Encephalocele: a combined experimental and clinical study of its development and prevention

Applicants: Prof Andrew Copp and Mr Dominic Thompson

Summary
Encephalocele is a serious condition of newborn babies in which part of the brain develops outside the skull, and often becomes damaged. It can lead to disability or death, and yet very little is known of what causes encephalocele, or how the brain tissue ends up outside the skull. If we could understand this, it might be possible to treat the fetus during pregnancy, to minimise damage and disability, as is already possible for children with spina bifida. In this project, we will conduct one of the first combined experimental and clinical studies of encephalocele. A new mouse model of encephalocele will provide original information on how the defect arises, while our clinical studies will reveal details of specific patients with encephalocele and what are the key risk factors. Our aim is to gather new information on how and when the brain defect arises, in order to pave the way towards future introduction of fetal surgery for encephalocele in the coming years.

Costing of the project (£)

	Year 1	**Year 2**	**Totals**
Salary			
Dawn Savery, 50% time	24,877.35	26,247.95	51,125.30
Vejay Vakharia, 15% time *	0	0	0
Research costs			
Laboratory consumables	5,000.00	5,000.00	10,000.00
Mouse strain breeding	6,439.06	6,696.62	13,135.68
Totals	**36,316.41**	**37,944.57**	**74,260.98**

* Mr Vakharia is already fully funded through his clinical appointment

Background
Encephalocele makes up around 10% of all neural tube defects (NTDs), alongside spina bifida and anencephaly. It is characterised by herniation of the meninges, with or without brain tissue, outside the skull (Figs A, B). This exposes the brain to potential damage both before and after birth and, despite surgical repair, later health problems are common including hydrocephalus, epilepsy and learning difficulties. Children with the most severe cases can fail to survive. Encephaloceles emerge along the skull midline, with fronto-ethmoidal, parietal, occipital and cervical forms regularly encountered (Figs C, D). Although the origin of encephalocele in the embryo has long been debated [1], our recent studies in a mouse genetic model show that the future brain is completed, but then part of the brain undergoes herniation due to rupture of the future skin layer [2]. A skull defect develops later, overlying the herniated region, to generate the appearance as seen in the 'mature' encephalocele lesion.

Prospects of improved outcome in encephalocele
Two new approaches may improve the clinical outlook in encephalocele. ***First***, there is the possibility that folic acid can prevent encephalocele from arising. Other NTDs can be prevented by folic acid [3], but it is unclear whether encephalocele is also prevented [1,4]. Better information on the interaction between folic acid and encephalocele is required, and we will study this in a human-like mouse model. ***Second***, we need to move towards surgical treatment of the fetus during pregnancy, to minimise damage and disability. Recent work at Great Ormond Street and UCLH has allowed fetuses with spina bifida to be repaired before birth [5], reducing several of the complications of spina bifida. In contrast, UK babies with encephalocele currently receive surgery only after they are born. However, a recent Brazilian study has suggested that fetal surgery could also be beneficial for babies with encephalocele [6]. To develop the necessary evidence

base for planning of fetal surgery in UK, we would need to understand much more about the origins of encephalocele in the embryo and fetus. This is the goal of the present project proposal.

Aims of the research

In this project, we will conduct one of the first combined experimental and clinical studies of encephalocele. Our aim is to gather important new information on how and when this brain defect arises, in order to pave the way towards future introduction of fetal surgery for this condition. Specific aims are:

1. Study a new, 'human-like' mouse model of encephalocele to understand the timing and sequence of events in the embryo/fetus that leads to the appearance of this birth defect.
2. Test whether encephalocele in the mouse model can be prevented, or reduced in severity, by treating the pregnant female with folic acid, or another related vitamin-like molecule.
3. Review and analyse cases of encephalocele at Great Ormond Street Hospital to determine the brain region affected, severity of the defect, time of first diagnosis, child's gender, and treatment outcomes.

Experimental study

The experimental part of the study will use a new 'FGF-deletion' strain of mice with encephalocele, whose features most closely resemble the commonest (occipito-parietal) type of human defect. This research is a collaboration with a molecular genetics team led by Dr Pedro Rocha at the National Institutes of Health (Bethesda, USA). The Rocha team inactivated a key DNA sequence that controls the expression of the genes for fibroblast growth factor (FGF) 4, 5 and 15. They found a brain defect in the deficient fetuses (Figs E, F) and, not being expert in brain development, asked Andrew Copp to collaborate on further studies. Currently, the mice are bred in USA and embryos/fetuses are sent to UK. Later, we will import the mouse strain so that more extensive studies can be done with the mice at ICH. The stages of the research will be:

(i) ***Analysis of the developing brain and skull***. This will be a stage-by-stage study, from the time the brain first forms until the encephalocele is fully present. This will reveal exactly how the abnormality arises, and which tissues are primarily abnormal. Preliminary sectioning of the fetal head shows a major abnormality in which one or both of the brain hemispheres protrude outside the developing skull (Figs G, H). Sections will be prepared at all stages, in both frontal and sagittal planes. Staining will be with H&E, and with stains for bone, to understand the relationship between the brain protrusion and the skull structures. Once we have identified which tissues are mainly affected, we will extend the analysis to the gene expression level, looking particularly at how the FGF gene disruption may cause the encephalocele.

(ii) Testing for folic acid prevention. If encephalocele can be prevented or made less severe by treating the pregnant female with folic acid, this would be a major step forward towards primary prevention. We will raise the FGF-deletion female mice on a folate-deficient diet, to mimic the human situation of nutritional folate deficiency which is a risk factor for NTDs. This will reveal whether the encephalocele is made worse when folate is limiting. Then we will supplement the females with folic acid to determine whether the rate or severity of the encephalocele is lessened, when the folate status is improved. Using a folate deficient diet and folic acid supplementation of mice are routine procedures in our lab [7].

Clinical study

All available cases of encephalocele at Great Ormond Street will be reviewed by the clinical research fellow. A previous survey of cases, conducted in 2010, showed 37 cases (15 female, 22 male; Figs C, D) that had been managed by Dominic Thompson in the Neurosurgery Dept at GOSH. Our updated review has identified over 100 cases of encephalocele referred to the paediatric neurosurgical unit at Great Ormond Street, which are currently under initial evaluation. For those with complete clinical and radiological data available, a detailed review will provide information on the distribution of cranial sites and brain regions affected, the severity of the defect, the time of first diagnosis, gender of the child, and outcome following treatment. This will provide baseline information on the range of defects encountered in clinical practice, and indicate whether fetal surgery could be developed for some or all of these fetuses.

Ethical aspects of the study

The mouse research in this project is covered by Project Licence PPL P8B3094F0, granted by the UK Home Office, which is held by Prof Nick Greene (ICH), with Andrew Copp as Deputy Licence Holder. This contains permission to breed genetically altered strains and to modify the nutritional status of the mice using diets and supplements. The clinical research in this project is a review of past patient case-notes, which does not require formal ethics committee approval. No patient identities will be revealed in the course of the study and all data will have patient names and addresses removed before dissemination or publication.

Staff to be employed on the project

Dawn Savery is Research Assistant at the Institute of Child Health, and has worked with Andrew Copp for over 15 years. During this time she has authored more than 30 scientific publications. She is experienced in analysis of embryos and fetuses, and most recently has specialised in the assessment of fetal skull defects, as a model of the human disease craniosynostosis. She is expert in mouse husbandry and genetics, and in 2020 was first author on a paper that described a new oral (drinking) method for administering tamoxifen, to induce transgene expression in embryos [8]. This is a significant contribution to the humane use of animals in research, as it avoids the need to inject pregnant female mice. Dawn will devote 50% time to this project over 2 years, and will conduct all the mouse studies, under supervision of Andrew Copp.

Vejay Vakharia is Neurosurgical Fellow at Great Ormond Street Hospital, training under the direction of Dominic Thompson. He graduated from Cambridge University Medical School and has a PhD in Neuroscience from University College London. He has worked at the National Hospital for Neurology and Neurosurgery and has published over 40 scientific papers on different aspects of neurosurgery including neuroimaging, incorporating machine-learning techniques, as well as the clinical translation of advanced computer-assisted planning for minimally invasive neurosurgical procedures. Highlights include a clinical randomised control trial of robotic stereotactic techniques, custom medical device translation and being awarded the Sir Hugh Cairn's prize for the development and clinical application of a dynamic learning algorithm for stereotactic trajectory planning in stereoelectroencephalography. Vejay will devote 15% time to this project over 2 years, and will be responsible for all of the clinical aspects of the research, under supervision of Dominic Thompson.

References

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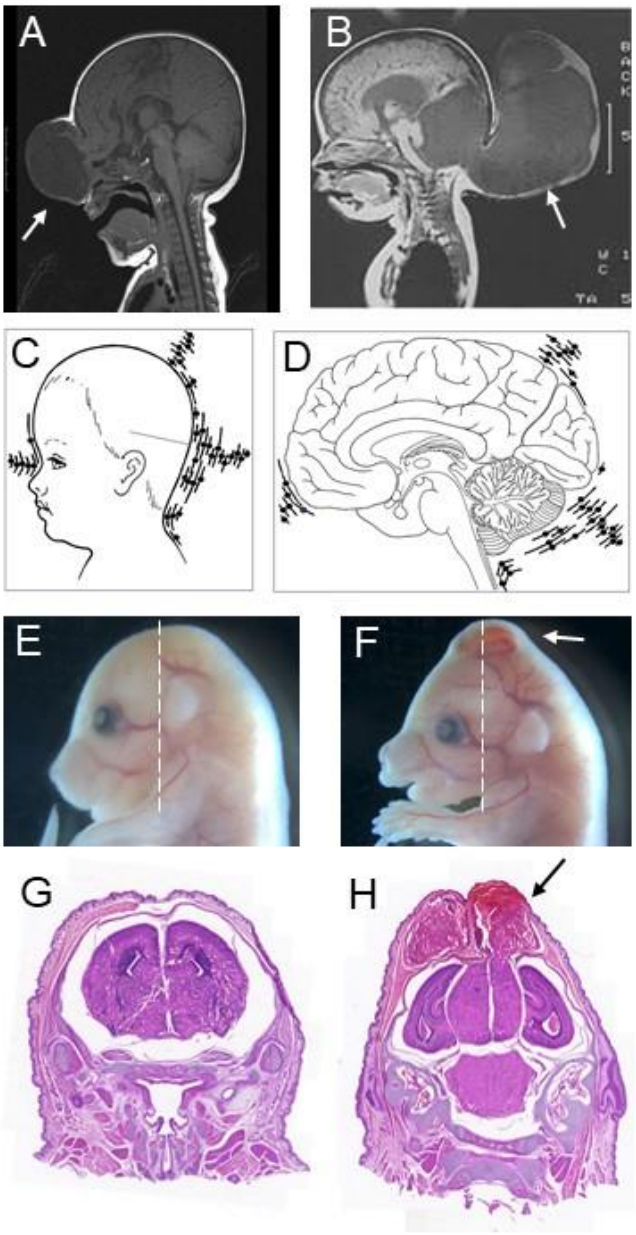

Figures to show the appearance of encephalocele in humans and mice.

(**A,B**) Protrusion of the front (A) and back (B) of the head, as seen in 'fronto-ethmoidal' and 'occipital' types of human encephalocele. Arrows point to the protrusions which can contain brain tissue

(**C,D**) Location of 37 encephalocele cases in Dominic Thompson's clinical practice. Note the location of the defect is variable around the head and brain. Diagrams from Samantha Grahame's project dissertation.

(**E,F**) The 'FGF deletion' mouse model of encephalocele, as will be studied in this project. The normal fetus (E) has a smooth head whereas there is a large protrusion of the brain in the abnormal fetus (arrow in F).

(**G,H**) Sections through the mouse heads, along the dashed lines in E,F. The normal brain (G) is enclosed within the head, whereas the encephalocele is an outward protrusion of part of the brain (arrow in H).