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Anatomical Insights into Cerebral and Craniofacial Morphology in ASD Using Anthropometric Measurements

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ABSTRACT

Background: The association of neurodevelopmental and craniofacial anomalies with autism spectrum disorder (ASD) is indicative of cerebral abnormalities. To examine cerebral and craniofacial anatomical variations in a rodent model of ASD using defined anthropometric measures.

Methods: This in vivo study was performed in an animal research facility, and analysis was done (Ref#18/05/223) at Liaquat University of Medical & Health Sciences (LUMHS), Jamshoro, and Continental Medical College, Lahore (CMC) from June to December 2023. Twenty Albino Wistar rats were allocated into two groups. Control (n=6) and ASD-imposed (n = 14) under the administration of propionic acid (PPA) using a random sampling technique. OpenEpi 3.0.0 was used for sample size calculation. The craniofacial and cerebral markers were measured using the calipers and stereotactic referencing after euthanasia. The skull length, intercanthal width, head circumference, and cranial index were considered. H&E staining was also performed to view histological patterns in certain brain tissues. Statistical analysis was done using SPSS version 26. Independent t-tests and Chi-square tests were performed. A p-value of <0.05 was considered statistically significant.

Results: The ASD-induced group exhibited significantly reduced skull length (29.3 ± 2.1 mm vs. 32.8 ± 1.5 mm; p = 0.002) and head circumference (40.2 ± 2.7 mm vs. 42.5 ± 2.2 mm; p = 0.041), alongside increased intercanthal distance (7.8 ± 0.6 mm vs. 6.2 ± 0.4 mm; p = 0.0001) and cranial index (81.9% vs. 75.3%; p = 0.003). Histologically, cortical layering was disrupted in 10 of 14 ASD rats, and ventricular enlargement was observed in 12 of 14, compared to none in controls (all p < 0.01).

Conclusion: Craniofacial morphometric studies of ASD rodent models show quantifiable anatomical differences associated with neurodevelopmental compromise. This strategy can act as a translational model for grasping structural markers of early ASD detection and pathogenesis.

Keywords: Autism Spectrum Disorder, Craniofacial Abnormalities, Anthropometry, Animal Model, Cerebrum & Propionic Acid.

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tINTRODUCTION

Autism Spectrum Disorder (ASD) is a neurological indicator of neurodevelopmental disorder, presenting a list of impairments, related to the social, communicative, and behavioral sphere [1]. The ASH prevalence is on the rise, and current estimations reveal that [1] in 36 children (aged 4-17) are thus affected worldwide [2]. The disorder is believed to have complex genetic, environmental, as well as neurobiological factors in its causation, which interfere with normal brain similarly resulting in behavioral and cognitive problems [3]. Although the etiology of ASD is still multifactorial, research has increasingly concentrated on the establishment of anatomical and physiological markers which could help provide clues on the organization of the disorder's etiology [4]. The craniofacial and cerebral anomalies are commonly reported in people with ASD. Dysmorphologies characterized by skull alterations in shape, brain structure asymmetry, and abnormalities of cortical layering have been demonstrated in animal as well as human research [5]. Such anatomical variations are believed to be indicative of neurodevelopmental disturbances that could underwrite the behavioral manifestations of ASD [6]. Even though human studies are hampered by such a complex linking together of the brain and craniofacial alterations, the use of animal models can provide a fruitful avenue for further study of anatomical deviations in detail [7]. The use of rodent models has become popular in ASD research over the last decade, especially with the use of propionic acid (PAA) to mimic ASD behaviors [8]. The administration of PPA in rodents is associated with similar neurodevelopmental and behavioral phenotypes that occur in ASD, and thus, it is an adequate model for ASD in which to study the anatomical changes underlying the disorder [9]. Therefore, a study was required on rat models to observe conformational changes in the skull region caused by ASD.

This study intended to close this gap by a combination of craniofacial measurement and histopathological approaches to evaluate cerebral morphology in a PPA-induced ASD rat model. The goal was to examine cerebral and craniofacial anatomical variations in a rodent model of ASD using defined anthropometric measures.

METHODS

This in vivo study was performed in an animal research facility, and analysis was done (Ref#18/05/223) at LUMHS and CMC from June to December 2023. Twenty male Wistar rats were used for this in vivo study. The rats were assigned to two groups: Control group, Group A (n=6), and Group B (PPA-induced ASD model, n=14). The samples were assigned using a random sampling technique, and OpenEpi 3.0.0 was employed for sample size calculation with 80% power and alpha of 0.05 [10]. All animals were kept under standard laboratory conditions with a 12-hour light/dark cycle in a room temperature maintained at 22°C ± 2°C, with food and water supplied ad libitum. Only healthy male Wistar rats were included in the study, with an age range of 8-10 weeks and weighing up to 250 to 300 grams. Rats showing signs of prior neurological, cranial, or behavioral abnormalities were excluded based on preliminary screening.

ASD- like behaviors in Group B were elicited using propionic acid (PPA, Sigma-Aldrich), dissolved in sterile saline, by oral administration at a dose of 250 mg/kg body weight. PPA was administered for five successive days every day. Group A got the same amount of saline as a control. Parameters of craniofacial and cerebral anatomies were determined after euthanasia by cervical dislocation. Skull length, Inter-canthal distance, head circumference, and cranial index were measured using digital calipers and stereotactic referencing. Measurements were performed after euthanasia to maintain uniformity. In addition, histology of the brain tissues was studied by means of Hematoxylin and Eosin (H&E) staining. Structural properties such as cortical layering, morphology of the surface of the brain, and size of the ventricles were examined.

The brains were preserved in 10% formalin and processed histopathologically. Tissues from the brain were dehydrated in a series of alcohols, cleared with xylene, placed in paraffin, and sliced into sections 5 microns thick. Brain convolutions, the symmetry of cortical symmetry, and the appearance of lateral vents were assessed. Histological sections were studied under light microscope for structural anomalies including disruption of the cortical layering and enlarged ventricles. The neuronal density in selected areas of the brain (pons, medulla, midbrain) was also determined. Statistical analysis was done using SPSS version 26. Independent t-tests were employed for continuous variables, whereas all categorical variables were analyzed by means of Chi-square tests between the control and ASD-induced groups. Below, a p-value of <0.05 was taken to be statistically significant.

RESULTS

A detailed morphometric analysis indicated significant craniofacial and cerebral differences between the ASD-induced groups and controls. Quantitative analysis showed changing skull sizes and interorbital size. Postmortem brain morphology revealed some marked deviation in cortical lamination and ventricular dimensions. These results collectively highlight structural aberrations linked with ASD phenotypes. Significant structural brain

abnormalities were identified for the ASD group. Ventricular dilation, cortical symmetry, and layering disruption were most apparent. The neurodevelopment is also supported by irregularity of the surface of the brain. These findings accentuate the relationship between cranial anomalies and internal cerebral anatomy in the pathology of ASD as given in **Figure 1.**

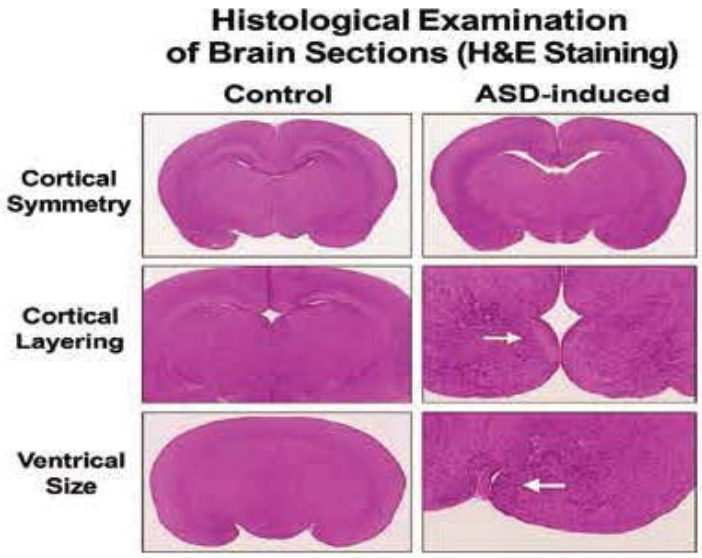

Figure 1: Histological Examination of Brain Sections (H&E Staining)

Table 1: Comparison of Craniofacial Anthropometric Parameters Between Control and ASD-Induced Groups

Parameter	Control (n=6)	ASD Group (n=14)	p-value	Statistical Test (t-value)
Skull Length (mm)	32.8 ± 1.5	29.3 ± 2.1	0.002 **	t = 3.65
Intercanthal Distance (mm)	6.2 ± 0.4	7.8 ± 0.6	0.0001 **	t = 7.11
Head Circumference (mm)	42.5 ± 2.2	40.2 ± 2.7	0.041 *	t = 2.22
Cranial Index (%)	75.3 ± 3.1	81.9 ± 2.8	0.003 **	t = 3.45
Facial Height (mm)	18.4 ± 0.7	16.9 ± 1.0	0.021 *	t = 2.62

Values are mean ± SD. *p < 0.05, **p < 0.01.

The ASD-induced group showed a statistically significant reduction in skull length and head circumference compared to controls, indicating microcephalic trends as shown in **Table 1**. Intercanthal distance and cranial index were significantly increased, suggestive of dysmorphologies often associated with neurodevelopmental disorders. These alterations in craniofacial measurements reflect disrupted cranial development. Such morphological parameters may serve as early phenotypic markers in ASD models.

Table 2: Gross Anatomical Observations and Cerebral Morphological Deviations

Parameter	Control Group (n=6)	ASD Group (n=14)	Statistical Test (Chi-square)	p-value
Cortical Symmetry (Present/Absent)	6 / 0	3 / 11	$\chi^2 = 9.79$	0.002 **
Ventricular Appearance (Normal/Enlarged)	6 / 0	2 / 12	$\chi^2 = 11.65$	0.0006 **
Cortical Layering (Organized/Disrupted)	6 / 0	4 / 10	$\chi^2 = 8.57$	0.003 **
Brain Surface (Smooth/Irregular)	6 / 0	5 / 9	$\chi^2 = 6.43$	0.011 *

Significant at p < 0.05, highly significant at p < 0.01.

The ASD group had a significantly reduced skull length (29.3 ± 2.1 mm) compared to controls (32.8 ± 1.5 mm, p = 0.002) and lower head circumference (40.2 ± 2.7 mm vs. 42.5 ± 2.2 mm, p = 0.041). Intercanthal distance was markedly increased in the ASD group (7.8 ± 0.6 mm) relative to controls (6.2 ± 0.4 mm, p = 0.0001). Cranial index was also significantly higher in ASD rats (81.9 ± 2.8%) than in controls (75.3 ± 3.1%, p = 0.003), indicating a broader cranial shape.

Similarly, the ASD group had remarkable structural brain abnormalities. Cortical symmetry and layering disruption and ventricle enlargement were core. The irregularity of the brain surface provides further reasons for disturbed neurodevelopment. These findings demonstrate the relationship between cranial abnormalities and internal cerebral structure in the pathology of ASD as can be observed from **Table 2**.

Cortical symmetry was preserved in all control rats (6/6), but absent in most ASD rats (3/14 symmetric, p = 0.002). Ventricular enlargement was observed in 12/14 ASD rats versus none in the control group (0/6 normal, p = 0.0006). Disruption of cortical layering (10/14 vs. 0/6, p = 0.003) and irregular brain surfaces (9/14 vs. 0/6, p = 0.011) were also significantly more common in the ASD group. These results showed that ASD induced rats had distinct conformational changes in craniofacial and cerebral structures that highlighted the underlying disruptions in neurodevelopmental processes.

DISCUSSION

This study identified substantial differences in craniofacial and cerebral morphologies in the PPA-induced autism spectrum disorder (ASD) rat model versus controls, in agreement with increasing findings relating morphological abnormalities to autism phenotypes [11,12]. The craniofacial dysmorphologies were observed, including shorter skull length, greater intercanthal distance, and different cranial index, have the same discovery from human ASD studies, which frequently report microcephaly, midfacial hypoplasia, and increased facial asymmetry. These findings support the hypothesis that neurodevelopmental anomalies in ASD go beyond neural circuits and include more general morphogenetic aberrations, especially those that are shared between forms of brain and face development and those that are shared between pathways that are conserved during brain and face development [13]. Morphologically, the noted deviations in brainstem and cerebral cortex found in the ASD group, including disrupted cortical layering, ventricular dilatation, and asymmetrical brain architecture, relate to the neuropathological side of ASD [14].

Enlarged ventricles, which constitute a common finding in ASD imaging studies, indicate impaired cerebrospinal fluid circulation, and may be associated with neuroinflammation or impaired neurogenesis [15,16]. What is more, the disrupted cortical layering observed further implicates altered neuronal migration, something known to contribute to the abnormal connectivity patterns and behaviour abnormalities observed in ASD [17]. Our histopathological findings support this structural observation by showing decreased neuronal density in the midbrain, pons, and medulla that might explain the common sensorimotor and autonomic dysfunctions noted in the ASD patient. This reduction may be related to excitotoxicity, inflammation, or oxidative stress caused by PPA, which imitates environmental exposures that cause ASD pathophysiology, in humans [18,19]. Behaviorally, deficits in social interaction and locomotor hyperactivity that the PPA model demonstrates match the core characteristics of ASD and confirm the construct validity of the model. These behavioral phenotypes are probably a result of cortical and subcortical disruption, especially in social reward, attention, and sensorimotor integration circuits [20,21].

Combined, our results thus support that it is useful to combine craniofacial anthropometry, behavioral testing, and histopathological analyses in models of ASD. Register of cranial abnormalities observed may be utilized as early phenotypic biomarkers for early diagnosis and intervention [22,23]. Additionally, this integrative approach is useful in bridging the divide between extrinsic phenotypes and intrinsic neuroanatomical pathology, thus improving translational knowledge from animal models to human diseases [24,25]. However, limitations must be acknowledged. The study sample was not large, especially for the control group; this may have made a difference in statistical power. Moreover, even though PPA is an extensive ASD inducer employed by the majority of animal models of ASD, it fails to replicate the genetic and environmental heterogeneity of human ASD. Neuroimaging, molecular profiling, and longitudinal follow-up are methods that should be integrated into future studies to continue to clarify the developmental trajectory of noted abnormalities.

CONCLUSION

To summarize, our results supported the PPA model as a useful paradigm of ASD-related neuroanatomical and behavioral changes. The close relationship between craniofacial dysmorphology and cerebral abnormalities presented an innovative opportunity for the

screening of early ASD and improved understanding of the neurodevelopmental disruption causing the syndrome. Such insights may facilitate the creation of specific diagnostics and interventions for ASD in a clinical environment.

LIST OF ABBREVIATIONS

ASD: Autism Spectrum Disorder
PPA: Propionic Acid
H&E: Hematoxylin and Eosin

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CONFLICT OF INTEREST

None

ETHICAL APPROVAL

This in vivo study was performed in an animal research facility, and analysis was done (Ref#18/05/223) at LUMHS and CMC from June to December 2023.

AUTHORS' CONTRIBUTION

All participants participated equally as per ICMJE.

REFERENCES

1. Maenner MJ, Warren Z, Williams AR, Amoakohene E, Bakian AV, Bilder DA, et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2020. MMWR Surveill Summ. 2023 Mar 24;72(2):1-14. doi: 10.15585/mmwr.ss7202a1.
2. Solmi M, Song M, Yon DK, Lee SW, Fombonne E, Kim MS, et al. Incidence, prevalence, and global burden of autism spectrum disorder from 1990 to 2019 across 204 countries. Mol Psychiatry. 2022 Oct;27(10):4172-4180. doi: 10.1038/s41380-022-01630-7.
3. Kaplan ZB, Pearce EN, Lee SY, Shin HM, Schmidt RJ. Maternal Thyroid Dysfunction During Pregnancy as an Etiologic Factor in Autism Spectrum Disorder: Challenges and Opportunities for Research. Thyroid. 2024 Feb;34(2):144-157. doi: 10.1089/thy.2023.0391.
4. Zhuang H, Liang Z, Ma G, Qureshi A, Ran X, Feng C, et al. Autism spectrum disorder: pathogenesis, biomarker, and intervention therapy. MedComm (2020). 2024 Mar 2;5(3):e497. doi: 10.1002/mco2.497.
5. Junaid M, Slack-Smith L, Wong K, Bourke J, Baynam G, Calache H, et al. Association between craniofacial anomalies, intellectual disability and autism spectrum disorder: Western Australian population-based study. Pediatr Res. 2022 Dec;92(6):1795-1804. doi: 10.1038/s41390-022-02024-9.
6. Pretzsch CM, Schäfer T, Lombardo MV, Warrier V, Mann C, Bletsch A, et al. Neurobiological Correlates of Change in Adaptive Behavior in Autism. Am J Psychiatry. 2022 May;179(5):336-349. doi: 10.1176/appi.ajp.21070711.
7. Silverman JL, Thurm A, Ethridge SB, Soller MM, Petkova SP, Abel T, et al. Reconsidering animal models used to study autism spectrum disorder: Current state and optimizing future. Genes Brain Behav. 2022 Jun;21(5):e12803. doi: 10.1111/gbb.12803.
8. Li Z, Zhu YX, Gu LJ, Cheng Y. Understanding autism spectrum disorders with animal models: applications, insights, and perspectives. Zool Res. 2021 Nov 18;42(6):800-824. doi: 10.24272/j.issn.2095-8137.2021.251.
9. Lagod PP, Abdelli LS, Naser SA. An In Vivo Model of Propionic Acid-Rich Diet-Induced Gliosis and Neuro-Inflammation in Mice (FVB/N-Tg(GFAPGFP)14Mes/J): A Potential Link to Autism Spectrum Disorder. Int J Mol Sci. 2024 Jul 25;25(15):8093. doi: 10.3390/ijms25158093.
10. Bhardwaj R, Agrawal U, Vashist P, Manna S. Determination of sample size for various study designs in medical research: A practical primer. J Family Med Prim Care. 2024 Jul;13(7):2555-2561. doi: 10.4103/jfmpc.jfmpc_1675_23.
11. Rahi S, Gupta R, Sharma A, Mehan S. Smo-Shh signaling activator purmorphamine ameliorates neurobehavioral, molecular, and morphological alterations in an intracerebroventricular propionic acid-induced experimental model of autism. Hum Exp Toxicol. 2021 Nov;40(11):1880-1898. doi: 10.1177/09603271211013456.
12. Quatrosi G, Genovese D, Galliano G, Zoppé H, Amodio E, Bonnet-Brilhault F, et al. Cranio-Facial Characteristics in Autism Spectrum Disorder: A Scoping Review. J Clin Med. 2024 Jan 26;13(3):729. doi: 10.3390/jcm13030729.
13. Exposito-Alonso D, Rico B. Mechanisms Underlying Circuit Dysfunction in Neurodevelopmental Disorders. Annu Rev Genet. 2022 Nov 30;56:391-422. doi: 10.1146/annurev-genet-072820-023642.
14. Guerra M, Medici V, La Sala G, Farini D. Unravelling the Cerebellar Involvement in Autism Spectrum Disorders: Insights into Genetic Mechanisms and Developmental Pathways. Cells. 2024 Jul 10;13(14):1176. doi: 10.3390/cells13141176.
15. Kyriakopoulou V, Davidson A, Chew A, Gupta N, Arichi T, Nosarti C, et al. Characterisation of ASD traits among a cohort of children with isolated fetal ventriculomegaly. Nat Commun. 2023 Mar 21;14(1):1550. doi: 10.1038/s41467-023-37242-0.
16. Ellingford RA, Panasiuk MJ, de Meritens ER, Shaunak R, Naybour L, Browne L, et al. Cell-type-specific synaptic imbalance and disrupted homeostatic plasticity in cortical circuits of ASD-associated Chd8 haploinsufficient mice. Mol Psychiatry. 2021 Jul;26(7):3614-3624. doi: 10.1038/s41380-021-01070-9.

17. Rozhkova IN, Okotrub SV, Brusentsev EY, Uldanova EE, Chuyko EA, Lipina TV, et al. Neuronal density in the brain cortex and hippocampus in Clsnt2-KO mouse strain modeling autistic spectrum disorder. Vavilovskii Zhurnal Genet Selektsii. 2022 Jul;26(4):365-370. doi: 10.18699/VJGB-22-44.
18. Usui N, Kobayashi H, Shimada S. Neuroinflammation and Oxidative Stress in the Pathogenesis of Autism Spectrum Disorder. Int J Mol Sci. 2023 Mar 13;24(6):5487. doi: 10.3390/ijms24065487. PMID: 36982559; PMCID: PMC10049423.
19. Sharma AR, Batra G, Saini L, Sharma S, Mishra A, Singla R, et al. Valproic Acid and Propionic Acid Modulated Mechanical Pathways Associated with Autism Spectrum Disorder at Prenatal and Neonatal Exposure. CNS Neurol Disord Drug Targets. 2022;21(5):399-408. doi: 10.2174/1871527320666210806165430.
20. Themistocleous CK, Andreou M, Peristeri E. Autism Detection in Children: Integrating Machine Learning and Natural Language Processing in Narrative Analysis. Behav Sci (Basel). 2024 May 29;14(6):459. doi: 10.3390/bs14060459.
21. Kasherman MA, Currey L, Kurniawan ND, Zalucki O, Vega MS, Jolly LA, et al. Abnormal Behavior and Cortical Connectivity Deficits in Mice Lacking Usp9x. Cereb Cortex. 2021 Feb 5;31(3):1763-1775. doi: 10.1093/cercor/bhaa324.
22. Nisar S, Haris M. Neuroimaging genetics approaches to identify new biomarkers for the early diagnosis of autism spectrum disorder. Mol Psychiatry. 2023 Dec;28(12):4995-5008. doi: 10.1038/s41380-023-02060-9.
23. Matthews H, de Jong G, Maal T, Claes P. Static and Motion Facial Analysis for Craniofacial Assessment and Diagnosing Diseases. Annu Rev Biomed Data Sci. 2022 Aug 10;5:19-42. doi: 10.1146/annurev-biodatasci-122120-111413.
24. Carroll L, Braeutigam S, Dawes JM, Krsnik Z, Kostovic I, Coutinho E, et al. Autism Spectrum Disorders: Multiple Routes to, and Multiple Consequences of, Abnormal Synaptic Function and Connectivity. Neuroscientist. 2021 Feb;27(1):10-29. doi: 10.1177/1073858420921378.
25. Derkowski W, Kędzia A, Dudek K, Glonek M. Morphometric evaluation of the anterior cranial fossa during the prenatal stage in humans and its clinical implications. PLoS One. 2024 Dec 27;19(12):e0309184. doi: 10.1371/journal.pone.0309184.