Expression Patterns of Ciliopathy Genes ARL3 and CEP120 Reveal Roles in Multisystem Development

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Abstract

Joubert syndrome and related disorders (JSRD) and Jeune syndrome are multisystem ciliopathy disorders with overlapping phenotypes. There are a growing number of genetic causes for these rare syndromes, including the recently described genes *ARL3* and *CEP120*. We sought to explore the developmental expression patterns of *ARL3* and *CEP120* in humans to gain additional understanding of these genetic conditions. We used an RNA *in situ* detection technique called RNAscope to characterise *ARL3* and *CEP120* expression patterns in human embryos and foetuses in collaboration with the MRC-Wellcome Trust Human Developmental Biology Resource. This study provides insights into the potential pathogenesis of JSRD by uncovering the spatial expression of two JSRD-causative genes during normal human development.

Introduction

Joubert syndrome and related disorders (JSRD) are a group of autosomal inherited ciliopathies that are characterised as a cerebello-retinal-renal phenotype, and have an incidence rate of 1:80,000-100,000 live births (1–3). The hallmark brain phenotype is a “molar tooth sign” shown on axial brain MRI, caused by cerebellar vermis hypoplasia and other mid and hindbrain malformations (4). These defects often cause symptoms of hypotonia, ataxia and intellectual disability in patients (5). The retinal and renal phenotypes associated with JSRD have a lower incidence rate and vary in severity. Renal disorders occur in ~ 25% of patients, often presenting as corticomedullary cysts, interstitial fibrosis, or tubulointerstitial kidney disease (5). The renal component is progressive and can lead to end-stage renal disease (6). Ocular phenotypes of retinitis dystrophy, retinitis pigmentosa, oculomotor apraxia, and ptosis are common in patients, and as with the renal aspects of JSRD are often progressive in nature (7).

Currently, there are more than 35 genes that are known to cause JSRD (https://www.omim.org/phenotypicSeries/PS213300). The syndrome is caused by defects of the primary cilia, which are found on most mammalian cells (8). Primary cilia act as a cellular antenna to transduce extracellular signals such as mechanical flow, chemical stimulation, and key signalling pathways (including Hedgehog, Wnt, and PDGF) into the cell (9–13). Due to the multi-organ involvement, varying phenotypes, and multitude of genes known to cause JSRD there is great heterogeneity within the syndrome and overlap with closely related ciliopathies including Bardet-Biedl syndrome (https://omim.org/phenotypicSeries/PS209900) and Jeune syndrome (https://omim.org/phenotypicSeries/PS208500) (14). Recently discovered genetic causes of JSRD include *ARL3* (15) and *CEP120* (16, 17); the fact that their encoded proteins have such divergent roles within the primary cilium demonstrates the complexity underlying this group of related disorders.

ADP-ribosylation factor-like 3 (ARL3), a RAS superfamily member, is a low molecular weight GTP-binding protein (18) that cycles between inactive GDP-bound and active GTP-bound states to release cargo from their carriers in the cilium (19). ARL3 interacts with its Guanine Exchange Factor ARL13B in the cilium (20) and GTPase Activating Protein RP2 at the basal body of the cell (21, 22). *Arl3* knockout studies in

mice demonstrate a multi-organ ciliopathy phenotype, including kidney cysts, liver fibrosis and retinal disease with photoreceptor cell degeneration (23–26). Recently, two families affected by JSRD have been identified, presenting with ciliopathy phenotypes (15). The underlying genetic cause was shown to be missense mutations in *ARL3*, which affect an amino acid residue involved in the interaction between ARL3 and ARL13B (15).

Centrosomal protein of 120 kDa (CEP120) is a centrosomal protein involved in centriole biogenesis, including centriole duplication, assembly (27, 28), elongation (29–31) and maturation (28). CEP120 also interacts with other centrosomal proteins including CPAP (29, 30), SPICE1 (29), Talpid3 (28, 31) and C2CD3 (31). CEP120 was found to be expressed ubiquitously in murine embryonic tissues such as the brain, kidney and lungs. Additionally, Cep120 was observed to be highly expressed in embryonic mouse brain compared to postnatal or adult mouse brain (32). Inactivation of *CEP120* in the mouse central nervous system results in hydrocephalous and cerebellar hypoplasia (28). *CEP120* mutations have been shown to cause JSRD and Jeune syndromes (16, 17), and overlapping ciliopathy phenotypes such as tectocerebellar dysraphia with occipital encephalocele (TCDOE), Meckel syndrome (MKS) and oral-facial-digital (OFD) syndromes (17).

The developmental expression patterns of *ARL3* and *CEP120* in humans is not known. In order to explore this, we used an RNA *in situ* detection technique called RNAscope to compare and contrast the developmental spatial expression of these new and divergent causes of JSRD. We successfully characterised *ARL3* and *CEP120* expression patterns in human embryos and foetuses in collaboration with the MRC-Wellcome Trust Human Developmental Biology Resource (HDBR). This study provides insights into the potential pathogenesis of JSRD by uncovering the expression pattern of two JSRD-causative genes during normal human development.

Materials And Methods

RNAscope studies

Characterisation of *ARL3* and *CEP120* expression patterns was performed in human embryonic tissue using samples obtained from the MRC-Wellcome Human Developmental Biology Resource (HDBR). Formalin fixed paraffin embedded (FFPE) sections of human embryonic and foetal tissue were used. FFPE sections were prepared using 10% neutral buffered formalin and fixed for 32 h at room temperature. Samples were then prepared for the RNAscope assay, a RNA *in situ* detection platform for detection of target RNA within intact cells as per manufacturers' instructions (33, 34). An RNAscope 2.5 Assay RED was employed with 20 paired probes across nucleotides 169–1570 (NM_004311.3) and 115–1133 (NM_001166226.1) for detection of *ARL3* and *CEP120*, respectively and counterstained with Methyl Green.

Whole human embryo sections of 8 post-conception weeks (8PCW, equivalent to Carnegie Stage 23) were analysed, along with hindbrain (14PCW and 19PCW), eye (14PCW), kidney and adrenal gland (14PCW

and 18PCW). A negative RNAscope 2.5 HD Assay Red control (*dapB*, a bacterial gene which is not expressed in human tissues) was performed (Supplementary Fig. 1). In addition, the RNAscope 2.5 HD Assay RED was performed with a positive control (*KI67*, a cell proliferation marker). KI67 is a nuclear protein commonly used as a proliferation marker, which is expressed in cycling cells and is associated with cellular proliferation. It is encoded by the gene: marker of proliferation Ki-67, *MKI67. ARL3* and *CEP120* human expression patterns were analysed using the HDBR image server (Leica Biosystems).

Clinical phenotypes and sequence analysis

Reported clinical phenotypes associated with *ARL3* and *CEP120* mutations were reviewed within OMIM (https://omim.org/). Putative Arl3 and Cep120 orthologues were identified using BLASTP, with human *ARL3* (isoform a, NP_004302.1) and *CEP120* (NP_694955.2) transcripts as the query sequences within NCBI (https://www.ncbi.nlm.nih.gov/). Additional databases including Flybase https://flybase.org/), Wormbase (https://www.wormbase.org/) and Phytozome (https://phytozome.jgi.doe.gov/) were also queried using BLAST.

Results

Clinical phenotypes of ARL3 and CEP120 patients

Biallelic mutations in both *ARL3* and *CEP120* mutations are rare causes of ciliopathy syndromes. A comparison of the known phenotypes associated with *ARL3* and *CEP120* mutations is shown in Table 1. This overview reveals that mutations in *CEP120* are at present associated with severe phenotypes including Meckel syndrome (MKS) but also that single heterozygous changes in *ARL3* are sufficient to cause retinal-limited phenotypes. *ARL3* is highly conserved, with homologs present in *C. elegans*, *C. reinhardtii* and *D. melanogaster* whereas *CEP120* appears not to have homologs within these lower organisms (Supplementary Table 1). Known protein localisation within the cell of both ARL3 within the ciliary axoneme, and CEP120 in the centrosomes, are consistent with their role in ciliopathy syndromes (Supplementary Table 2).

Table 1
Comparison of the known phenotypes associated with *ARL3* and *CEP120* mutations

	Patients with *ARL3* related ciliopathy	**Patients with *CEP120* related ciliopathy**
Number of affected patients reported and presenting phenotypes	4 patients reported with JSRD secondary to biallelic changes (15) 4 patients with retinitis pigmentosa secondary to monoallelic changes (48, 49)	4 patients with JSRD 4 patients with JATD 1 foetus with MKS 1 foetus with TCDOE (16, 17)
Brain imaging findings	Molar tooth sign	Molar tooth sign
Intellect	Developmental delay Psychomotor delay	Developmental delay Cognitive impairment
Skeletal	No known associated phenotypes	Severely narrow chest Skeletal dysplasia Small and horizontal ribs Short limbs Polydactyly Synpolydactyly
Mobility	Ataxic gait Hypotonia	Truncal ataxia Hypotonia

JSRD, Joubert syndrome and related disorders; JATD, Jeune asphyxiating thoracic dystrophy; MKS, Meckel syndrome; TCDOE, tectocerebellar dysraphia with occipital encephalocele.

Abbreviations: Ganglion cell layer (GCL), Inner nuclear layer (INL), Inner plexiform layer (IPL), Nerve fibre layer (NFL), Outer nuclear layer (ONL), Outer plexiform layer (OPL) Outer neuroblastic layer (ONbL), Retinal pigment epithelium (RPE).

	Patients with *ARL3* related ciliopathy	Patients with *CEP120* related ciliopathy
Eye	Night blindness	Microphthalmia
	Bilateral vision loss	Duane syndrome
	Retinal dystrophy	Strabismus
	Ocular motor apraxia	
Kidney	Cystic dysplastic kidney	Cystic dysplastic kidney
	Bilateral renal scarring	
	Recurrent urinary tract infections	
JSRD, Joubert syndrome and related disorders; JATD, Jeune asphyxiating thoracic dystrophy; MKS, Meckel syndrome; TCDOE, tectocerebellar dysraphia with occipital encephalocele.		
Abbreviations: Ganglion cell layer (GCL), Inner nuclear layer (INL), Inner plexiform layer (IPL), Nerve fibre layer (NFL), Outer nuclear layer (ONL), Outer plexiform layer (OPL) Outer neuroblastic layer (ONbL), Retinal pigment epithelium (RPE).		

ARL3 and CEP120 are expressed in early human brain development

In 8PCW human brain tissue, the expression of *ARL3* and *CEP120* is remarkably similar. There is expression of both genes in the choroid plexus (Fig. 1Ai and 1Bi), which appears to favour the luminal facing surface of the tissue. The cell proliferation marker *KI67* does not share this same expression pattern in the choroid plexus (Fig. 1 Ci). This specific expression pattern of *ARL3* and *CEP120* in luminal-facing cells is continued throughout the developing brain where both genes exhibit expression throughout the ventricular zone of the ganglionic eminence, cortical plate, and the hindbrain (Fig. 1Aii-Aiv and 1Bii-Biv). There is specific expression of *ARL3* and *CEP120* on the outermost layer of cells in each tissue, facing into the ventricular space. Expression of *KI67* is seen throughout these tissues (Fig. 1Cii-Civ), with specific expression in the outermost cells consistent with proliferation of cells in the ventricular zone (35).

Expression of ARL3 and CEP120 is maintained in the developing cerebellum

In the human cerebellum at 14PCW there is expression of *ARL3* and *CEP120*. Both genes have strong expression in all cells of the developing cerebellum (Fig. 2Ai and Ci). Expression in cell bodies is also seen at 19PCW for *ARL3* (Fig. 2Bi) however, *CEP120* expression is seen in the molecular layer (ML) of the cerebellum, as well as in the cell bodies at 19PCW (Fig. 2Di). Expression of *KI67* is seen throughout the cerebellum at 19PCW, indicating the tissue is proliferative (Fig. 2Ei). *ARL3* and *CEP120* are therefore widely expressed in the cerebellum during development, with specific expression of *CEP120* in the

molecular layer which is where the dendritic trees of Purkinje cells and their interacting parallel fibres are found; suggesting a role for *CEP120* in these cell types (36).

Expression of ARL3 and CEP120 in the developing eye

The human retina can be divided into nine layers based upon the cell types that occupy each layer (Fig. 3A), with the retinal pigment epithelial (RPE) and photoreceptor layers at the outermost part of the eye (37). At 8PCW, the retinal layers are not well defined with only a ganglion cell layer separated from a layer of mostly immature neuroblasts with a few photoreceptor cells by a thin inner plexiform layer (38). At this stage *ARL3* and *CEP120* show expression throughout the developing retina, with high expression within the retinal ganglion cells and the photoreceptor layer (Fig. 3Bi and Di). At 14PCW, the retinal layers are maturing (37) which is reflected in the expression pattern of both *ARL3* and *CEP120.* Clear expression of both genes is still seen in all layers of the retina, although to a lesser extent in the plexiform and nerve fibre layers due to reduced cell density in these areas (Fig. 3 Ci and Ei).

Expression of ARL3 and CEP120 in the developing dorsal root ganglia

The dorsal root ganglia are formed by migrating neural crest cells and contain most of the body's sensory neurones (39, 40). Both *ARL3* and *CEP120* show expression in cells of the dorsal root ganglia, which are post-mitotic primary sensory neurons (Fig. 4Ai-Aii and Bi-Bii). There is strong expression of *KI67* within the dorsal root ganglia (Fig. 4 Ci-Cii).

Expression of ARL3 and CEP120 in the developing kidney

In the developing human kidney at 8PCW, where there is strong renal cortical staining of *KI67* indicating cell proliferation (Supplementary Fig. 2), there is expression of *ARL3* in cells within the developing cortical nephrons; this expression appears to be specifically oriented to the lumen of the structures (Fig. 5Ai). This expression pattern is maintained at 14PCW (Fig. 5Bi) and 18PCW, though overall expression of *ARL3* appears to have decreased at this time point (Fig. 5 Ci). Expression of *CEP120* is also seen in developing nephrons at 8PCW, however there is also expression in the renal cortex (Fig. 5Di). This expression pattern of *CEP120* is maintained at 14PCW (Fig. 5Ei) and 18PCW, although overall expression appears to have decreased at this time point (Fig. 5Fi).

Expression of ARL3 and CEP120 in other major organs

In the developing human heart, lung and gut at 8PCW, there is very low levels of expression of *ARL3* and *CEP120* (Supplementary Fig. 3). Expression of *ARL3* and *CEP120* is seen around the developing alveoli and at low levels in the developing bowel epithelia. The remaining organs of the developing embryo did not reveal prominent expression patterns.

Discussion

Mutations in *ARL3* and *CEP120* are rare and relatively new causes of JSRD and other related ciliopathies. Human protein atlas data suggests that tissue expression of ARL3 protein is widely expressed, with highest expression scores seen in cerebellum and lowest in heart and skeletal muscle (https://www.proteinatlas.org/ENSG00000138175-ARL3/tissue). RNA expression is high in cerebral cortex, cerebellum, retina and kidney consistent with its known phenotypes. CEP120 protein expression is not annotated within the human protein atlas, whereas RNA is strongly expressed in the cerebellum (https://www.proteinatlas.org/ENSG00000168944-CEP120/tissue). We aimed to define expression of *ARL3* and *CEP120* during human development using the HDBR tissue bank employing a relatively new in situ hybridisation assay called RNAscope for the detection of target RNA within intact cells. Our data provide an insight into the developmental expression of *ARL3* and *CEP120*. We show that both of these genes are expressed in key tissues (including retina, cerebellum and kidney) during development. This expression pattern fits with the multisystem disease phenotypes seen in patients with *ARL3* and *CEP120* mutations (Table 1). A similar approach, using the valuable HDBR tissue bank has been performed, using *in situ* hybridisation for studying the expression of *ARL13B* (41), another cause of Joubert syndrome. Here *ARL13B* was detected at stage CS16 in the alar and basal plate of the myelencephalon, the mesencephalon and the metencephalon. At CS19 *ARL13B* was seen in the ventricular layer of the diencephalon and myelencephalon, the tegmentum of the pons and the cerebellar rhombic lips as well as the dorsal root ganglia. This pattern of expression is remarkably similar to the *CEP120* and *ARL3* data described here.

Expression of both *ARL3* and *CEP120* was minimal in developing cardiac, lung and gut tissues, consistent with lack of known phenotypes affecting these organ systems (Supplementary Fig. 3). *ARL3* and *CEP120* encode proteins that are expressed in the primary cilia and basal body respectively (Supplementary Table 2) and pathogenic variants result in similar and overlapping phenotypes, including the cerebello-retinal-renal syndrome JSRD (Table 1). The number of patients with pathogenic variants in either *ARL3* or *CEP120* remains small, allowing a limited comparison of phenotypes, although skeletal manifestations (in particular short ribs/asphyxiating thoracic dystrophy phenotypes) seen in patients with *CEP120* mutations have not been documented in patients with *ARL3* mutations.

There were notable differences in evolutionary conservation between *ARL3* and *CEP120* (Supplementary Table 1). The ARL3 human protein shares greater than 90% identity with its two orthologous sequences (there is genomic duplication of *arl3*) in *Danio renio* (zebrafish), a well-studied model species in vertebrates. In contrast, CEP120 human protein only shares 57% identity with its single orthologous sequence found in zebrafish. Moreover, human ARL3 protein shares > 60% identity with its orthologues found in *Drosophila melanogaster*, *Caenorhabditis elegans* and *Chlamydomonas reinhardtii*. CEP120 is conserved in some vertebrate organisms but orthologues were not readily identified in invertebrates. There is a putative *CEP120* orthologue, UNI2, found in *Chlamydomonas reinhardtii*, but this has not as yet been confirmed as a functional ortholog (27, 42). ARL3 is described in diverse eukaryotic organisms such as *Leishmania donovani* (43) and *Caenorhabditis elegans* (44, 45) where it has a functional role in the cilium/flagella. Despite these differences in evolutionary conservation, our results show that *ARL3* and *CEP120* have similar expression patterns during human development, specifically in the eye and dorsal

root ganglia as well as during early brain development. Both genes are expressed throughout the retina during development, with expression in the RPE and photoreceptor layers, suggesting a role for both genes during retinal development. This is further supported by the numerous retinal phenotypes associated with mutations in *ARL3* (15–17). Similarly, the specific expression of *ARL3* and *CEP120* in the dorsal root ganglia hints at a role for both genes in primary sensory neurone migration and differentiation. A recurring pattern was the expression of both mRNAs on the luminal facing surface of the cerebral tissue (seen in the choroid plexus, cortical plate, ganglionic eminence, and hindbrain) which could suggest a sensory role for both the genes and within the cilium of the ventricular lining of the brain.

Expression of *ARL3* and *CEP120* changes during development notably in the cerebellum and kidney. *ARL3* and *CEP120* are expressed throughout the cerebellum at 14PCW however, at 19PCW *CEP120* was also expressed in the ML of the cerebellum. This could imply that *ARL3* and *CEP120* are expressed in different cell populations of the cerebellum as the ML contains parallel fibres and the dendrites of Purkinje cells, whereas the rest of the cerebellum is largely made up of granule cells (36). It has been previously reported in mouse studies that *Cep120* is required for proliferation of cerebellar neural progenitor cells (28) and is required for correct development of the embryo. Taken with these results, it suggests that *CEP120* is required for correct development of the cerebellum in humans.

Expression of *ARL3* and *CEP120* also differed in the developing kidney. The results showed that *ARL3* was specifically expressed in cells of the nephrons whereas *CEP120* was expressed in the nephrons as well as within cells in the developing renal cortex. This difference in expression could imply that *ARL3* has a more sensory/signalling function in luminal structures of the kidney, whereas *CEP120* has a more universal role in all cells as it is expressed more ubiquitously throughout the tissue.

The differences in gene expression may reflect the divergent functions of ARL3 and CEP120 proteins (Supplementary Table 2). As ARL3 is a trafficking protein involved in ciliary signalling (19, 46), it may only be expressed in actively signalling cells during certain points in development such as nephron progenitors and cells in the IGCL. In contrast, CEP120 is involved in building the centriole, and therefore cilium, (27, 29) and so will be expressed more widely within tissues, especially those with ciliated epithelia (47, 48).

In conclusion, we establish in human embryonic tissue expression patterns of *ARL3* and *CEP120* during development and provide insights into the wide phenotypic spectrum of mutations affecting *ARL3* and *CEP120* in humans. These studies will allow further investigations into tissue-specific mechanistic roles of *ARL3* and *CEP120* in human health and disease.

Declarations

Ethics approval and consent to participate

This study was conducted with full ethical approval. For human embryonic and foetal tissue samples, the samples were collected with appropriate maternal consents and ethical approval by the Newcastle and North Tyneside 1 Research Ethics Committee, UK.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

LP and MB-G analysed and interpreted the data regarding the RNA expression studies. GJC, LAD, SAR and CGM performed data analysis and contributed in writing the manuscript. JAS conceived the project. All authors read and approved the final manuscript.

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Tables

Table 1. Comparison of the known phenotypes associated with *ARL3* and *CEP120* mutations

JSRD, Joubert syndrome and related disorders; JATD, Jeune asphyxiating thoracic dystrophy; MKS, Meckel syndrome; TCDOE, tectocerebellar dysraphia with occipital encephalocele.

	Patients with *ARL3* related ciliopathy	Patients with *CEP120* related ciliopathy
Number of affected patients reported and presenting phenotypes	4 patients reported with JSRD secondary to biallelic changes(15) 4 patients with retinitis pigmentosa secondary to monoallelic changes(48, 49)	4 patients with JSRD 4 patients with JATD 1 foetus with MKS 1 foetus with TCDOE (16, 17)
Brain imaging findings	Molar tooth sign	Molar tooth sign
Intellect	Developmental delay Psychomotor delay	Developmental delay Cognitive impairment
Skeletal	No known associated phenotypes	Severely narrow chest Skeletal dysplasia Small and horizontal ribs Short limbs Polydactyly Synpolydactyly
Mobility	Ataxic gait Hypotonia	Truncal ataxia Hypotonia
Eye	Night blindness Bilateral vision loss Retinal dystrophy Ocular motor apraxia	Microphthalmia Duane syndrome Strabismus
Kidney	Cystic dysplastic kidney Bilateral renal scarring Recurrent urinary tract infections	Cystic dysplastic kidney

Figures

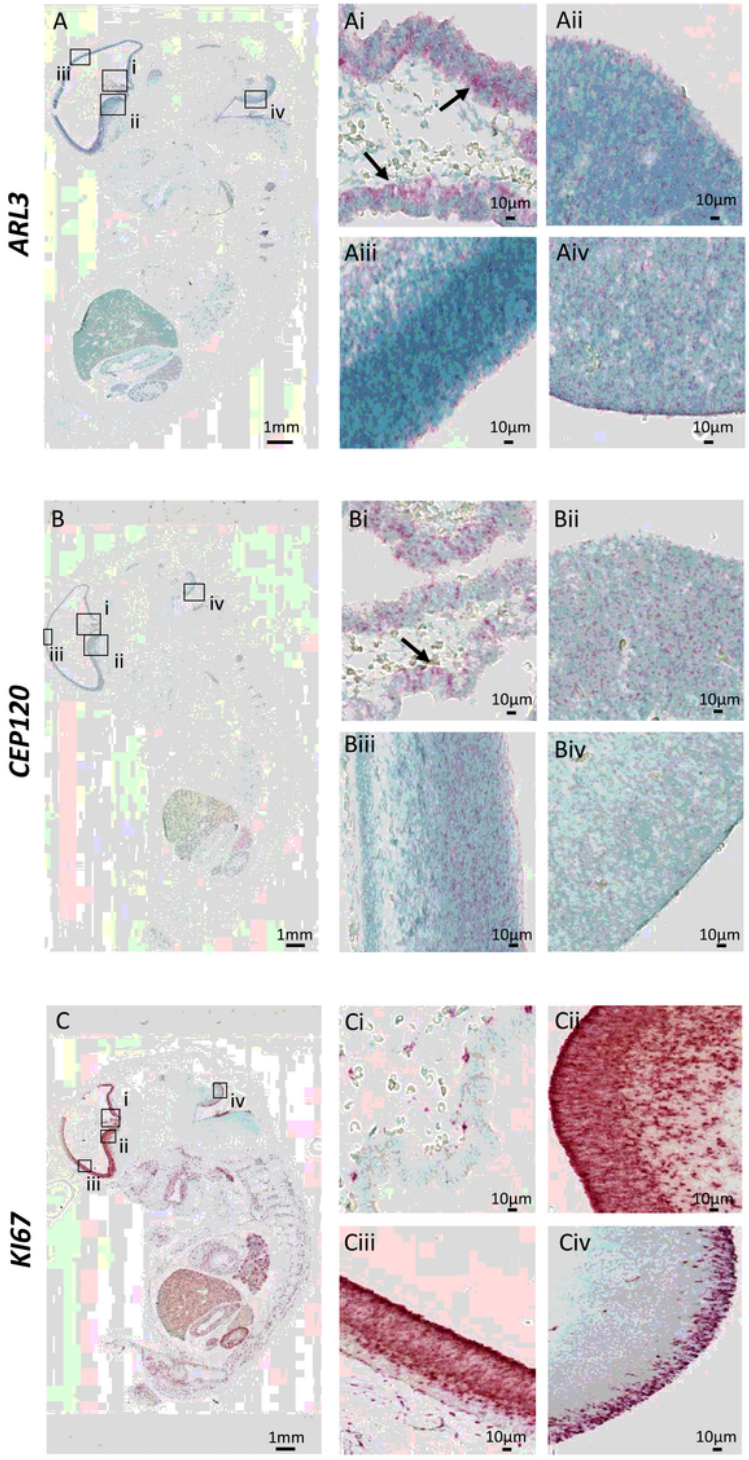

Figure 1

Expression pattern of ARL3 and CEP120 in the human brain during early development Sagittal sections of 8PCW-stage human embryos stained using RNAscope to show expression of ARL3 (A) (red), CEP120 (B) (red) and KI67 (C) (red), counterstained with Methyl Green. (Ai and Bi) ARL3 and CEP120 are

expressed within cells of the choroid plexus (arrow). (Ci) KI67 expression is minimal in the choroid plexus. (Aii-Aiv) and (Bii-Biv) Expression of ARL3 and CEP120 is seen in the ventricular radial glia progenitor cells including the ventricular zone of ganglionic eminence (Aii and Bii), cortical plate (Aiii and Biii), and hindbrain (Aiv and Biv). (Cii-Civ) Expression of KI67 is seen in the ventricular zone of the ganglionic eminence, cortical plate, and hindbrain.

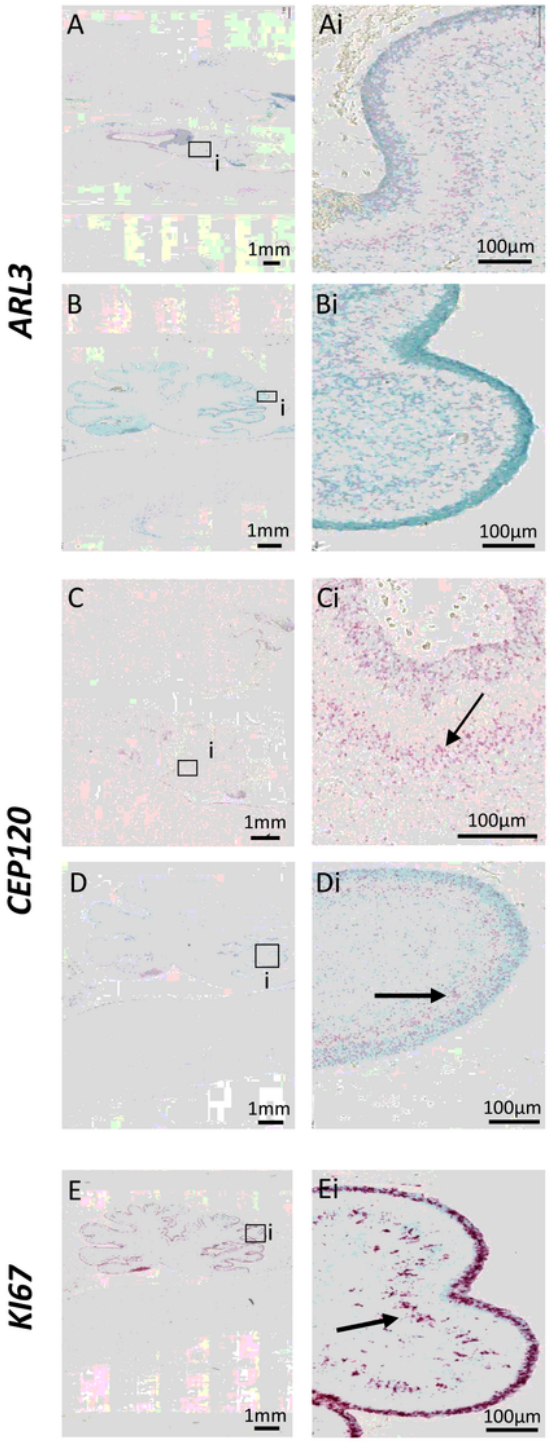

Figure 2

Expression of ARL3 and CEP120 in the developing human hindbrain Sagittal sections of 14PCW (A and C) and 19PCW (B, D and E) human brain stained using RNAscope to show ARL3 expression (A and B) (red), CEP120 (C and D) (red) and KI67 (E) (red), countered stained with Methyl Green. (Ai and Ci) Expression of ARL3 and CEP120is evident in the cerebellumat 14PCW.(B and D) ARL3 and CEP120 expression in the cerebellum at 19PCW. (Bi) Cerebellar expression of ARL3.(Di)Cerebellar molecular layer (arrow) expression of CEP120. (E and Ei) Hindbrain KI67 expression at 18PCW shows tissue is proliferative.

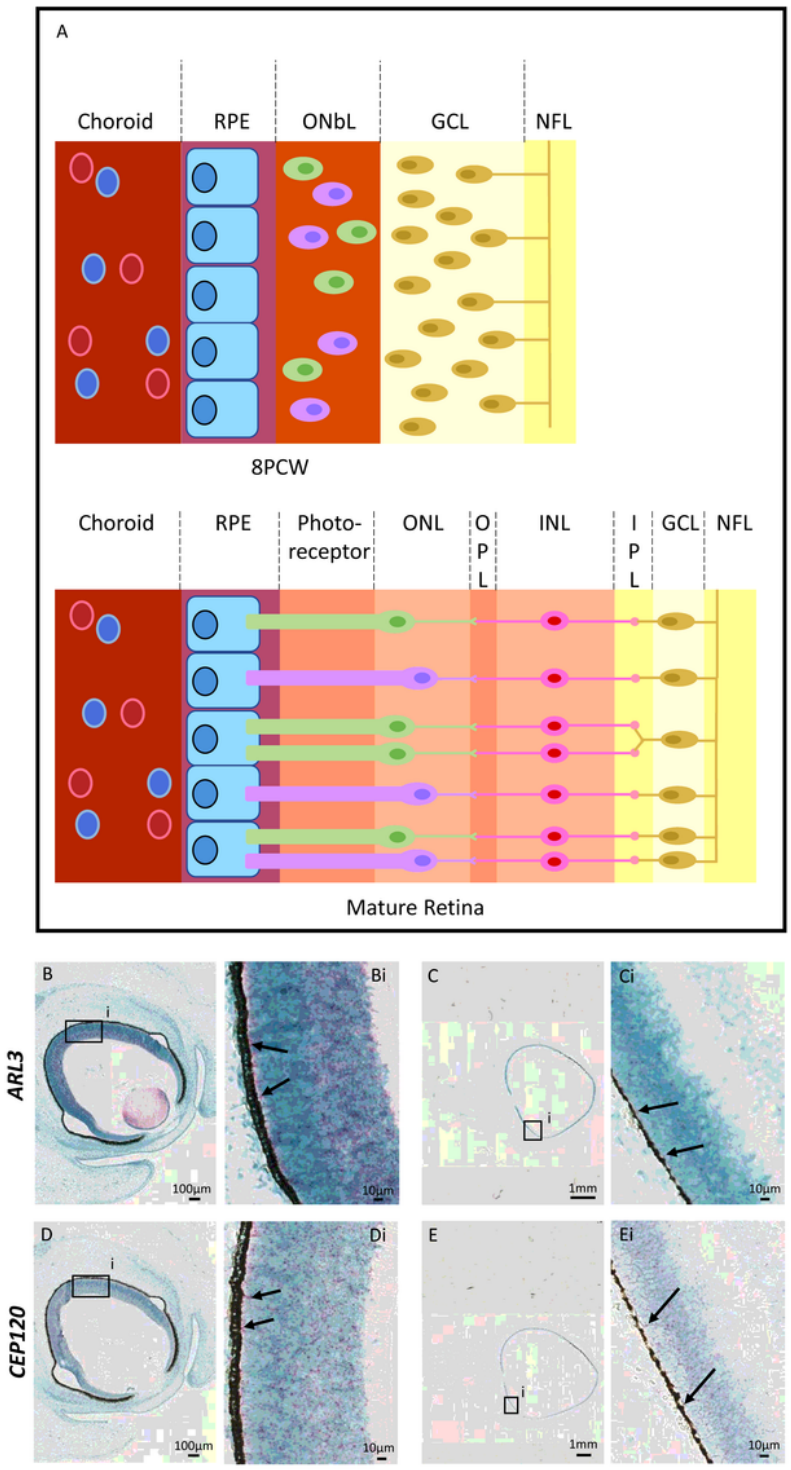

Figure 3

Expression of ARL3 and CEP120 in the developing human retina (A) Schematic diagram of the development of the layers of the retina from 8PCW to the mature form (adapted from (38)). At 8PCW, not all of the layers are present in the retina. The ONbL is a transitionary layer containing retinal progenitor cells that will develop into various cell types such as photoreceptors, amacrine and bipolar cells; separating into the ONL, OPL, INL, and IPL (the IPL is sometimes visible at 8PCW). The GCL is thicker at 8PCW due to the migration of cells. The mature retina can be divided into 8 layers. The RPE is at the very back of the eye and assists in the removal of waste products from the photoreceptor cells, which transduce light. The ONL, OPL, INL and IPL layers house intermediary cell bodies and dendrites that interact with ganglion cells in the GCL to convey the signal through the optic nerve, formed in the NFL, to the brain (reviewed in (51)). (B)Human sections of developing eye at 8PCW (B and D) and 14PCW (C and E) stained using RNA Scope to show ARL3 expression (B and C) (red) and CEP120 (D and E) (red) counterstained with Methyl Green(Bi and Di).There is a gradient of ARL3 (Bi) and CEP120(Di) expression in the retina at 8PCWacross multiple retinal layers including the ONbL. The RPE layer, a black pigmented layer is show by arrows.At 14 PCW, ARL3 (Ci) and CEP120(Ei)expression is localised across multiple layers including the photoreceptor cell layer, just below the RPE layer (arrows). Abbreviations: Ganglion cell layer (GCL), Inner nuclear layer (INL), Inner plexiform layer (IPL), Nerve fibre layer (NFL), Outer nuclear layer (ONL), Outer plexiform layer (OPL) Outer neuroblastic layer (ONbL), Retinal pigment epithelium (RPE).

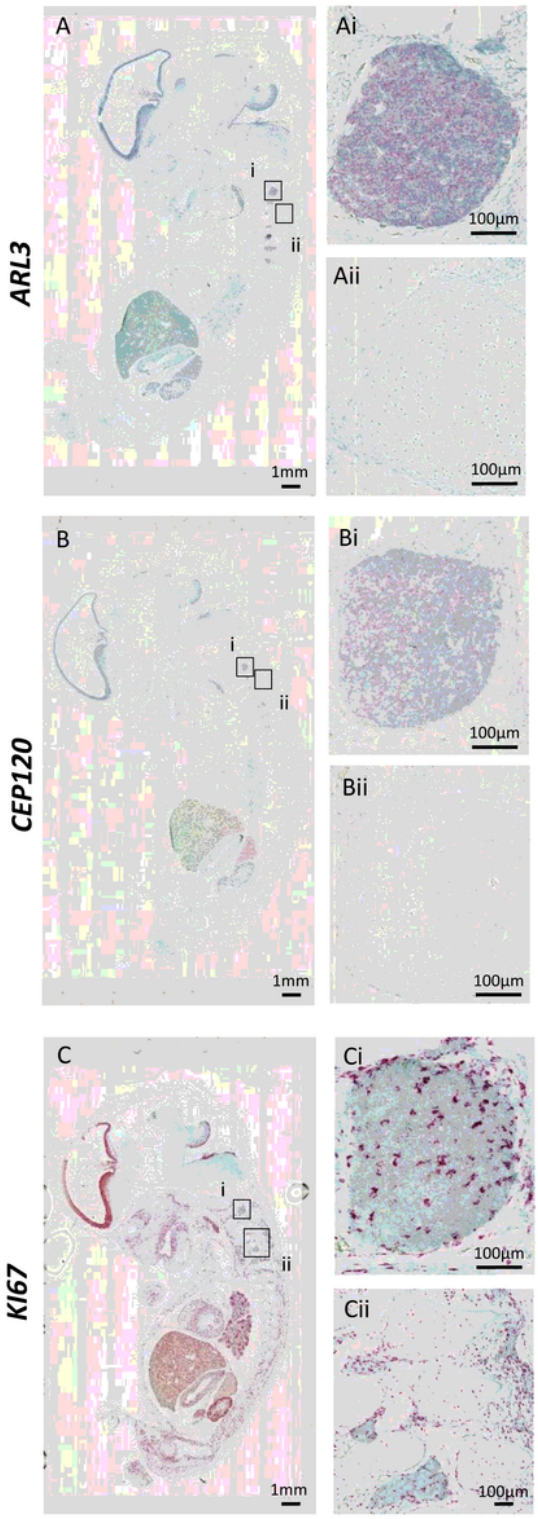

Figure 4

Expression of ARL3 and CEP120 in the developing human dorsal root ganglia Sagittal sections of 8PCW human embryos stained using RNAscope to show expression of ARL3 (A) (red), CEP120 (B) (red) and KI67 (C) (red) counterstained with Methyl Green. ARL3 and CEP120 expression is shown within the DRG (Ai and Bi respectively), whereas surrounding tissue has low level expression of these genes (Aii and Bii). KI67 expression is seen in the dorsal root ganglia (Ci) and surrounding tissues (Cii).

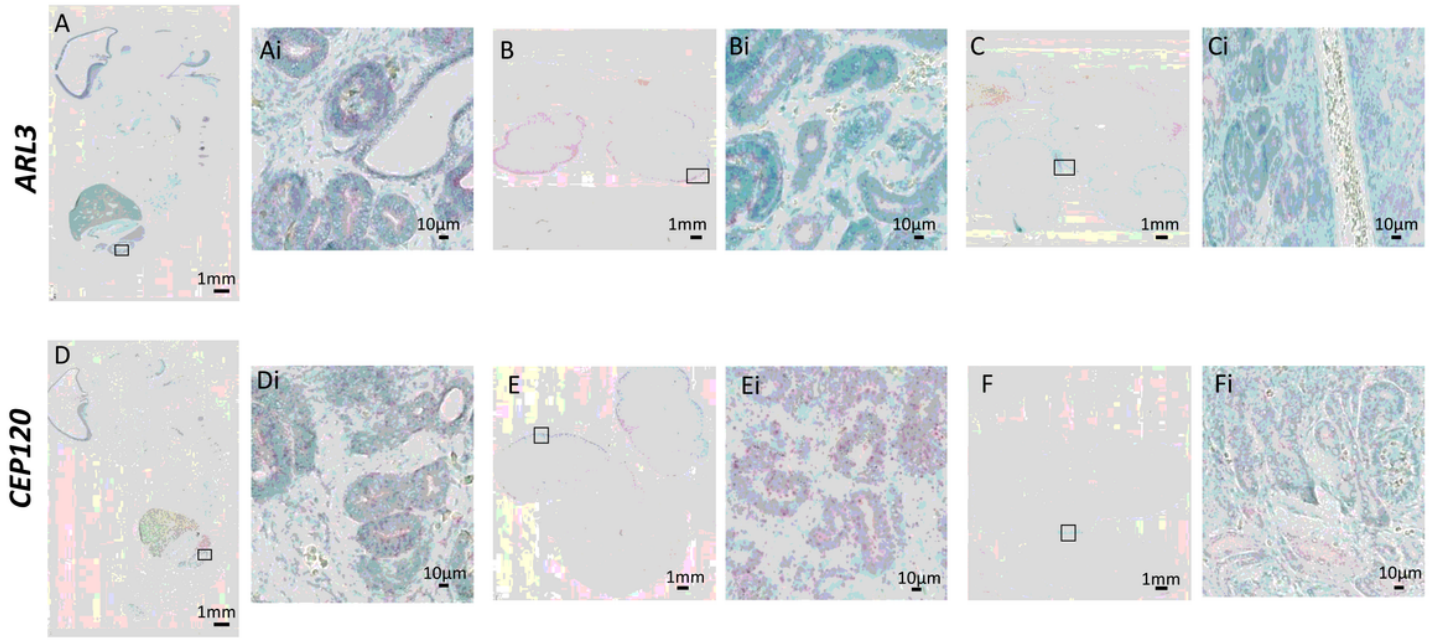

Figure 5

Expression of ARL3 and CEP120 in the developing human kidney Sagittal sections of human kidney at 8PCW (A and D), 14PCW (B and E), and 18PCW (C and F) stained using RNAscope to show ARL3 expression (A, B, C) (red) and CEP120 (D, E, F) (red) and counterstained with Methyl Green. ARL3 and CEP120 expression at 8PCW (Ai and Di) is seen in cells surrounding the developing nephrons and in the cortex. ARL3 and CEP120 expression patterns remain the same at 14PCW (Bi and Ei respectively). Ci and Fi shows ARL3 and CEP120 expression at 18PCW have decreased in the kidney, with expression seen in the outer cortex.

Supplementary Files

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