://doi.org/10.1007/978-1-4939-8651-4_8

Lewis, W. R., Bales, K. L., Revell, D. Z., Croyle, M. J., Engle, S. E., Song, C. J., & Yoder, B. K. (2019). Ms6 mutations reveal tissue- and cell type-specific roles for the cilia transition zone. *The FASEB Journal, 33*(1), 1440–1455. https://doi.org/10.1096/fj.201801149R

Mahjoub, M. R., Xie, Z., & Stearns, T. (2010). Cep120 is asymmetrically localized to the daughter centriole and is essential for centriole assembly. *Journal of Cell Biology, 191*(2), 331–346. https://doi.org/10.1083/jcb.20100309

Mejeacse, C., Hummel, A., Mohand-Said, S., Andrieu, C., El Shami, S., Antonio, A., & Audo, I. (2019). Whole exome sequencing resolves complex phenotype and identifies CC2D2A mutations underlying non-syndromic rod-cone dystrophy. *Clinical Genetics, 95*(2), 329–333. https://doi.org/10.1111/ceg.13453

Mitchison, H. M., & Valente, E. M. (2017). Motile and non-motile cilia in human pathology: From function to phenotypes. *The Journal of Pathology, 241*(2), 294–309. https://doi.org/10.1002/path.4843

Molinari, E., Decker, E., Mabillard, H., Tellez, J., Srivastava, S., Raman, S., & Sayer, J. A. (2018). Human urine-derived renal epithelial cells provide insights into kidney-specific alternate splicing variants. *European Journal of Human Genetics, 26*(12), 1791–1796. https://doi.org/10.1038/s41431-018-0212-5

Molinari, E., Ramsbottom, S. A., Srivastava, S., Booth, P., Alkanderi, S., McLaufferty, S. M., & Sayer, J. A. (2019). Targeted exon skipping rescues ciliary protein composition defects in Joubert syndrome patient fibroblasts. *Scientific Reports, 9*(1), 10828. https://doi.org/10.1038/s41598-019-47243-z

Molinari, E., Srivastava, S., Sayer, J. A., & Ramsbottom, S. A. (2017). From disease modelling to personalised therapy in patients with CEP290 mutations. *F1000Research, 6*, 669. https://doi.org/10.12688/f1000research.11553.1

Mougou-Zerelli, S., Thomas, S., Szenker, E., Audollent, S., Elkhartoufi, N., Barbati, C., & Attie-Bitach, T. (2009). CC2D2A mutations in Meckel and Joubert syndromes indicate a genotype-phenotype correlation. *Human Mutation, 30*(11), 1574–1582. https://doi.org/10.1002/humu.21116
Ojeda Naharros, I., Gesemann, M., Mateos, J. M., Barmettler, G., Oberbauer, R., Schreiner, G. F., & Meyer, T. W. (1995). Renal up
Parisi, M. A. (2019). The molecular genetics of Joubert syndrome and
Pardridge, W. M. (2002). Drug and gene delivery to the brain: the vascu
Pan, Q., Shai, O., Lee, L. J., Frey, B. J., & Blencowe, B. J. (2008).
Pauli, S., Altmuller, J., Schroder, S., Ohlenbusch, A., Dreha-
Ramsbottom, S. A., Molinari, E., Srivastava, S., Silberman, F., Henry,
Reiter, J. F., & Leroux, M. R. (2017). Genes and molecular pathways underpinning ciliopathies. Nature Reviews Molecular Cell Biology, 18(9), 533–547. https://doi.org/10.1038/nrm.2017.60
Romani, M., Micalizzi, A., & Valente, E. M. (2013). Joubert syndrome: congenital cerebellar ataxia with the molar tooth. The Lancet Neurology, 12(9), 894–905. https://doi.org/10.1016/s1474
Roosig, S., Romani, M., Isrie, M., Rosti, R. O., Micalizzi, A., Musaev, D., & Valente, E. M. (2016). Mutations in CEP120 cause Joubert syndrome as well as complex ciliopathy phenotypes. Journal of Medical Genetics, 53(9), 608–615. https://doi.org/10.1136/jmedgenet-2016-103382
Rozet, J. M., & Gerard, X. (2015). Understanding disease pleiotropy: From puzzle to solution. Science Translational Medicine, 7(291), 291fs224. https://doi.org/10.1126/scitranslmed.aac6504
Scherue, M., Halbritter, J., Phelps, I. G., Braun, D. A., Otto, E. A., Porath, J. D., & Hildebrandt, F. (2016). Large-scale targeted sequencing comparison highlights extreme genetic heterogeneity in nephronophthisis-related ciliopathies. Journal of Medical Genetics, 53(3), 208–214. https://doi.org/10.1136/jmedgenet-2015-103304
Serais, L., Montus, M., Guiner, C. L., Ben Yau, R., Annoussamy, M., Moraux, A., & Voit, T. (2015). Non-ambulant duchenne patients theoretically treatable by exon 53 skipping have severe phenotype. Journal of Neuromuscular Diseases, 2(3), 269–279. https://doi.org/10.3233/jnd-150100
Shaheen, R., Schmidt, M., Faqeih, E., Hashem, A., Lausch, E., Holder, I., & Alkuraya, F. S. (2015). A founder CEP120 mutation in Jeune asphyxiating thoracic dystrophy expands the role of centriolar proteins in skeletal ciliopathies. Human Molecular Genetics, 24(5), 1410–1419. https://doi.org/10.1093/hmg/ddu555
Shaheen, R., Szymanska, K., Basu, B., Patel, N., Ewida, N., Faqeih, E., & Alkuraya, F. S. (2016). Characterizing the morbid genome of ciliopathies. Genome Biology, 17(1), 242. https://doi.org/10.1186/s13059-016-1099-5
Shamseldin, H. E., Shaheen, R., Ewida, N., Bubshait, D. K., Alkuraya, H., Almardawi, E., & Alkuraya, F. S. (2020). The morbid genome of ciliopathies: An update. Genetics in Medicine, 22, 1051–1060. https://doi.org/10.1038/s41436-020-0761-1
Srou, M., Hamdan, F. F., Schwartzentruber, J. A., Patry, L., Osipa, L. H., Shevell, M. I., & Michaud, J. L. (2012). Mutations in TMEM231 cause Joubert syndrome in French Canadians. Journal of Medical Genetics, 49(10), 636–641. https://doi.org/10.1136/jmedgenet-2012-101132
Stenson, P. D., Mort, M., Ball, E. V., Evans, K., Hayden, M., Heywood, S., & Cooper, D. N. (2017). The Human Gene Mutation Database: Towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. Human Genetics, 136(6), 665–677. https://doi.org/10.1007/s00439-017-1779-6
Szymanska, K., Berry, I., Logan, C. V., Cousins, S. R., Lindsey, H., Jafri, S., & Johnson, C. A. (2012). Founder mutations and genotype-phenotype correlations in Meckel-Gruber syndrome and associated ciliopathies. Cilia, 1(1), 18. https://doi.org/10.1186/2046-2530-1-18
Tabrez, S. S., Sharma, R. D., Jain, V., Siddiqui, A. A., & Mukhopadhyay, A. (2017). Differential alternative splicing coupled to non-sense-mediated decay of mRNA ensures dietary restriction-induced longevity. Nature Communications, 8(1), 306. https://doi.org/10.1038/s41467-017-00370-5
Tallila, J., Jakkula, E., Peltonen, L., Salonen, R., & Kestila, M. (2008). Identification of CC2D2A as a Meckel syndrome gene adds an
important piece to the ciliopathy puzzle. *American Journal of Human Genetics*, 82(6), 1361–1367. https://doi.org/10.1016/j.ajhg.2008.05.004

Tallila, J., Salonen, R., Kohlschmidt, N., Peltonen, L., & Kestila, M. (2009). Mutation spectrum of Meckel syndrome genes: one group of syndromes or several distinct groups? *Human Mutation*, 30(8), E813–E830. https://doi.org/10.1002/humu.21057

Tsai, J. J., Hsu, W. B., Liu, J. H., Chang, C. W., & Tang, T. K. (2019). CEP120 interacts with C2CD3 and Talpid3 and is required for centriole appendage assembly and ciliogenesis. *Scientific Reports*, 9(1), 6037. https://doi.org/10.1038/s41598-019-42577-0

Uhlén, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., & Pontén, F. (2015). Proteomics. Tissue-based map of the human proteome. *Science*, 347(6220), 1260419. https://doi.org/10.1126/science.1260419

Veleri, S., Manjunath, S. H., Fariss, R. N., May-Simera, H., Brooks, M., Foskett, T. A., & Swaroop, A. (2014). Ciliopathy-associated gene Cc2d2a promotes assembly of subdistal appendages on the mother centriole during cilia biogenesis. *Nature Communications*, 5, 4207. https://doi.org/10.1038/ncomms5207

Vilboux, T., Doherty, D. A., Glass, I. A., Parisi, M. A., Phelps, I. G., Cullinane, A. R., & Gunay-Aygun, M. (2017). Molecular genetic findings and clinical correlations in 100 patients with Joubert syndrome and related disorders prospectively evaluated at a single center. *Genetics in Medicine*, 19(8), 875–882. https://doi.org/10.1038/gim.2016.204

Watson, C. M., Crinnion, L. A., Berry, I. R., Harrison, S. M., Lascelles, C., Antanaviciute, A., & Bonthron, D. T. (2016). Enhanced diagnostic yield in Meckel-Gruber and Joubert syndrome through exome sequencing supplemented with split-read mapping. *BMC Medical Genetics*, 17, 1. https://doi.org/10.1186/s12881-015-0265-z

Xiao, D., Lv, C., Zhang, Z., Wu, M., Zheng, X., Yang, L., & Chen, J. (2017). Novel CC2D2A compound heterozygous mutations cause Joubert syndrome. *Molecular Medicine Reports*, 15(1), 305–308. https://doi.org/10.3892/mmr.2016.6007

Xie, Z., Moy, L. Y., Sanada, K., Zhou, Y., Buchman, J. J., & Tsai, L. H. (2007). Cep120 and TACCs control interkinetic nuclear migration and the neural progenitor pool. *Neuron*, 56(1), 79–93. https://doi.org/10.1016/j.neuron.2007.08.026

Yates, A. D., Achuthan, P., Akanni, W., Allen, J., Allen, J., Alvarez-Jarreta, J., & Flicek, P. (2020). Ensembl 2020. *Nucleic Acids Research*, 48(D1), 688–688. https://doi.org/10.1093/nar/gkaa966

Yu, R. Z., Kim, T. W., Hong, A., Watanabe, T. A., Gaus, H. J., & Geary, R. S. (2007). Cross-species pharmacokinetic comparison from mouse to man of a second-generation antisense oligonucleotide, ISIS 301012, targeting human apolipoprotein B-100. *Drug Metabolism and Disposition*, 35(3), 460–468. https://doi.org/10.1124/dmd.106.012401

Zhao, Q., Zhou, R., Temsamani, J., Zhang, Z., Roskey, A., & Agrawal, S. (1998). Cellular distribution of phosphorothioate oligonucleotide following intravenous administration in mice. *Antisense and Nucleic Acid Drug Development*, 8(6), 451–458. https://doi.org/10.1089/oli.1.1998.8.451

Zhou, X., Edmonson, M. N., Wilkinson, M. R., Patel, A., Wu, G., Liu, Y., & Zhang, J. (2016). Exploring genomic alteration in pediatric cancer using ProteinPaint. *Nature Genetics*, 48(1), 4–6. https://doi.org/10.1038/ng.3466

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.