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ORIGINAL ARTICLE

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

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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.

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 dystrophy (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 multiorgan 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-Gagescu et al., 2012; Mougou-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-Gagescu 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-Gagescu 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 multiexon 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

now been well-established (Aartsma-Rus & van Ommen, 2007; Bennett & Swayze, 2010). The treatment of patients with Duchenne muscular dystrophy (DMD, MIM PS310200) by targeting 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., 2017; 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 JBTS, 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 encephalocele (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 JBTS (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.(Glu229del) (including 528 homozygous individuals) and 0.002 for p.(Pro721Ser) (including three homozygous carriers) (Table S5), suggesting that these are hypomorphic alleles

TABLE 1 Patients with biallelic *CEP120* variants and associated phenotypes (ranked by publication)

Family ID	Patient ID	Phenotype	Kidney phenotype (1)	Allele 1 (Ex,Int)	Allele 2 (Ex,Int)	Reference
1	Family 1_II:2	JATD (2)	n/a	c.595G>C; p.(Ala199Pro) (Ex6)	c.595G>C; p.(Ala199Pro) (Ex6)	Shaheen et al. (2015)
2	Family 2_II:4	JATD (2)	n/a	c.595G>C; p.(Ala199Pro) (Ex6)	c.595G>C; p.(Ala199Pro) (Ex6)	Shaheen et al. (2015)
3	Family 3_II:1	JATD (2)	yes	c.595G>C; p.(Ala199Pro) (Ex6)	c.595G>C; p.(Ala199Pro) (Ex6)	Shaheen et al. (2015)
4	COR391	JBTS	no	c.581T>C; p.(Val194Ala) (Ex6)	c.581T>C; p.(Val194Ala) (Ex6)	Roosing et al. (2016)
5	MTI-143	JBTS	no (3)	c.2177T>C; p.(Leu726Pro) (Ex16)	c.2134C>T; p.(Leu712Phe) (Ex16)	Roosing et al. (2016)
6	MTI-991	JBTS	no	c.49+5_49+10del; p.(Gly+1AspfsTer14) (Int2)	c.49+5_49+10del; p.(Gly+1AspfsTer14) (Int2)	Roosing et al. (2016)
7	MTI-1516	JBTS	no	c.1138_1139insA; p.(Ser380TyrfsTer19) (Ex9)	c.1646C>T; p.(Ala549Val) (Ex12)	Roosing et al. (2016)
8	MKS-2930	MKS/OFD	yes	c.2924T>G; p.(Ile975Ser) (Ex21)	c.2924T>G; p.(Ile975Ser) (Ex21)	Roosing et al. (2016)
9	SW-476410	TCDOE	no	c.451C>T; p.(Arg151Ter) (Ex5)	c.595G>C; p.(Ala199Pro) (Ex6)	Roosing et al. (2016)

Note: CEP120 transcript: NM_153223.3. JATD, Jeune asphyxiating thoracic dystrophy; JBTS, Joubert syndrome; MKS, Meckel syndrome; OFD, oro-facial-digital syndrome; TCDOE, tectocerebellar dysraphia with occipital encephalocele. (1) Designated as n/a, unless renal phenotype clearly stated. (2) A fourth JATD case was described (Shaheen et al., 2015), however DNA from the proband was not available. Both parents presented the *CEP120*: p.(Ala199Pro) variant in heterozygosis. (3) For this patient Roosing et al. (Roosing et al., 2016) reported grade II-III hydronephrosis detected at birth that spontaneously resolved after few months. No kidney problems have been reported since then.

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

TABLE 2 Patients with truncating *CC2D2A* variants in potentially skippable exons and associated phenotypes (ranked by publication)

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	UM10	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)	Mougou-Zerelli et al. (2009)
29	MKS-977	MKS	yes	c.3084del; p.(Lys1029ArgfsTer3) (Ex25)	c.3084del; p.(Lys1029ArgfsTer3) (Ex25)	Mougou-Zerelli et al. (2009)
33	MKS-365	MKS	yes	c.2773C>T; p.(Arg925Ter) (Ex22) (3)	c.2486+1G>C (Int20)	Mougou-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_1264insGGCATGTTTTGGC; p.(Ser422GlyfsTer19) (Ex13) (4)	c.3452T>C; p.(Val1151Ala) (Ex28)	Bachmann-Gagescu et al. (2012)
43	UW79-4	JBTS	no	c.1263_1264insGGCATGTTTTGGC; 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)

(Continues)

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_4InsGGCATGTTTTGGC; c.1268G>A; p.(Ser423Glyfs*19). (5) Study does not state potential extra-CNS manifestations. (6) Variant initially reported as c.4258G>A; p.(Arg1528His). This variant was corrected as c.4583G>A; p.(Arg1528His) (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).

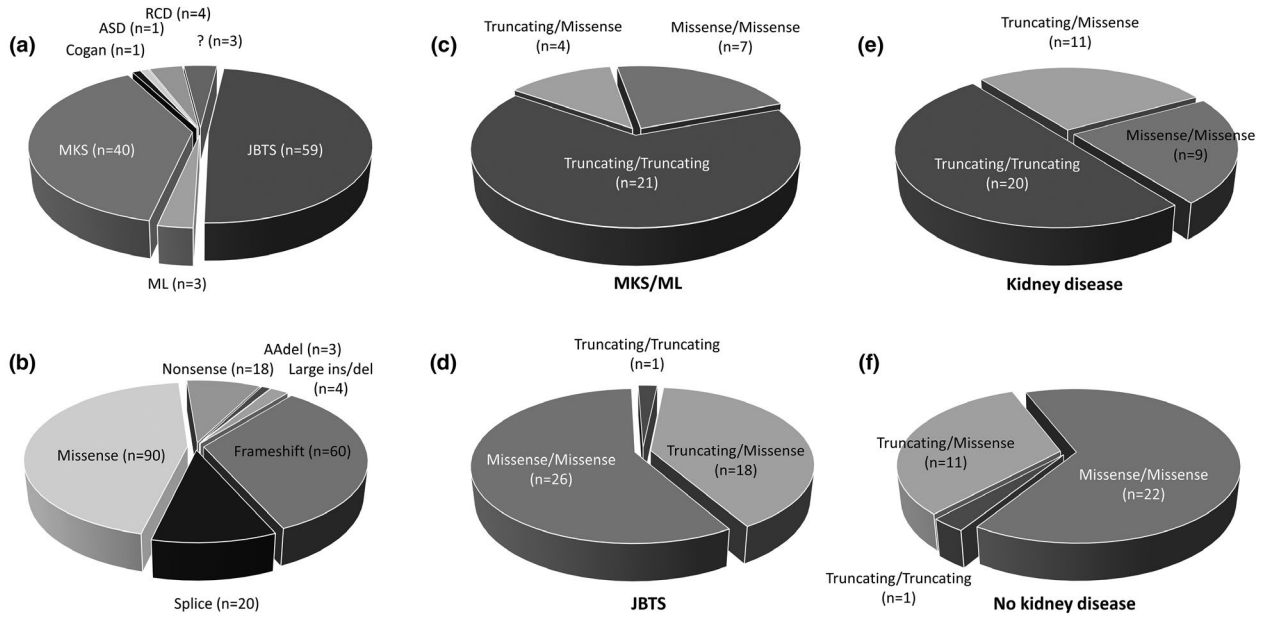

FIGURE 1 Phenotypes and genotypes in patients carrying biallelic *CC2D2A* variants. (a) Distribution of phenotypes associated with *CC2D2A* biallelic variants in reported patients. n indicates total number of patients. ASD, autism spectrum disorder; Cogan, Cogan-type congenital oculomotor apraxia; JBTS, Joubert syndrome; MKS, Meckel syndrome; ML, Meckel-like syndrome; RCD, rod cone dystrophy; ?, not unequivocally described. (b) Distribution of *CC2D2A* variant consequences detected in index patients. n indicates total number of alleles. Of note, in one family three different variants were reported with two different compound heterozygote combinations (see Table S3). AAdel, single amino acid deletion; Large ins/del, large insertions/deletion including retrotransposon insertion. (c) Distribution of *CC2D2A* allelic status detected in patients with Meckel syndrome or Meckel-like syndrome. (d) Distribution of *CC2D2A* allelic status detected in patients with Joubert syndrome. (e) Distribution of *CC2D2A* allelic status detected in patients with kidney disease. (f) Distribution of *CC2D2A* allelic status detected in patients without kidney disease. Truncating indicates either a nonsense or a frameshift variant

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 and cerebellum. 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(ii)). 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

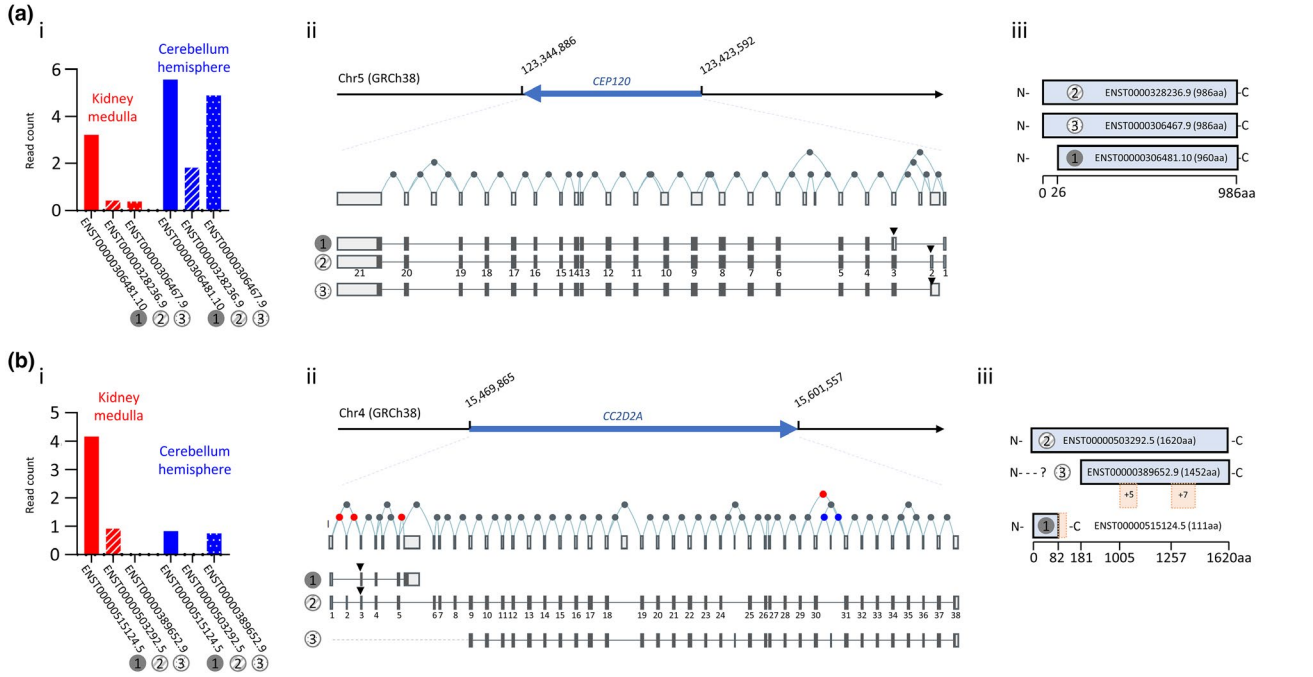

FIGURE 2 Exon usage and tissue specific transcript expression of *CEP120* and *CC2D2A*. (a(i)). *CEP120* predicted protein coding transcript isoforms with highest expression levels in kidney medulla (red) und cerebellar hemisphere (blue) based on RNA sequencing data from the Genotype-Tissue Expression (GTEx) Project. (a(ii)) *CEP120* genomic localization, the different exons detected in GTEx data with the different imputed splice junctions and the three transcripts detected at highest levels in kidney medulla and cerebellar hemisphere. Exons are labelled with respect to transcript ENST00000328236.9. The open reading frame is shown in dark grey with the start codon marked with an arrowhead. (a(iii)) Predicted protein products from the three analysed transcripts. (b(i)) *CC2D2A* predicted protein coding transcript isoforms with highest expression levels in kidney medulla (red) and cerebellar hemisphere (blue) based on RNA sequencing data from the Genotype-Tissue Expression (GTEx) Project. (b(ii)) *CC2D2A* genomic localization, the different exons detected in GTEx data with the different imputed splice junctions and the three transcripts detected at highest levels in kidney medulla and cerebellar hemisphere. Exons are labelled with respect to transcript ENST00000503292.5. The open reading frame is shown in dark grey with the start codon marked with an arrowhead. Junction reads enriched in the kidney medulla (compared to cerebellum) are marked in red and junction reads enriched in the cerebellum (compared to kidney) are marked in blue. (b(iii)) Predicted protein products from the three analysed transcripts, sequences deviating from reference sequence are depicted in orange. 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 15/05/2020

tissue-specific transcripts for *CEP120* and *CC2D2A*. Exons that are predicted to undergo organ-specific splicing events, such as exon 30 of *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 *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 *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 *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 *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

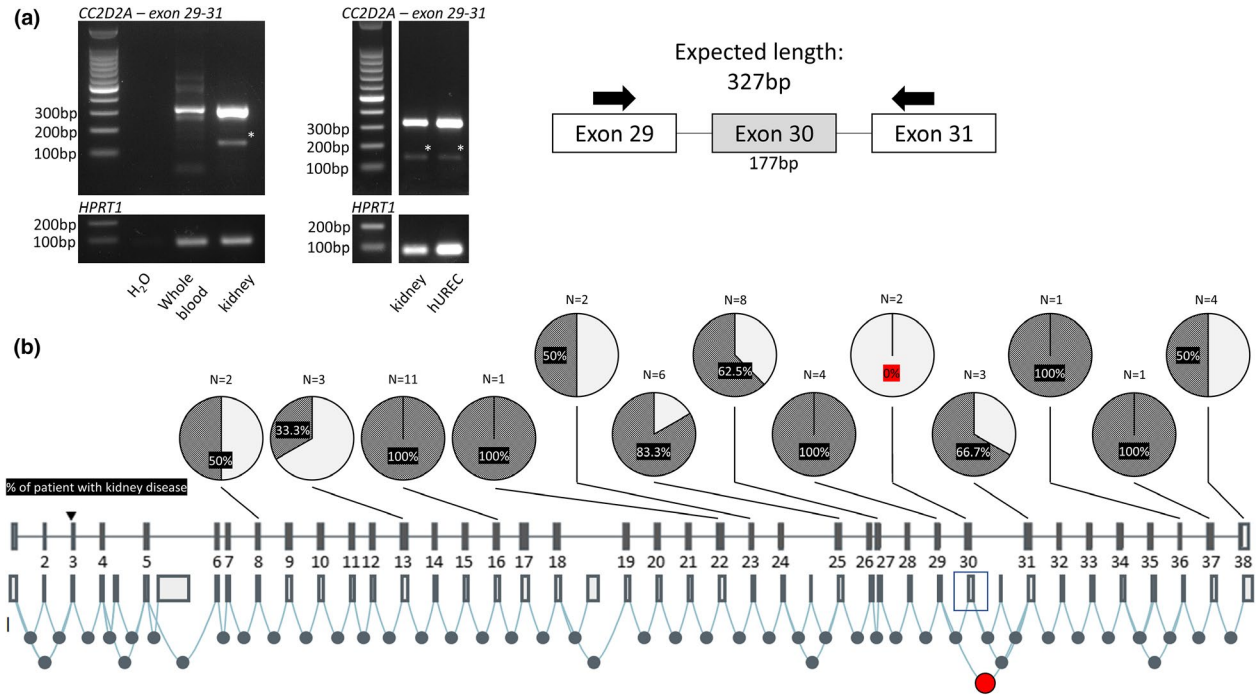

FIGURE 3 Basal exon skipping of *CC2D2A* exon 30 in kidney and human urine-derived renal epithelial cells (hURECs) and correlation with tissue specific disease expression. (a) RT-PCR using RNA isolated from human kidney, whole blood and hURECs. *CC2D2A* primer pair (arrows) designed to detect exon 30 skipping illustrated on the right. Note shortened transcript at ~150 bp (*asterisk) detected in kidney and hURECs suggesting basal exon 30 skipping. (b) Prevalence of kidney disease associated with truncating *CC2D2A* variants in different exons. Exons are labelled according to transcript ENST00000328236.9. The different exons and possible splice junctions detected in GTEx are shown below transcript ENST00000328236.9. The specific splice junction leading to basal exon 30 skipping in the kidney is marked in red. N indicates the total number of patients (with and without kidney disease) harbouring at least one truncating variants in the corresponding exons. 35/48 (73%) present with kidney disease. Note that both patients with a truncating variant in exon 30, undergoing basal exon skipping in the kidney, have no reported kidney disease (0%)

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 ENST00000306481.10) 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.

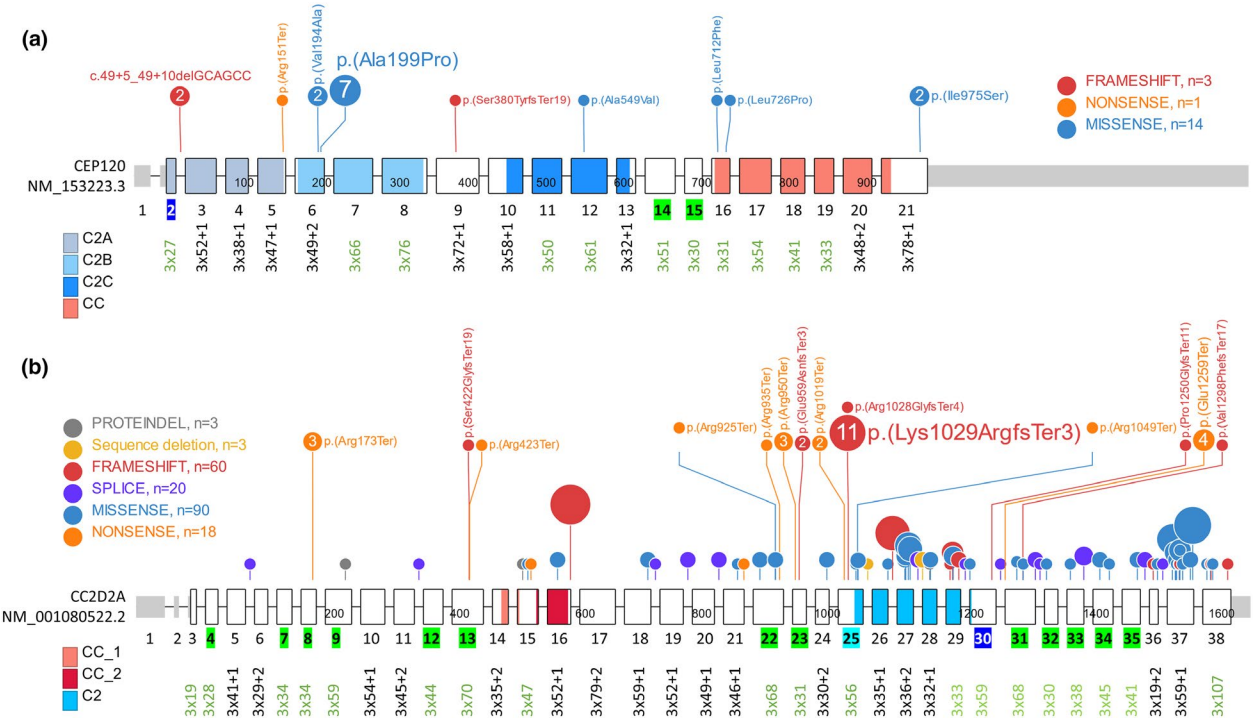

FIGURE 4 Distribution of mutations in *CEP120* and *CC2D2A* and identification of potential targets for exon skipping. (a) *CEP120* mRNA (NM_153223.3) and exon structure with UTR in grey. Exon numbers are shown below exons with nucleotide numbers in multiples of three below the exon numbers. Exact multiples of three are shown in green. Protein domains are shown in colour-code for coiled-coil domain (CC) and the 3 C2 domains. Detected *CEP120* variants are painted above the mRNA structure with respect to their location and allelic frequency in index patients (disc size reflecting allelic frequency). Variant consequences are colour-coded as indicated. Exon numbers that appear as candidates for exons skipping based on nucleotide numbers and domain functions are shaded in green, while candidates arising from tissue-specific transcript analysis are shaded in blue. (b) *CC2D2A* mRNA (NM_001080522.2) and exon structure with UTR in grey. Exon numbers are shown below exons with nucleotide numbers in multiples of three below the exon numbers. Exact multiples of three are shown in green. Protein domains are shown in colour-code for coiled-coil domain 1 and 2 (CC) and the C2 domain. Exon numbers that appear as candidates for exons skipping based on nucleotide numbers and domain functions are shaded in green, candidates arising from tissue-specific transcript analysis are shaded in blue and possible candidate based on conflicting domain annotation shaded in turquoise. Only truncating *CC2D2A* variants that are reported in candidate exons for exon skipping are painted above the mRNA structure with respect to their location and allelic frequency in index patients (disc size reflecting allelic frequency). Variant consequences are colour-coded as indicated. The retrotransposon insertion described in one family is not represented

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 truncating variants, in any of the *CC2D2A* skippable exons. Furthermore, all *CC2D2A* truncating variants falling into any of the predicted skippable exons are represented in Figure 4b. 14 different truncating variants in *CC2D2A* represent potential targets for exon skipping and are located in exons 8, 13, 22, 23, 25, 30 and 31 (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 *TMEM67* 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 extend 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 (Zhou et al., 2016).

PubMed: https://pubmed.ncbi.nlm.nih.gov/

SMART: http://smart.embl-heidelberg.de/

Varsome®: https://varsome.com/

Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), (Hamosh et al., 2000). World Wide Web URL: https://omim.org/

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- AGGCACTGACGATTTGGAAAC -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- TACAGCAGTAGTGCGCTTGC -3 and 5- GGGAAATGCCGACCTCACAG -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.

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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).

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SUPPORTING INFORMATION

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

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