Received:
28 November 2016

Revised:
20 January 2017

Accepted:
25 January 2017

<https://doi.org/10.1259/bjr.20160902>

Cite this article as:

Williams F, Griffiths PD. *In utero* MR imaging in fetuses at high risk of lissencephaly. *Br J Radiol* 2017; **90**: 20160902.

FULL PAPER

In utero MR imaging in fetuses at high risk of lissencephaly

FIONN WILLIAMS, FRCR and PAUL D GRIFFITHS, PhD, FRCR

Academic Unit of Radiology, University of Sheffield, Royal Hallamshire Hospital, Sheffield, UK

Address correspondence to: Professor Paul D Griffiths

E-mail: p.griffiths@sheffield.ac.uk

Objective: Lissencephaly is a rare disorder of cortical developmental, which usually carries increased risk of recurrence in future pregnancies. In this prospective observational study, we wished to test the hypothesis that sulcation patterns can be used to diagnose lissencephaly successfully on *in utero* MR (iuMR) imaging in the third-trimester but not in the late second-trimester fetus.

Methods: Pregnant females were recruited into this study if they had an increased risk of fetal lissencephaly based on a fetus or child with lissencephaly in an earlier pregnancy. All females were offered serial iuMR examinations at one centre and are reported whether they had at least two examinations. The overall recurrence rate of lissencephaly was recorded along with the sulcation patterns of non-affected fetuses.

Results: 19 females were recruited with 23 pregnancies. In 3/23 (13%) fetuses, lissencephaly was diagnosed on iuMR and not detected on ultrasonography. In two cases, the

diagnosis of lissencephaly was made on second-trimester iuMR imaging—with certainty in one and described as “possible” in the other. Confident diagnoses of lissencephaly were made by 28-week gestation in all three cases. Four fetuses, ultimately shown not to have lissencephaly, were judged to have minor sulcation delay on second-trimester imaging but became gestational age appropriate in the third trimester.

Conclusion: iuMR imaging can identify fetal lissencephaly between 20 and 24 weeks, but false positives should be expected, particularly in the second trimester, and follow-up imaging later in pregnancy may be required.

Advances in knowledge: It is possible to detect fetal lissencephaly between 20- and 24-week gestational age; but, it is considerably easier in the third trimester. As a result, if a fetus has an increased risk of lissencephaly on the basis of family history, it may be necessary to do serial iuMR studies to confirm normality (or abnormality) of the fetal brain.

INTRODUCTION

Lissencephaly is a rare developmental malformation of the brain with an estimated prevalence of 1 in 100,000 births and is classified as a failure of cortical formation, specifically of abnormal neuroglial migration.^{1,2} Macroscopically, this produces marked widening of the cortical gyri and a reduction in the number and complexity of sulci, producing abnormally smooth cerebral hemispheres. Microscopically, there is often disruption of the normal six-layer structure of the neocortex. The prognosis for children affected by lissencephaly is generally poor, with a high likelihood of seizures and severe neurodevelopmental problems.

The prenatal diagnosis of lissencephaly is theoretically challenging, and this appears to be borne out in clinical practice using ultrasonography. *In utero* MR (iuMR) imaging provides a supplemental method of assessing the fetus and may provide additional clinically relevant information compared with ultrasound alone. This is predominantly due to the superior tissue contrast and resolution of iuMR when compared with ultrasound and

so is likely to be particularly valuable in showing fetal brain pathology.^{3,4} There are, however, fundamental difficulties in diagnosing abnormalities such as lissencephaly *in utero* with any imaging modality. The fetal brain normally undergoes significant developmental change in the second and third trimesters, particularly in terms of gyration/sulcation. Most centres do not perform iuMR before 18 weeks and at that stage, the normal fetal brain is smooth and relatively featureless. Over the next 20 weeks, the major sulci and gyri form in a reasonably predictable fashion producing the usual highly complex surface features associated with the term fetus.^{5,6} Reduced sulcation is a major part of the diagnosis of lissencephaly in children; so, in the fetus, it is likely that there is a gestational age before which a diagnosis of lissencephaly is not possible with certainty.

The purpose of this study was to establish early data about sulcation patterns of brain in fetuses at a high risk of lissencephaly because of lissencephaly in an earlier pregnancy. We wished to test the hypothesis that sulcation patterns can

be used to diagnose lissencephaly successfully in the third-trimester but not in the late second-trimester fetus.

METHODS AND MATERIALS

Pregnant females were eligible to come into the study if they had a history of confirmed lissencephaly in a fetus or child from an earlier pregnancy, had no known or suspected contraindications to MR and were willing to consider serial iuMR studies in the second and/or third trimesters of pregnancy. Most females were recruited as part of a research study and provided fully informed written consent under the guidance and with the approval of our regional ethics committee. Those females were not paid for their involvement in the study, but travel expenses were offered for themselves and a companion. Relevant review was sought, and approval obtained, from the Institutional Clinical Effectiveness Unit and Research Department in order to allow the small number of MR examinations performed for clinical purposes to be reported in this study as well. Females were recruited from nine regional fetal maternal centres in England between 2004 and 2012. All of the females underwent a detailed sonographic assessment at their base hospital by an experienced fetal maternal consultant.

The females were invited to attend for three iuMR studies, the first between 20- and 24-week gestation, the second at 27–28 weeks and the third at 30–34 weeks. Females are included in the analysis if they attended for at least two studies or if the pregnancy was interrupted after one iuMR examination. All of the iuMR examinations were performed at the Academic Unit of Radiology, University of Sheffield, on a 1.5-T system (Infinion 1.5T, Philips Medical Systems—2004 to 2007, Signa 1.5T HDx, GE Healthcare—2008–12). The following sequences were performed as a minimum: 4–5-mm-thick T_2 weighted sequences in three orthogonal planes using single shot fast spin echo, 5-mm gradient-echo T_1 weighted imaging in the axial plane and 5-mm diffusion-weighted imaging ($b = 700$) also in the axial plane. The examinations were interpreted and a clinical-style report issued at the time of the iuMR; but, for the purposes of this study, the examinations were re-evaluated by two neuroradiologists (PDG and FW).

Along with their general assessment of the fetal brain, the neuroradiologists made an assessment of sulcation of the cerebral hemispheres in comparison with the gestational age using a standard reference text.⁶ Sulcation patterns were classified as either normal or definitely abnormal—lissencephaly, minor sulcation delay (uncertain significance) or delayed sulcation—possibly lissencephaly (based on other features to suggest abnormal brain development).

RESULTS

19 females with 23 pregnancies (4 females attended with different pregnancies) met the entrance criteria for inclusion in the study. A summary of the timings and findings of each iuMR examination is shown in Table 1 along with the findings of the late second-trimester ultrasound examination results. 16 fetuses had a normal ultrasound examination, and at least 2 normal iuMR brain examinations, including age-appropriate sulcation patterns. 12 of those cases had postnatal clinical follow-up available ranging between 2 and 6 years, and all of those had

normal neurodevelopmental status. Four fetuses had a normal ultrasound examination but mild sulcation delay (uncertain significance) on the 20–24-week iuMR study. Concern was based on possible undersulcation of the sylvian fissures; but, iuMR studies performed in the third trimester showed normal sulcation patterns in all four cases. Postnatal follow-up information was available in all four of those cases (ranging from 1 to 4 years), and there was no concern about neurodevelopmental progress in any of the four (they were not under the care of paediatricians for neurological problems). We have assumed that those children did not have lissencephaly. It is important to underline that our outcome reference standard did not include postnatal MRI in children with no suspicion of lissencephaly. This is because a fetus at high risk of lissencephaly on the basis of family history but with normal antenatal ultrasound and iuMR studies and having no neurodevelopmental problems as a child is unlikely to get a postnatal MR examination for clinical reasons.

Three fetuses were reported as having lissencephaly on iuMR. One fetus (Case 4 in Figure 1) had the first iuMR study at 23 weeks following an antenatal ultrasound examination showing borderline ventriculomegaly. The first iuMR examination was reported as “definitely abnormal—lissencephaly”, and hypogenesis of the corpus callosum was found as well. A repeat iuMR examination at 28 weeks redemonstrated and reaffirmed the findings of the earlier study, and pregnancy was terminated. Autopsy confirmed the structural findings of the iuMR study, and a diagnosis of Miller–Dieker syndrome was made. The second fetus with iuMR findings of lissencephaly (Case 17 in Figure 2) had a normal second-trimester ultrasound examination but did not have a second-trimester iuMR study. The first iuMR examination at 28 weeks was reported as “definitely abnormal—lissencephaly”. The pregnancy ended as a stillbirth 2 weeks later, but no post-mortem studies were performed. The third fetus (Case 22 in Figure 3) had a normal second-trimester ultrasound examination, but the first iuMR study at 23 weeks showed “delayed sulcation—possibly due to lissencephaly”, although germinolytic cysts were also present. A repeat examination at 28 weeks was reported as “definitely abnormal—lissencephaly”. Those findings were confirmed on postnatal imaging.

DISCUSSION

The formation of the normal cerebral cortex is a complicated process that can be thought of as three, sequential but overlapping processes namely neuroglial; proliferation, migration and organization.^{1,2} Neuroglial proliferation occurs within the ventricular zone (or “germinal matrix”) of the second-trimester fetus and is followed by targeted migration of cells to a pre-determined site in the developing cerebral cortex (fate mapping). On reaching the future cortex, the cells must form a layered structure associated with mature cerebral cortex; for example, the six-layered pattern of neocortex. Disruption of those processes leads to a group of brain abnormalities termed “malformations of cortical development” of which lissencephaly is one. Lissencephaly is considered to result from failure of neuroglial migration,^{1,2,7,8} and although abnormal migration is the root cause of the malformation, its manifestations in the

Table 1. A summary of the timing of antenatal MRI and imaging findings in 23 fetuses at increased risk of lissencephaly

Case	20–24-week ultrasound	20–24-week iuMR	27–28-week iuMR	30–34-week iuMR	Outcome
1	Normal	Normal	Not performed	Normal	Normal at 3 years
2	Normal	Normal	Not performed	Normal	Normal at 2 years
3	Normal	Normal	Not performed	Normal	Normal at 2 years
4 (Figure 1)	Ventriculomegaly only	Definitely abnormal—lissencephaly, hypogenesis of corpus callosum	Definitely abnormal—lissencephaly hypogenesis of corpus callosum	TOP 29 weeks	PM diagnosis Miller–Dieker syndrome
5	Normal	Minor sulcation delay	Normal	Normal	Normal at 2 years
6	Normal	Minor sulcation delay	Normal	Normal	Normal at 2 years
7 (Figure 4)	Normal	Minor sulcation delay	Normal	Normal	Normal at 4 years
8	Normal	Normal	Not performed	Normal	NFA
9	Normal	Minor sulcation delay	Normal	Not performed	Normal at 1 year
10	Normal	Normal	Not performed	Normal	NFA
11	Normal	Normal	Not performed	Normal	Normal at 1 year
12	Normal	Normal	Not performed	Normal	Normal at 5 years
13	Normal	Normal	Not performed	Normal	Normal at 6 years
14	Normal	Normal	Not performed	Normal	Normal at 5 years
15	Normal	Normal	Not performed	Normal	Normal at 6 years
16	Normal	Normal	Not performed	Normal	Normal at 6 years
17 (Figure 2)	Normal	Not performed	Definitely abnormal—lissencephaly	Stillborn 30 weeks	No autopsy
18	Normal	Normal	Not performed	Normal	Normal at 5 years
19	Normal	Normal	Not performed	Normal	Normal at 3 years
20	Normal	Normal	Not performed	Normal	NFA
21	Normal	Not performed	Normal	Normal	NFA
22 (Figure 3)	Normal	Delayed sulcation—possible lissencephaly, germinolytic cysts	Definitely abnormal—lissencephaly, germinolytic cysts	Not performed	Lissencephaly confirmed on postnatal MR
23	Normal	Normal	Not performed	Normal	Normal at 3 years

iuMR, *in utero* MR; NFA, no follow-up available; PM, post-mortem examinations; TOP, termination of pregnancy.

adult brain are wide ranging. The typical postnatal pathological and radiological features of lissencephaly are the absence or widening of cerebral gyri (agyria or pachygyria) associated with no or fewer cortical sulci, which are frequently abnormal. Microscopically, the neocortex often shows abnormal thickening and lack of the normal six-layered pattern.

Historically, lissencephaly has been subclassified as either “classical” (Type 1) or “cobblestone” (Type 2).^{1,2} In classical lissencephaly, there is a thickened, four-layered neocortex, and the abnormality may be isolated (isolated lissencephaly sequence) or associated with other features such as facial dysmorphism, as in Miller–Dieker syndrome. Other brain abnormalities such as failure of commissuration, cerebellar hypoplasia and microcephaly can be found in association with classical lissencephaly. Cobblestone lissencephaly consists of a disorganized unlayered

cortex with leptomeningeal neuronal and glial ectopia and is often associated with muscular dystrophy or eye malformations (e.g. Fukuyama congenital muscular dystrophy, Walker–Warburg syndrome and muscle–eye–brain disease).

Barkovich et al⁹ have extended the classification of Malformations of cortical development based on the identification of causative genetic defects. Type IIA abnormalities include lissencephaly and subcortical band heterotopia (incorporating classical lissencephaly) and Type IIB refers to the cobblestone complex of disorders. It is apparent that single-gene defects may be associated with >1 phenotype (depending on the exact nature and severity of the defect), and conversely a particular phenotype may be caused by abnormalities in a number of different genes. Mutations of LIS1 and DCX genes account for 77% of cases of lissencephaly (65% and 12%, respectively).¹⁰

Figure 1. Case 4: axial (a) and coronal (b) single shot fast spin echo images of a fetus at 23-week gestation show marked sulcation delay and hypogenesis of the corpus callosum. A diagnosis of “definitely abnormal—lissencephaly” was made, and the findings were supported by *in utero* MR at 28 weeks (c, d) and confirmed post-mortem. Hypogenesis of the corpus callosum was also present, and the ultrasound scan diagnosis of mild ventriculomegaly was confirmed with both trigones measuring 10 mm.

The inheritance pattern of these genes is autosomal dominant (LIS1—chromosome 17) and X-linked (DCX); however, the majority of cases are a result of *de novo* mutations. In a small

number of cases, there may be an identifiable parental genetic defect; for example, in cases where a female with a mild phenotype carries a defective copy of the DCX gene, the recurrence risk

Figure 2. Case 17: axial (a) and coronal (b) single shot fast spin echo images of a fetus imaged with *in utero* MR (iuMR) at 28-week gestation show “definitely abnormal—lissencephaly”. Second-trimester iuMR was not performed in this case, and there were no post-mortem studies.

Figure 3. Case 22: axial (a, b) and coronal (c) single shot fast spin echo images of a fetus at 23-week gestation show “delayed sulcation —possibly due to lissencephaly” and germinolytic cysts. Repeat examination at 28 weeks (d-f) shows a typical figure of eight configuration allowing a confident diagnosis of “definitely abnormal—lissencephaly” to be made. The germinolytic cysts persist.

may be as high as 50%. Alternatively, when one parent has a balanced translocation involving the LIS1 gene, the recurrence risk for isolated lissencephaly sequence is thought to be around 10–15%.¹⁰ The recurrence risk for cobblestone lissencephaly is 25% (autosomal recessive inheritance), as is the case for lissencephaly with cerebellar hypoplasia.

There is no doubt that the *in utero* diagnosis of lissencephaly is challenging using ultrasonography, and anecdotal experience based on our paediatric neuroradiological practice suggests that nearly all children with lissencephaly are not detected by antenatal ultrasonography. Advances in ultrasonography may change this limitation, and two recent publications have suggested that improved diagnostic accuracy with ultrasound is possible.^{11,12} Our present study lends support to that suggestion, as the 3/23 (13%) fetuses with lissencephaly did not have the diagnosis made on ultrasonography, although borderline ventriculomegaly was found in 1 case. The inability to detect (or raise the possibility of) lissencephaly on ultrasound, coupled with the rarity of the disorder (prevalence approximately 1:100000), makes it virtually impossible to perform a prospective study of diagnostic

accuracy of antenatal imaging methods in *de novo* cases. This problem led us to the design of the present study, in which we recruited pregnant females who had an increased risk of fetal lissencephaly because of a fetus/child with lissencephaly in an earlier pregnancy. Even in this situation, it was not possible to recruit large numbers (19 females and 23 pregnancies), in spite of working with 9 regional centres (combined population of approximately 16 million) over a long period of time (recruited over 8 years). This problem was compounded by the relatively low recurrence rate in our study (13%). Few of the females in this study had had formal genetic testing performed on the previously effected fetus; therefore, it was impossible to quote formal recurrence risks in most cases. It is more likely that any future study will have the advantage of improved genetic information, and this would be coupled with a formal review of imaging or autopsy data from previous pregnancies.

On the practical side, iuMR was generally well tolerated by the females, although only three of the females in the study had all three iuMR examinations as suggested in the original protocol. We have not investigated this issue formally, but our impression

Figure 4. Case 7: a case in which sulcation was considered to be mildly delayed at 22 weeks [axial (a) and coronal (b) single shot fast spin echo images], although there were no other suspicious findings, *i.e.* the cortical plate has normal thickness and the transient layers of the cerebral hemispheres are normal. Follow-up *in utero* MR imaging at 26 weeks (c, d) and 31 weeks (e, f) shows a normal brain with normal sulcation.

is that it is related to the distances some females had to travel to have the iuMR examinations. This is important because the timing of the iuMR examination is central to the issue, as we have predicted that diagnostic accuracy and confidence are related to gestational age. All three fetuses identified as having lissencephaly had iuMR examinations at 27–28-week gestational age, and confident diagnoses were made in all three cases at that time. Only two of those had iuMR examinations in the second trimester (both at 23 weeks) and at that stage, the position was less certain. Although a confident diagnosis of lissencephaly was made on iuMR at 23 weeks in Case 4, it was decided to repeat the examination at 28 weeks and only at that stage was the decision made to terminate the pregnancy. In the other fetus (Case 22), the sulcation abnormalities at 23 weeks were much more subtle, and a possible diagnosis of lissencephaly was only entertained because of the presence of germinal cysts, which have been shown in conditions such as Zellweger syndrome.¹³ At 28 weeks, diagnosis of lissencephaly was much clearer.

4/20 (20%) fetuses who did not have lissencephaly on third-trimester iuMR and had normal postnatal neurodevelopmental outcomes had non-specific undersulcation of the cerebral hemispheres reported on the 20–24-week iuMR. The major sulci and gyri form in a recognized pattern, between 20- and 30-week gestation,^{6,7} but there is often marked interindividual variability. As a result, it is often clear whether those surface features are

pathologically absent or merely delayed in their appearance because of biological variability in the late second trimester. This highlights the pressing need for more iuMR studies on normal pregnancies. We restate that our study only had three cases of recurrent lissencephaly; so, our conclusions must be guarded. What appears to be true from our study is that it is easier to be confident about the diagnosis of lissencephaly from 28-week gestation when compared with late second-trimester imaging. There seems to be a significant false positive rate and at that stage. This presents significant clinical problems, particularly if the female would consider termination of pregnancy if lissencephaly was confirmed in the pregnancy. Many countries and some institutions in countries do not offer “late” termination of pregnancy and indeed the mechanism for performing termination of pregnancy (by fetocide) is a much more complicated and interventional procedure after 23 weeks. This is why we recommend that looking for lissencephaly in an at-risk fetus should commence at 21–22-week gestation, although further examinations later in pregnancy are likely to be required.

Is it possible that there are radiological signs on iuMR other than sulcation patterns that will improve the quality of diagnosis of lissencephaly in the late second trimester? Postnatally, great weight is put on the thickness of the cerebral cortex when diagnosing some varieties of lissencephaly. It must be remembered that the outer part of the cerebral hemispheres of the second-trimester fetus is not the cerebral cortex but

a temporary structure called the cortical plate. This does not appear to be abnormally thickened in the few cases of second-trimester lissencephaly we have detected on iuMR. It is possible, however, that there is more information in the other “transient layers” of the second-trimester cerebral hemispheres, possibly in the intermediate layer and cortical subplate regions. Those regions are relatively poorly shown on current iuMR methods, but future developments including fast, high-resolution diffusion-weighted images hold potential because of the different diffusivity of the cell-rich and cell-sparse portions of the transient layers.

In conclusion, there are problems with accurate and confident diagnosis of fetal lissencephaly in the second trimester, although it is possible in some cases. Diagnosis at 28-week gestational age

or more appears to be easier, but we stress that larger studies need to be performed in order to provide formal descriptive statistics such as sensitivity and positive- and negative-predictive values. This may prove exceptionally difficult because of the rarity of the condition. We also stress the relatively high rate of false-positive findings on iuMR examinations performed at 20–24 weeks based on mild delays in sulcation and the requirement for interval imaging in such cases.

ACKNOWLEDGMENTS

The authors would like to recognize the work of Dr M Reeves in the early part of this study.

FUNDING

This study was funded by a grant from the Newlife Foundation.

REFERENCES

1. Barkovich AJ, Kuzniecky RI, Dobyns WB, Jackson GD, Becker LE, Evrard P. A classification scheme for malformations of cortical development. *Neuropediatrics* 1996; **27**: 59–63. doi: <https://doi.org/10.1055/s-2007-973750>
2. Barkovich AJ, Kuzniecky RI, Jackson GD, Guerrini R, Dobyns WB. Classification system for malformations of cortical development: update 2001. *Neurology* 2001; **57**: 2168–78. doi: <https://doi.org/10.1212/WNL.57.12.2168>
3. Griffiths PD, Reeves MJ, Morris JE, Mason G, Russell SA, Paley MN, et al. A prospective study of fetuses with isolated ventriculomegaly investigated by ante-natal ultrasound and *in utero* MR. *AJNR Am J Neuroradiol* 2010; **31**: 106–11. doi: <https://doi.org/10.3174/ajnr.A1767>
4. Whitby EH, Paley MN, Sprigg A, Rutter S, Davies NP, Wilkinson ID, et al. Comparison of ultrasound and magnetic resonance imaging in 100 singleton pregnancies with suspected brain abnormalities. *BJOG* 2004; **111**: 784–92. doi: <https://doi.org/10.1111/j.1471-0528.2004.00149.x>
5. Levine D, Barnes PD. Cortical maturation in normal and abnormal fetuses as assessed with prenatal MR imaging. *Radiology* 1999; **210**: 751–8. doi: <https://doi.org/10.1148/radiology.210.3.r99mr47751>
6. Fogliarini C, Chaumoitte K, Chapon F, Fernandez C, Levrier O, Figarella-Branger D, et al. Assessment of cortical maturation with prenatal MRI. Part I: normal cortical maturation. *Eur Radiol* 2005; **15**: 1671–85. doi: <https://doi.org/10.1007/s00330-005-2782-1>
7. Barkovich AJ, Kuzniecky RI, Dobyns WB. Radiologic classification of malformations of cortical development. *Curr Opin Neurol* 2001; **14**: 145–9. doi: <https://doi.org/10.1097/00019052-200104000-00002>
8. Dobyns WB, Truwit CL. Lissencephaly and other malformations of cortical development: 1995 update. *Neuropediatrics* 1995; **26**: 132–47. doi: <https://doi.org/10.1055/s-2007-979744>
9. Barkovich AJ, Kuzniecky RI, Jackson GD, Guerrini R, Dobyns WB. A developmental and genetic classification for malformations of cortical development. *Neurology* 2005; **65**: 1873–87. doi: <https://doi.org/10.1212/01.wnl.0000183747.05269.2d>
10. Guerrini R, Dobyns WB, Barkovich AJ. Abnormal development of the human cerebral cortex: genetics, functional consequences and treatment options. *Trends Neurosci* 2008; **31**: 154–62. doi: <https://doi.org/10.1016/j.tins.2007.12.004>
11. Lacalm A, Nadaud B, Massoud M, Putoux A, Gaucherand P, Guibaud L. Prenatal diagnosis of cobblestone lissencephaly associated with Walker-Warburg syndrome based on specific sonographic pattern. *Ultrasound Obstet Gynecol* 2016; **47**: 117–22. doi: <https://doi.org/10.1002/uog.15735>
12. Tonni G, Pattacini P, Bonasoni MP, Araujo Junior E. Prenatal diagnosis of lissencephaly type 2 using three-dimensional ultrasound and fetal MRI: case report and review of the literature. *Rev Bras Ginecol Obstet* 2016; **38**: 201–6. doi: <https://doi.org/10.1055/s-0036-1582126>
13. Russel IM, van Sonderen L, van Straaten HL, Barth PG. Subependymal germinolytic cysts in Zellweger syndrome. *Pediatr Radiol* 1995; **25**: 254–5. doi: <https://doi.org/10.1007/bf02011090>