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BRIEF REPORT OPEN ACCESS

Fetal and Perinatal Brain MRI Findings in Adaptor Protein Complex 4–Associated Hereditary Spastic Paraplegia

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ABSTRACT

Purpose: Adaptor protein complex 4–associated hereditary spastic paraplegia (AP-4-HSP) is a rare childhood-onset neurogenetic disorder. With gene replacement therapies advancing, early—potentially prenatal—diagnosis holds significant clinical promise. We aimed to characterize fetal and perinatal brain MRI features of AP-4-HSP to assess whether early imaging can prompt timely diagnosis, counseling, and interventions.

Methods: In this retrospective analysis, we reviewed prenatal imaging from 303 individuals with genetically confirmed AP-4-HSP enrolled in the Registry and Natural History Study for Early Onset Hereditary Spastic Paraplegia (NCT04712812). Four patients (covering SPG47, SPG50, SPG52) with fetal, perinatal, or early postmortem imaging available were selected for detailed neuroradiologic evaluation. Systematic assessment documented several structural anomalies, correlated with genotype and clinical progression.

Results: Fetal imaging between 22 and 38 weeks' gestation revealed ventriculomegaly, corpus callosum hypoplasia, reduced periventricular white matter, and hippocampal under-rotation across all subtypes. The SPG52 patient exhibited additional severe features, including gyral immaturity and pontine/vermis hypoplasia. Postnatal follow-up demonstrated progressive white matter volume reduction and delayed myelination.

Conclusions: This study demonstrates that fetal and perinatal brain MRI can detect early, consistent neurodevelopmental abnormalities in AP-4-HSP, reinforcing its classification as both a neurodevelopmental and neurodegenerative disorder. Integration of prenatal neuroimaging with molecular diagnostics could enable earlier recognition, family counseling, and access to emerging gene therapies. These findings support the incorporation of fetal brain MRI into diagnostic protocols for suspected neurogenetic conditions.

1 | Introduction

The hereditary spastic paraplegias (HSPs) encompass a group of more than 80 monogenic neurological disorders, primarily characterized by progressive lower limb spasticity and gait impairment. HSPs are broadly classified into "pure" forms, involving isolated motor dysfunction, and "complex" forms, which include additional central or peripheral nervous system manifestations. Among the childhood-onset HSPs, those caused by biallelic loss-of-function variants in the adaptor protein complex 4 (AP-4) subunit genes—*AP4B1* (SPG47), *AP4M1* (SPG50), *AP4E1* (SPG51), and *AP4S1* (SPG52)—represent a

complex form of HSP characterized by early-onset global developmental delay, secondary microcephaly, and epilepsy, followed by progressive lower limb spasticity that eventually leads to loss of ambulation and other secondary complications [1, 2]. This trajectory classifies AP-4-related HSP as both a neurodevelopmental and progressive neurodegenerative disease [2], a finding that is further supported by the constellation of abnormalities identified on postnatal brain magnetic resonance imaging (MRI), including periventricular white matter changes, thinning of the corpus callosum, absence or thin anterior commissure, and abnormal signal in the forceps minor ("ears of the grizzly sign") [3]. Here, we present the first systematic investigation of prenatal imaging findings in AP-4 deficiency, shedding light on its early impact on brain development. With gene therapies for AP-4 deficiency, namely SPG50 and SPG47, advancing into late-stage preclinical [4, 5] and clinical development [6] (NCT05518188, NCT06948019), these findings highlight the importance of early diagnosis through prenatal genetic testing, offering critical insights into the therapeutic window for potential interventions.

2 | Patients and Methods

This study was conducted with the approval of the Institutional Review Board at Boston Children's Hospital (IRB-P00033016). Postmortem imaging of patient #1 was obtained under research protocol EK Nr. 2027/2024 at the University of Vienna. Informed consent was obtained. We analyzed perinatal histories and neuroimaging findings from a cohort of 303 individuals with a genetically confirmed diagnosis of AP-4-HSP, enrolled in the Natural History Study for Early-Onset Hereditary Spastic Paraplegia (NCT04712812) [7]. Among these, we identified four previously unreported patients, bringing the total to six fetal, perinatal, and postmortem brain MRI studies acquired during routine clinical care. The imaging data, spanning fetal to postmortem stages, were systematically evaluated by a board-certified neuroradiologist with extensive expertise in fetal MR imaging (E.Y.).

3 | Results

We systematically reviewed imaging and clinical data from 303 individuals with genetically confirmed AP-4-HSP. Prenatal ultrasound reports were available for 180 individuals, among whom 23.3% exhibited abnormalities—most commonly ventriculomegaly (73.8% of those with findings).

Fetal or perinatal brain MRI was available for four individuals, representing three of the most prevalent AP-4-HSP subtypes: SPG47, SPG50, and SPG52. These included one postmortem fetal MRI (22 weeks), three fetal MRIs from two individuals (at 24/32 weeks and 34 weeks), and one early postnatal MRI obtained at 35 weeks corrected gestational age in a preterm infant. Postnatal follow-up MRIs were also available for two individuals at 21 and 24 months, providing longitudinal insight into evolving radiologic features (Table 1, Figure 1).

Patient 1: A female fetus with *AP4B1*-associated SPG47 (NM_001253852.3, c.114-2A>C, p.(?)/c.114-2A>C, p.(?)) underwent postmortem MRI at 22 weeks and 3 days' gestation following stillbirth. Imaging was obtained under a research protocol and was largely unremarkable but demonstrated subtle findings, including mild ventriculomegaly, corpus callosum hypoplasia, and underrotated hippocampi. Signal abnormalities were observed in the periventricular crossroads, transient structures of the fetal brain where growing cortical pathways intersect [8]. These results suggest that radiographic hallmarks of AP-4-HSP may be minimal or only subtly present at this early gestational age.

Patient 2: A male infant with *AP4M1*-associated SPG50 (NM_004722.4, c.1012C>T, p.(Arg338Ter)/c.330C>G, p.(Tyr110Ter)) underwent fetal MRI at 24 and 32 weeks' gestation following detection of ventricular enlargement on screening ultrasound. Both studies showed minimally reduced periventricular white matter and slight lateral ventricular enlargement without significant progression between timepoints. However, postnatal MRI at 21 months revealed more pronounced abnormalities, including severe corpus callosum hypoplasia, an absent anterior commissure, reduced white matter volume, delayed myelination with frontal periventricular white matter gliosis (ears of grizzly bear sign) [3], and mild pontine hypoplasia. These findings indicate that abnormalities are detectable prenatally and continue to evolve significantly after birth in SPG50.

Patient 3: A male infant with *AP4B1*-associated SPG47 (NM_001253852.3, c.664del, p.(Leu222CysfsTer31)/c.664del, p.(Leu222CysfsTer31)) underwent fetal MRI at 34 weeks' gestation following detection of ventriculomegaly on ultrasound. Prenatal imaging showed mild corpus callosum hypoplasia, lateral ventricle size at the upper limits of normal for gestational age, slight white matter volume loss, and underrotated hippocampi. Postnatal MRI at 24 months demonstrated more extensive CNS changes, including severe posterior corpus callosum hypoplasia, absence of the anterior commissure, mild-to-moderate white matter reduction, delayed myelination with frontal periventricular gliosis [3], and minimal inferior vermian hypoplasia, reflecting progressive supratentorial and infratentorial brain involvement. As in SPG50 with patient 2, the findings in patient 3 indicate that abnormalities are detectable prenatally and continue to evolve significantly after birth in SPG47.

Patient 4: A male infant with *AP4S1*-associated SPG52 (NM_001128126.3, c.294+1G>T, p.(?)/c.294+1G>T, p.(?)) underwent early postnatal MRI at 35 weeks' corrected gestational age as part of a diagnostic evaluation for significant neonatal hypotonia. Imaging revealed the most severe findings in the cohort, including severe corpus callosum hypoplasia, markedly reduced white matter volume, severe lateral ventriculomegaly, pontine hypoplasia, gyral immaturity, and frontal gliosis/dysmyelination. These abnormalities suggest profound prenatal disruption of structural brain development in SPG52.

Discussion

This study provides the first systematic characterization of fetal and perinatal brain MRI findings in individuals with AP-4-HSP, expanding our understanding of this condition as both a neurodevelopmental and progressive neurodegenerative disorder. Across four individuals with genetically confirmed diagnoses—representing

TABLE 1 | Summary of pre- and perinatal MR imaging findings in four individuals with AP-4 deficiency.

Patient	#1	#2	#3	#4
AP-4-HSP	SPG47	SPG50	SPG47	SPG52
AP-4 gene variant	*AP4B1* (NM_001253852.3): c.114-2A>C, p.?/c.114-2A>C, p.?	*AP4M1* (NM_004722.3): c.1012C>T, p.(Arg338Ter)/c.330C>G, p.(Tyr110Ter)	*AP4B1* (NM_001253852.3): c.664del, p.(Leu222CysfsTer31)/3:c.664del, p.(Leu222CysfsTer31)	*AP4S1* (NM_007077.4): c.294+1G>T, p.?/c.294+1G>T, p.?
Sex	Female	Male	Male	Male
Age at MRI	22 weeks and 3 days of gestation (postmortem MRI)	24 weeks and 6 days of gestation 32 weeks and 6 days of gestation 21 months of age	34 weeks of gestation 24 months of age	35 weeks of gestation
White matter volume (qualitative)	Grossly normal white matter volume	*Prenatal:* Minimally depressed left periatrial white matter volume (no change between 24 and 32 weeks' gestation) *Postnatal:* Moderately depressed periatrial white matter	*Prenatal:* Grossly normal *Postnatal:* Mildly to moderately depressed white matter volume with a gradient (posterior>anterior)	*Postnatal:* Severely depressed white matter with a gradient (posterior>anterior)
Gray matter volume (qualitative)	Normal	Normal	Normal	Normal
Cerebellar volume	Normal	Normal	Normal	Normal
Hippocampi	Underrotated	*Prenatal:* Normal *Postnatal:* Underrotated	*Prenatal:* Underrotated *Postnatal:* Underrotated	*Postnatal:* Underrotated
Basal ganglia	Normal	Normal	Normal	Normal
Mid pons diameter	Normal	*Prenatal:* Normal *Postnatal:* < 3rd percentile	*Prenatal:* Normal *Postnatal:* < 3rd percentile	*Postnatal*: < 3rd percentile
Frontal and occipital horn ratio	0.41	*Prenatal:* 0.40, 0.40 *Postnatal:* 0.37	*Prenatal:* 0.43 *Postnatal:* 0.47	*Postnatal:* 0.56
Myelination	Normal	*Prenatal:* Normal *Postnatal:* Immature/delayed	*Prenatal:* Normal *Postnatal:* Immature/delayed	*Postnatal:* Normal
Ears of the grizzly bear sign	No	*Prenatal:* No *Postnatal:* Yes	*Prenatal:* No *Postnatal:* Yes	*Postnatal:* No
Absent anterior commissure	Unable to determine	*Prenatal:* Unable to determine *Postnatal:* Absent	*Prenatal:* Unable to determine *Postnatal:* Absent	*Postnatal:* Absent
Corpus callosum	Within normal limits	*Prenatal:* Within normal limits *Postnatal:* Severe posterior hypoplasia	*Prenatal:* Borderline hypoplastic *Postnatal:* Severe posterior hypoplasia	*Postnatal:* Hypoplastic

(Continues)

TABLE 1 | (Continued)

Patient	#1	#2	#3	#4
Polymicrogyria	None	None	None	Yes (bilateral perisylvian dysgyria)
Summary of MRI findings	Grossly normal fetal MRI with minimal ventriculomegaly and underrotated hippocampi	*Prenatal:* Minimally reduced periventricular white matter with minimal enlargement of the lateral ventricles. No change between 24 weeks and 32 weeks' gestation. *Postnatal:* Severe posterior hypoplasia of the corpus callosum, absent anterior commissure, reduced periventricular white matter, abnormal myelination, mild pontine hypoplasia	*Prenatal:* Corpus callosum with mild hypoplasia. No obvious white matter depletion of ventriculomegaly. *Postnatal:* Severe posterior hypoplasia of the corpus callosum, reduced periventricular white matter, abnormal myelination, minimal inferior vermian hypoplasia	*Postnatal:* Severe hypoplasia of the corpus callosum, severely reduced white matter volume with severe lateral ventriculomegaly with septal degenerationationfrontal periventricular gliosis and gyral immaturity, pontine hypoplasia

three major AP-4-HSP subtypes—we observed early and consistent structural brain abnormalities on imaging, most notably ventriculomegaly, reduced periventricular white matter, severe hypoplasia of the posterior corpus callosum, and absence of the anterior commissure. These findings suggest that AP-4 deficiency disrupts brain development *in utero* and that its imaging manifestations may be detectable as early as the second trimester.

Our results underscore the utility of fetal MRI in identifying early radiologic markers of AP-4-HSP. Notably, abnormalities were apparent in individuals later confirmed to have SPG47, SPG50, and SPG52, with the most severe prenatal findings observed in SPG52. In this patient, significant white matter reduction, pontine hypoplasia, and gyral immaturity were evident at 35 weeks' gestation, reflecting a profound prenatal disruption of early neurodevelopmental processes. Conversely, in SPG47 and SPG50, antenatal findings were subtler but were much more pronounced on imaging obtained during the first few years of life, supporting the notion of a dynamic disease course in which structural abnormalities may become more evident over time [3].

These findings have important clinical implications. First, they raise the possibility that certain MRI features—particularly ventriculomegaly, corpus callosum hypoplasia, and white matter reduction—could serve as early imaging biomarkers that prompt prenatal genetic testing. Currently, most prenatal diagnostic algorithms rely on karyotyping or microarray analysis, which are insufficient to detect the single-nucleotide and small indel variants that underlie AP-4-HSP [1]. Integrating fetal MRI findings with molecular diagnostics such as whole-exome or whole-genome sequencing could improve diagnostic yield, particularly when abnormalities are detected in families without a known history of neurogenetic disease. Second, early diagnosis opens a critical window for genetic counseling, anticipatory guidance, and potentially, early access to emerging gene therapies. Both SPG47 and SPG50 are the focus of ongoing translational efforts, with AAV-mediated gene replacement therapies entering clinical trials [4–6]. While these therapies have shown promise in preclinical models, their effectiveness in addressing the neurodevelopmental aspects of the disease—particularly those initiated *in utero*—remains unclear. Nevertheless, identifying affected individuals before symptom onset provides an optimal therapeutic window and may offer an opportunity to delay or attenuate disease progression. Lastly, our study contributes to the broader understanding of the developmental timing of AP-4-related pathology. Even though the *in utero* manifestations may be mild, the presence of callosal, white matter volume, and pontine abnormalities in the fetal period indicates that AP-4 function is essential for normal brain morphogenesis and maturation. This is consistent with prior work demonstrating impaired neurite outgrowth, axonal trafficking, autophagy defects, and altered neurogenesis in cellular and animal models of AP-4 deficiency [8–13]. Longitudinal imaging of affected individuals from fetal to postnatal stages, as included in this study, may provide a valuable framework for understanding the timing and trajectory of these pathophysiological changes.

Despite the strengths of this study, including systematic neuroradiologic review and integration of genetic data, several

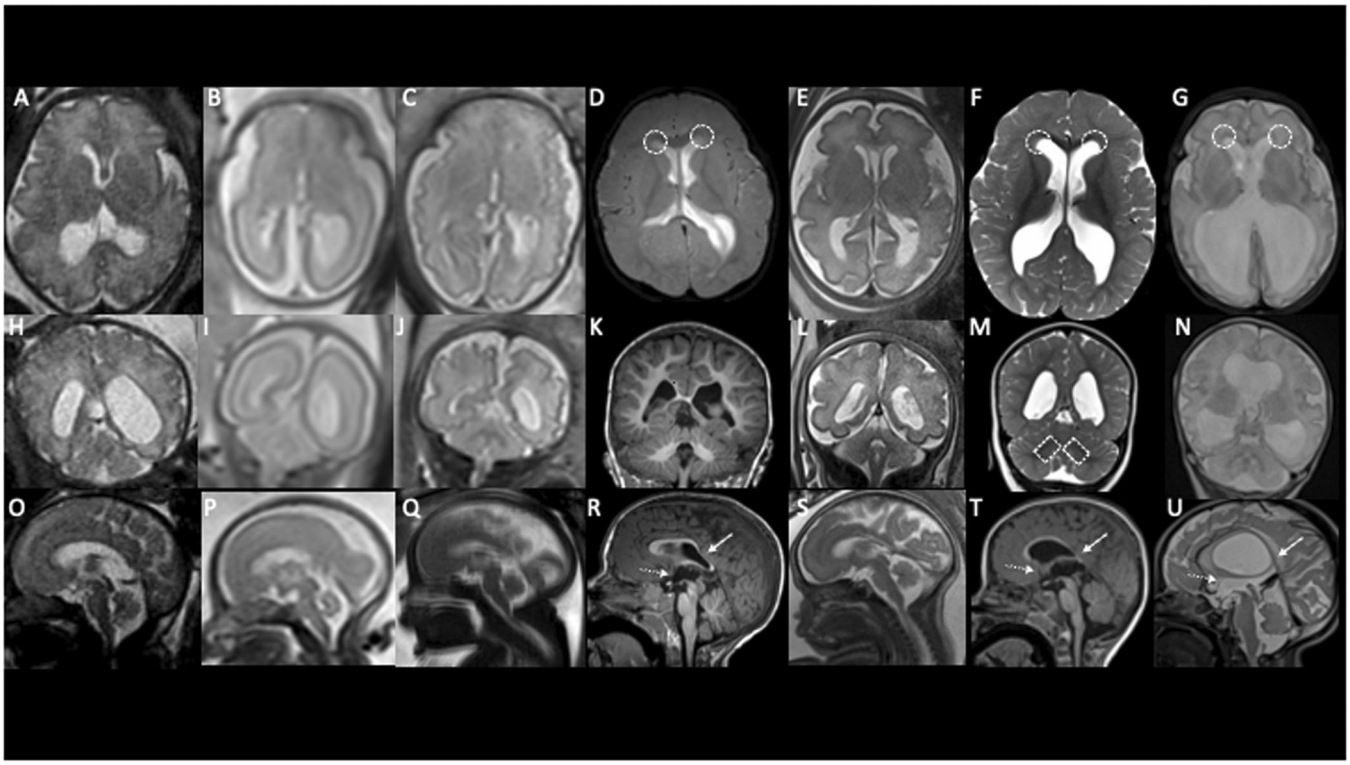

FIGURE 1 | Prenatal and postnatal manifestations of adaptor protein complex 4–associated hereditary spastic paraplegia (AP-4-HSP). Axial (A–G), coronal (H–N), and sagittal (O–U) images of patient 1 (A, H, O), patient 2 at 24 weeks gestational age (GA) (B, I, P), patient 2 at 32 weeks GA (C, J, Q), patient 2 at 21 months of age (D, K, R), patient 3 at 34 weeks' GA (E, L, S), patient 3 at 24 months (F, M, T), and patient 4 (G, N, U) demonstrate mild, nonspecific abnormalities in utero and more specific hallmarks of AP-4-HSP on postnatal imaging. Specifically, the ventriculomegaly and depressed cerebral white matter volume are more pronounced on postnatal imaging. Additionally, severe posterior callosal hypoplasia (solid stem arrow), absence of the anterior commissure (dashed stem arrow), frontal periventricular gliosis (dashed circle), and dentate gyrus dysmyelination (dashed box) are only callable on the postnatal imaging. Additionally, the delayed myelination in patient 2 at 21 months (D) and patient 3 at 24 months (F) is only visible on the imaging obtained in the second year of life. All shown fetal imaging utilizes T2 HASTE imaging. Postnatal imaging utilizes T2-weighted imaging except for coronal/sagittal T1-weighted imaging for patient 2 (K, R) and a sagittal T1 for patient 3 (T).

limitations should be acknowledged. First, the sample size is small, reflecting both the rarity of AP-4-HSP and the infrequent availability of high-quality fetal or perinatal imaging in affected individuals. This limits the generalizability of our findings and precludes genotype-phenotype correlations beyond qualitative observations. Second, imaging protocols varied across patients due to their acquisition in different clinical settings and at different gestational stages, potentially introducing variability in sensitivity for detecting subtle abnormalities. Third, not all patients had longitudinal imaging available, limiting our ability to fully characterize the evolution of radiographic findings over time; however, future studies incorporating serial imaging will be critical to elucidate the radiologic progression of AP-4-HSP and correlation with clinical outcomes. Fourth, the *in utero* abnormalities serve as indicators of abnormal brain development, but the specific findings of AP-4-HSP were only manifest on postnatal imaging. Therefore, advanced imaging or integration of additional biomarkers will likely be needed to suggest the diagnosis of AP-4-HSP prenatally. Finally, postmortem fetal imaging may be influenced by technical or postmortem artifacts that can complicate interpretation. Future studies incorporating standardized fetal MRI protocols and prospective recruitment will be essential to validate findings in larger cohorts and refine diagnostic criteria for prenatal detection of AP-4 deficiency.

In conclusion, this study provides evidence that prenatal MRI can reveal early structural brain abnormalities in AP-4-HSP, offering a potential avenue for earlier diagnosis and intervention. As sequencing technologies become more integrated into prenatal care, the combination of fetal imaging and molecular diagnostics may significantly advance the clinical management of HSP. Future prospective studies will be critical to validate these findings in larger cohorts and to determine the extent to which early interventions, including gene replacement therapies that are entering clinical testing, can alter the natural history of disease.

Author Contributions

Alexandra K. Brooks: writing – original draft, writing – review and editing, visualization, data curation, formal analysis, investigation. **Vicente Quiroz:** conceptualization, investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, data curation. **Luca Schierbaum:** investigation, writing – review and editing. **Julian E. Alecu:** investigation, data curation, writing – review and editing. **Katerina Bernardi:** investigation, data curation, writing – review and editing. **Nicole Battaglia:** investigation, project administration, data curation, writing – review and editing. **Amy Tam:** project administration, investigation, data curation, writing – review and

editing. **Joshua Rong:** investigation, project administration, data curation, writing – review and editing. **Gregor Kasprian:** conceptualization, investigation, writing – review and editing, data curation. **Edward Yang:** investigation, writing – review and editing, visualization, validation, methodology, formal analysis, data curation. **Darius Ebrahimi-Fakhari:** conceptualization, investigation, funding acquisition, writing – original draft, methodology, writing – review and editing, formal analysis, project administration, data curation, supervision, resources.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

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