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Clinical value of transfontanellar ultrasonography for neonatal insular development

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Summary

Objectives: The aims of this study were to assess the morphological characteristics and to establish ultrasonographic standards of normal neonatal insula size using transfontanellar ultrasonography, and to evaluate the clinical value of this technique. *Materials and Methods:* The authors performed transfontanellar ultrasonography in 481 single-birth cases at 28-43 weeks' gestation. Ultrasonographic examinations were performed in the parasagittal plane at the level of the insula through the anterior fontanelle, measuring area, and perimeter of the insula. Regression analyses were used to evaluate the relationship between insula size and gestational age (GA), and 60 cases were randomly selected for assessment of intra-observer and inter-observer reliability of ultrasonographic measurements. The authors obtained standard values of normal insula size and used them to assess and follow-up 40 cases with suspected insular malformations. Additionally, 30 late-onset neonates who were determined as small for gestational age (SGA) and 45 normally growing neonates were examined and tested using the Neonatal Behavioral Assessment Scale (NBAS). *Results:* The neonatal insula appeared as an inverted triangle, with the insular gyri extending radially in an anterior-inferior to posterior-superior direction. The area and perimeter of the normal neonatal insula significantly increased with GA ($p < 0.01$ for both), and these measurements were highly reliable. This assessment of cases with suspected insular malformation showed that five out of 40 cases presented abnormalities. Late-onset SGA neonates presented a significantly smaller area and perimeter in the insula compared to controls. In addition, the measured values of the insula significantly correlated with NBAS scores. *Conclusion:* Evaluation of the neonatal insula using transfontanellar ultrasonography can be performed and is clinically useful.

Key words: Insula development; Late-onset neonates; Gestational age; Insular abnormalities.

Introduction

The insula, or insular lobe, is located deep within the lateral fissure and accounts for only a small volume of the cerebral hemispheres. The insula is covered by the opercula, which are part of the frontal, parietal, and temporal lobes [1]. The anterior, superior, and inferior limiting sulci, which are located on the medial surface of the fronto-orbital, frontoparietal, and temporal opercula, separate the insula from the opercular cortex. The insular lobe, which is shaped like an inverted pyramid, is composed of a number of gyri. The insula is involved in sensory, motor, cognitive, emotive, and visceral functions [2-9]. Obsessive-compulsive disorder, epilepsy, Parkinson's disease, anxiety, drug addiction, and other neuropsychiatric disorders are associated with insular abnormalities [10-14]. Recent studies performed using MRI and functional MRI (fMRI) have provided information on the function of the insula. Gousias *et al.* [15] used three-dimensional reconstructions of MRI scans to measure the volume of the insula. By comparing the insular volumes of preterm infants with intrauterine growth restriction to those of normal-term infants, Padilla *et al.* [16] found that the grey and white matter in preterm infants was smaller. Although the use of MRI has obvious advantages for the assessment of the brain, cranial ultrasonography is a convenient, inexpensive, and applicable technique for bedside consultation, which makes it invaluable for the screening of neonatal cerebral diseases. Prenatal screening by ultrasonography can be used to examine abnormalities in multiple brain regions and structures such as the cerebellum, corpus callosum, sulci, gyri, and ventricles. Brain gyri can be observed in the 17th week of pregnancy, while the cingulate sulcus can be observed in the 24th week of pregnancy. Govaert *et al.* [17], who first described the use of cranial ultrasonography to examine the insula, expounded the performance of this technique for the examination of normal and abnormal anatomy, but a quantitative index is still not available. Currently, there are few studies addressing normal development of the insula in subjects that are normal for gestational age (GA). The aims of this study were to assess the morphological characteristics and to establish ultrasonographic standards of normal neonatal insula size using transfontanellar ultrasonography, and to evaluate the clinical value of this technique.

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Materials and Methods

This study was performed at the Department of Ultrasonography of the Second Affiliated Hospital of Fujian Medical University from June 2012 to December 2013. The study protocol was approved by the Ethics Committee at the Second Affiliated Hospital of Fujian Medical University, and written consent was obtained from the parents or legal guardians prior to enrollment in the study.

Using systematic sampling, 481 neonatal pediatric inpatients (285 males and 196 females) were selected for this study. The inclusion criteria were as follows: 1) mothers with regular menstruation who knew the exact date of their last menstrual period, 2) a singleton pregnancy with GA between 28 and 43 weeks and confirmed by ultrasonic estimation of the crown-rump length early in the first trimester, 3) no medical history of congenital central nervous system disorders, 4) no other risks or medical history that might cause fetal central nervous system diseases, and 5) the GA estimated using ultrasonography had to be consistent with the gestational weeks calculated according to the menstrual history. In addition, a sample of 75 singleton neonates, including 30 neonates who were classified as late-onset small for gestational age (SGA) and 45 appropriate for gestational age (AGA) infants were tested using the Neonatal Behavioral Assessment Scale (NBAS). These singleton neonates were between 34-37 weeks' gestation.

All examinations were carried out using an ultrasonographic machine equipped with a convex array probe with a frequency of 3.5 MHz, and an ultrasonographic machine equipped with a 7.5 MHz linear probe.

All neonates were examined three to seven days after birth using transfontanellar ultrasonography. The authors attempted to maintain the newborn in a quiet state in order to avoid interferences during examination, while the probe was placed on the anterior fontanelle. After performing routine scanning in the coronal and parasagittal planes to exclude intracranial lesions, the morphological features of the insula in the parasagittal plane were examined. All images were saved to the device for off line measurements. The perimeter (cm) and area (cm^2) of the insula were measured, and the same doctor obtained three separate measurements and averaged them (Figure 1). In addition, the authors identified fetal perimeter and area of insula at a specific GA (28 to 43 weeks) with a regression model.

For the reliability analysis, 60 cases were collected by systematic sampling. Each of the observers performed two consecutive image collections from each subject, and images were saved for offline analysis. The measurements that were conducted by X.K. Chen were used to evaluate intra-observer repeatability. The measurements that were obtained by X.K. Chen and S.H. Chen were used to evaluate inter-observer reliability. The method of measurement was the same as that described earlier.

The authors screened an additional 40 newborns with suspected insular malformations to evaluate if the established criteria were helpful in clinical diagnosis. Additionally, 30 SGA and 45 AGA neonates were respectively screened using the above method and evaluated by NBAS.SPSS 17.0 software package that was used to analyze the measurement data of normal newborns of different GAs in order to determine the normal reference values of the insular area and perimeter, which were expressed as mean ± standard deviation. The relation between measured values and gestational weeks was determined using regression analysis. Data for AGA and SGA newborns measured by two different physicians were analyzed for repeatability and consistency using the intraclass correlation coefficient and the Bland-Altman method. *P* values less than 0.05 were considered statistically significant. Student's *t*-tests for independent samples and Pearson's χ^2 tests were used to compare the quantitative and qualitative data, respectively.

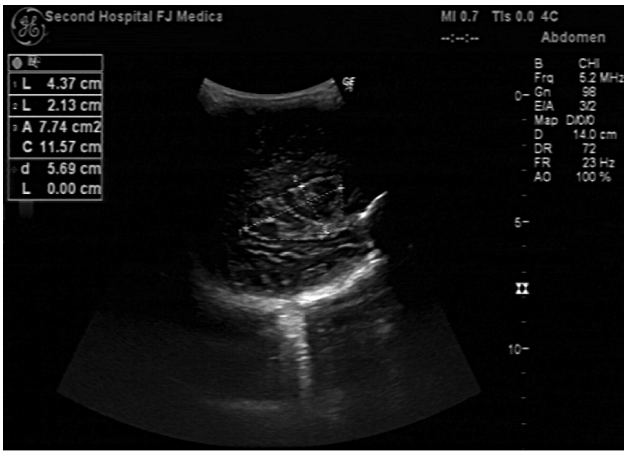

Figure 1. — Triangular shape of the insula lobe is seen in parasagittal insula plane at 39 gestational weeks.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

Results

The neonatal insula is located on the facies medialis of the temporal lobe and deep within the lateral fissure. The insular lobe, which looks like an inverted triangle, is separated from the surrounding brain lobes by the anterior, superior, and inferior limiting sulci. The insular gyri are petal-shaped and extend radially in an anterior-inferior to posterior-superior direction. The central sulcus of the insula, which divides it into a larger anterior and a smaller posterior insula, is clearly seen in the majority of newborns. The anterior insular lobe, which is associated with the frontal lobe, is mainly composed of three short gyri. The posterior insula, which is in close contact with the temporal lobe, is mainly composed of the anterior long insular gyrus and the posterior long insular gyrus (Figure 2).

Figures 3 and 4 show the relation between the area and perimeter of the neonatal insula and gestational age in weeks. The area and perimeter of the normal neonatal insula increased with GA. The regression equations for the insular area and perimeter were as follows:

$$\text{Area } (cm^2) = 2.28 - 0.307GA + 0.011GA^2$$
$$\text{Perimeter } (cm) = -2.85 + 0.316GA + 0.001GA^2$$

The intraclass correlation coefficient and its 95% confidence intervals for the measurement of the area and perimeter of the neonatal insula and the Bland-Altman analysis of measurements of the area and perimeter of the insula performed by different physicians is shown in Figures 5a and

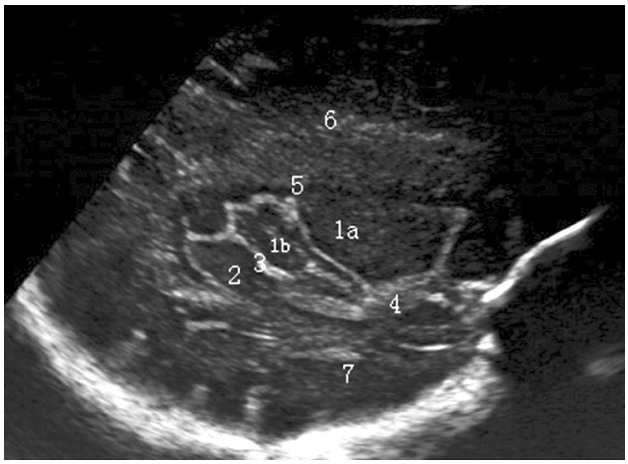

Figure 2. — Parasagittal plane of insula at 40 gestation weeks. 1a and 1b: short gyri of insula; 2: long gyrus of insula; 3: central sulcus of insula; 4: limen of insula; 5: limiting sulci; 6: frontal lobe; 7: temporal lobe.

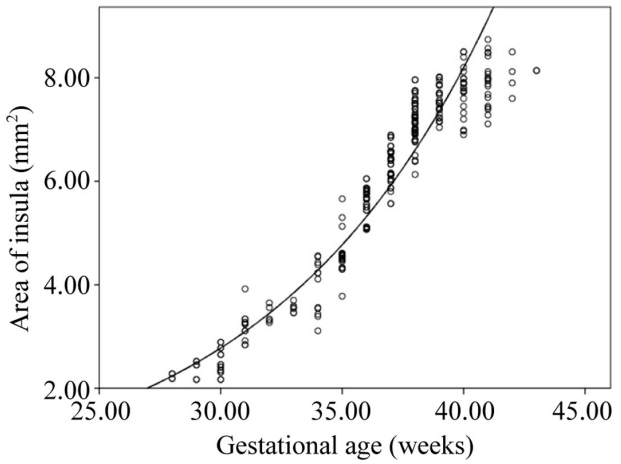

Figure 3. — The area of the neonatal insula against gestational age.

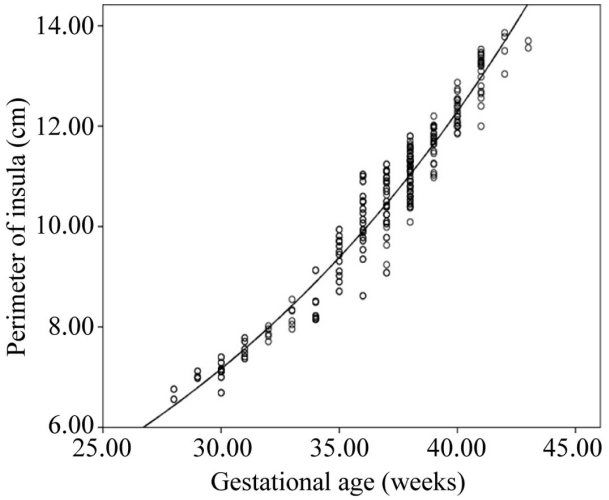

Figure 4. — The perimeter of the neonatal insula against gestational age.

5b. The repeatability of these measurements was very high, regardless of whether the measurements were performed by the same or by different physicians. As observed in the figures, these measurements were very consistent.

In the present study, the secondary gyri of the insula were almost visible at 28 weeks' gestation, and became clearly visible at 34 weeks (Figure 6). However the abnormal morphology of the neonatal insula appeared as long irregular strips without gyrus which clearly delineated the insula. In addition, the area and perimeter of the insula were at least two standard deviations less than the corresponding values in newborns of normal GA. Five cases had insular abnormalities. In addition to insular abnormalities, three new-

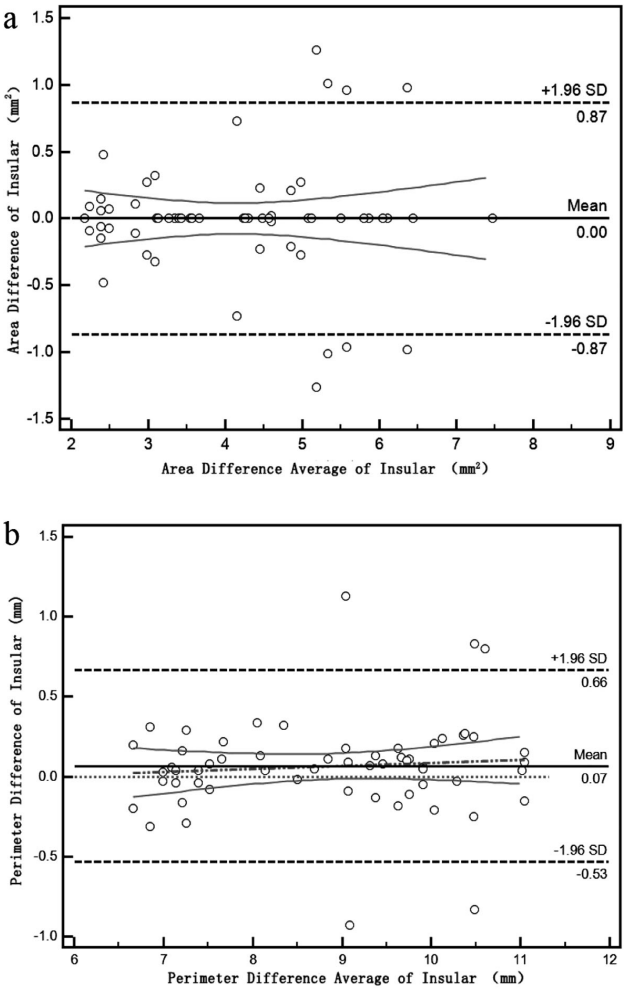

Figure 5. — A: Bland-Altman Plots of the area of insula measured by different physicians; B: Bland-Altman Plots of the perimeter of insula measured by different physicians.

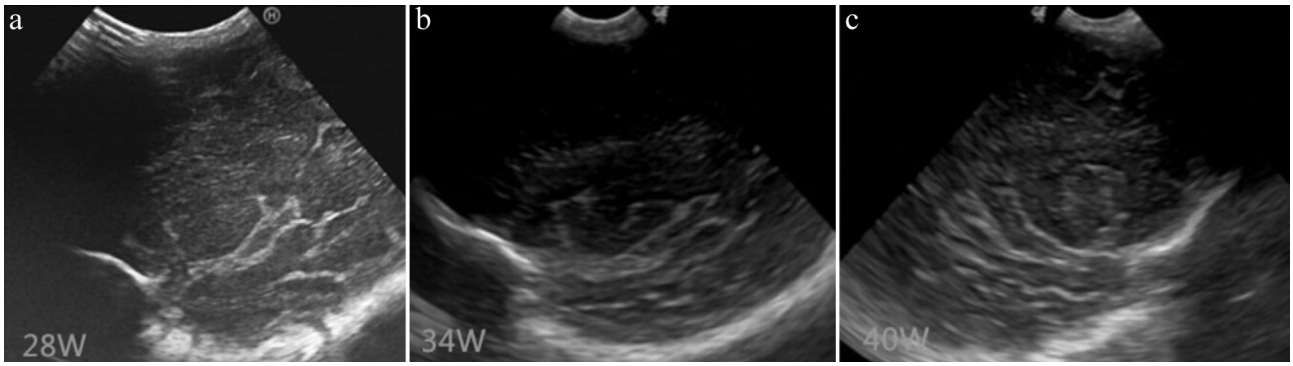

Figure 6. — Development of insular gyration in insular plane. The secondary gyri of the insula are almost visible at the 28th week of gestation, and they become much clearer at 34 and 40 weeks gestation.

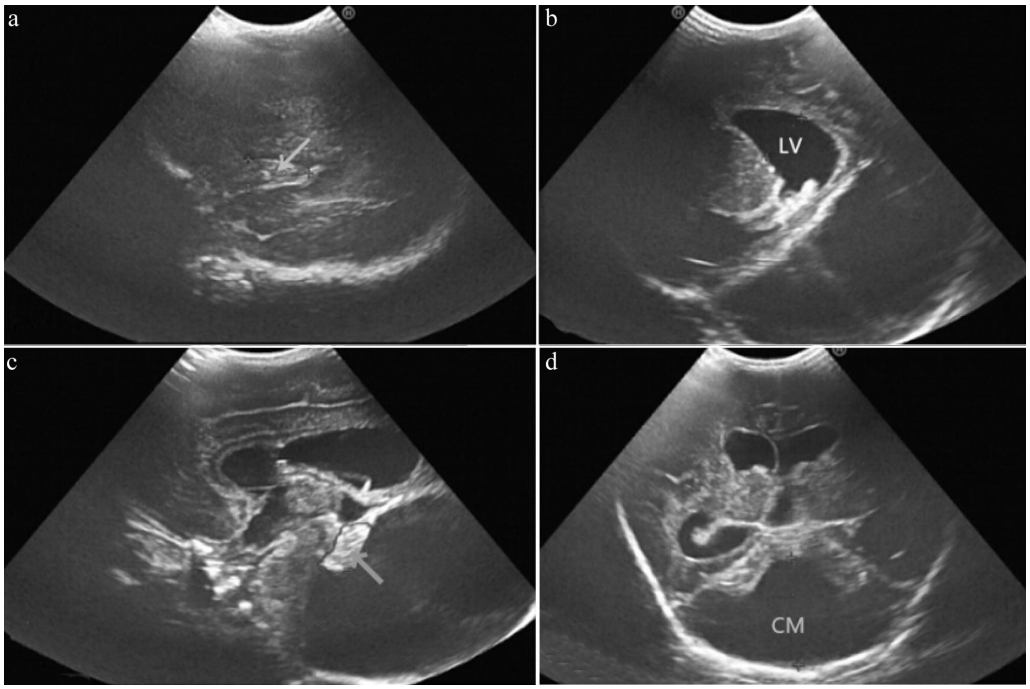

Figure 7. — Ultrasonogram of the newborn with abnormal insula associated with severe hydrocephalus at 38 gestation weeks. a) (arrows) Insula shows a small unclear triangle lacking secondary gyri in insular plane. (b) Combined with severe dilatation of the lateral ventricles. LV: lateral ventricles. c) (arrows) The cerebellum with clear dysplasia. (d) The cisterna magna is severely expand. CM: cistema magna.

borns also exhibited bilateral ventricular enlargement with severe ventricular dilation, expansion of the cavity of the septum pellucidum, and unclear cerebellar structure. Furthermore, one newborn showed slight dilation of the lateral ventricles, two presented leukomalacia near the anterior horn of the left ventricle, one had leukomalacia near the anterior horn of both lateral ventricles, and two showed agenesis of the cerebellar vermis (Figure 7).

The present results showed that SGA and AGA neonates exhibited very different perimeters and areas of the insula. In addition, NBAS scores of SGA neonates were significantly lower than those of AGA newborns (Table 1).

Discussion

Overall, the size of the neonatal insula increased with GA, and the area and perimeter were parameters with a high degree of reliability. Using these values the present authors found five cases of insular abnormalities. An abnormally developed insular lobe can appear as a small triangle lacking secondary gyri, an area lacking the normal triangular shape, or an abnormal secondary gyrus that is supported by the cerebral lateral fissure [17]. Because the insular lobe is composed of gyri, and is surrounded by three limiting sulci, the abnormal development of sulci and gyri may therefore influence the size of the insula. Previous studies have reported that insular abnormalities are common in polymicrogyria syndrome, dilated lateral ventricles,

Table 1. — *Perinatal outcome of the study groups.*

	SGA (n=30)	AGA (n=45)	*p*
GA at birth (weeks)	35.6 ± 1.4	36.3 ± 1.1	0.58
Birthweight (grams)	1689 ± 236	1863 ± 268	< 0.01
Male gender	51.5%	48.9%	0.68
NBAS	40.6 ± 2.5	45.3 ± 2.3	0.04
Area of insular	419.2 ± 36.4	469.7 ± 49.5	< 0.01
Perimeter of insular	86.1 ± 4.9	93.5 ± 5.2	< 0.01

glutaricacidemia type II, and other diseases and etiologies [17]. In cases of serious ventricular dilatation, the brain parenchyma may be compressed and sometimes is not detected. In addition to compression factors, serious ventricular dilation with retardation of the development of cerebral sulci and gyri may be related to other factors. For example, a fetus with ischemic cerebrovascular disease with dilated lateral ventricles often exhibits cerebral abnormalities such as agyria and necrotic lesions such as periventricular leukomalacia [18]. Furthermore, intrauterine growth restriction affects the development of the fetus and its brain. Dysplasia of the insula in the present five cases were associated with the presence of severely dilated ventricles or leukomalacia. In addition, this comparative assessment of 30 SGA and 45 AGA newborns showed that they presented different insula size (i.e., perimeter and area). These findings are in agreement with previous MRI studies [19]. Previous studies have shown that SGA neonates had a significantly thinner and smaller cortex compared to AGA newborns. Previous reports on the neurodevelopmental outcomes of late-onset SGA infants have shown decreased attention, sociability, self-regulation, communication, problem-solving, and memory function scores [20-22]. All these functions are closely related to the insula and the limbic system [8]. Egaña-Ugrinovic *et al.* found significant association between insular size and neurobehavioral parameters in the NBAS test. In term SGA infants, the thinner and smaller insular were, the worse the neurobehavioral outcomes were [18]. Accordingly, in the present study, NBAS scores of SGA infants were significantly lower than those of AGA newborns, supporting that insular abnormalities affect newborn neural function. The cause of SGA is still unclear, although it is possible that pathogenic factors such as chronic intrauterine hypoxia originate from the mother, the fetus, or the placenta. SGA is the main complication in pregnant women with high blood pressure, diabetes, or cardiovascular disease. Through observations of the neonatal insula at 28-43 weeks' gestation, the present authors found that each sulcus and gyrus of the insular lobe was visible using transfontanellar ultrasonography after 34 weeks' gestation, and the area and perimeter of the insula positively correlated with increases in GA.

The present study had some limitations. Although the present normal reference values of neonatal insular size covered a large majority of GAs, reference ranges for GAs before 28 weeks are still needed. Moreover, it will be necessary to replicate the present findings in future studies with a larger sample size for each GA. In addition, further research is needed to explore the effects of Doppler ultrasonography on insular blood flow.

Although CT and MRI are more frequently used in the diagnosis of brain diseases, these methods have a number of problems. They are expensive, radioactive, and extremely inconvenient to use in serious cases and in children who are not easy to calm. Transfontanellar ultrasonography has the advantages of being non-invasive, quick, and convenient. It provides an effective means for observing neonatal insular development, and it can better track and assess neonatal insular development over time.

Acknowledgments

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