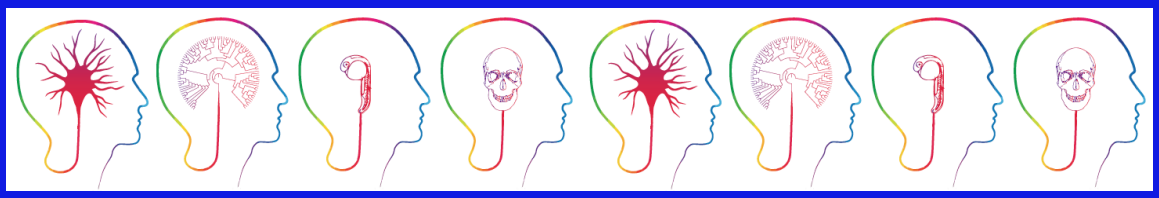
PROGRAMME AND ABSTRACTS

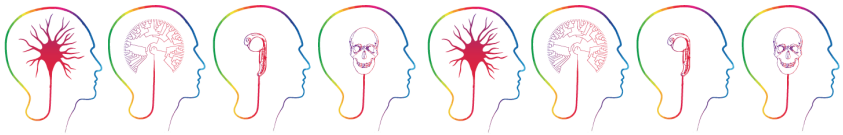
The 33rd HEAD GROUP MEETING

Craniofacial and Neural Development



Peter Thorogood Memorial Lecture

29th January, 2021
UCL GOS Institute of Child Health, Lndon, UK
via Zoom



33rd Head Group Meeting

29th January, 2021, UCL GOS Institute of Child Health via Zoom

Organizers:

Patrizia Ferretti
Paula Alexandre

Contact: Jonathan McCree
Email: ich.dbc.admin@ucl.ac.uk
Website: TBC

Sponsored by UCL GREAT ORMOND STREET INSTITUTE OF CHILD HEALTH

PROGRAMME

Session 1

Panel	***Lynda Erskine (****University of Aberdeen* ***) and Jeremy Green (****King's College London) and Speakers*	*abstract N°*
14:00 - 14:05	***Tengyang Qiu*****,** Department of Craniofacial Development & Stem Cell Biology, King's College London, UK *The mechanism of vestibular lamina formation in human embryos*	***1***
14:05 - 14:10	***Sami Leino*****,** King's College London and The Crick Institute, London, UK *Plzf regulates the temporal pattern of Fgf signalling in the developing hindbrain and inner ear*	***2***
14:10 - 14:15	***Mahbubeh Hejazi*****,** GOS Institute of Child Health and Department of Mechanical Engineering, UCL, London, UK *Mechanobiology of craniosynostosis*	***3***
14:15 - 14:20	***Afnan Alzarmrooni*****,** University of Portsmouth, Portsmouth, UK *Cardiac competence of the head mesoderm fades concomitant with a shift towards the head skeletal muscle programme*	***4***
14:20 - 14:25	***Zoe Crane-Smith,*** GOS Institute of Child Health, UCL, London, UK *Overexpression of Grhl2 causes midline craniofacial defects*	***5***
14:25 - 14:45	***Q&A – General Discussion***	
14:45- 15:00	***Tea Break 1: Breakout Rooms (posters)***	

Session 2

Panel	***Andrew Copp (****UCL)* ***and Karen Liu (****King's College London) and Speakers*	*abstract N°*
15:00 - 15:05	***Eunchai Kang*****,** University of Aberdeen, Aberdeen, UK *DISC1 Regulates Neurogenesis via Modulating Kinetochore Attachment of Ndel1/Nde1 during Mitosis*	***6***

15:05 - 15:10	***Rita Sousa-Nunes*****,** Centre for Developmental Neurobiology, King's College London, London, UK *Neural stem cells alter nucleocytoplasmic partitioning and accumulate nuclear polyadenylated transcripts during quiescence*	***7***
15:10 - 15:15	***Lewis Evans,*** GOS Institute of Child Health, UCL, London, UK *Genome-wide CRISPR screen for genes regulating human cortical progenitor proliferation and differentiation*	***8***
15:15 - 15:20	***Daniel Berg*****,** University of Aberdeen, Aberdeen, UK *Generation of non-ventricular neural stem cells in the developing brain*	***9***
15:20 -15:25	***Matthew Dawson*** Centre for Developmental Neurobiology, King's College London, London, UK *Studies in Cntnap2 mutant mice uncover sex differences in cortex development - implications for autism research*	***10***
15:25 - 14:45	***Q&A – General Discussion***	
15:45- 16:00	***Tea Break 2: Breakout Rooms (posters)***	

Peter Thorogood Memorial Lecture

Chairperson	***Patrizia Ferretti (****UCL)*
16:00 - 17:00	**Professor Robert Kelsh** *Department of Biology & Biochemistry*, University of Bath, Bath, UK ***Painting by numbers – zebrafish neural crest and pigment pattern formation?***
	BEST TALK & ENGAGEMENT PRIZES

PRESENTATIONS
ABSTRACTS

1.

The mechanism of vestibular lamina formation in human embryos

Tengyang Qiu1, Tathyane H. Teshima1, Maria Hovorakova2, 3, Abigail S. Tucker4,

31King's College London, United Kingdom, 2Charles University, Czechia, 3Institute of Experimental Medicine (ASCR), Czechia, 4Department of Craniofacial Development and Department of Orthodontics, King's College London

The vestibular lamina (VL) is a transient developmental structure that forms the lip furrow, creating a gap between the lips/cheeks and teeth (oral vestibule). Surprisingly little is known about the development of the VL and its relationship to the adjacent dental lamina (DL), which forms the teeth. In some congenital disorders, such as Ellis-van Creveld syndrome, development of the VL is disrupted and multiple supernumerary frenulum form, physically linking the lips and teeth. Here we assess the normal development of the VL in human embryos from 6.5 weeks (CS19) to 13 weeks of development, showing the close relationship between the VL and DL, from initiation to differentiation. In the anterior lower region, the two structures arise from the same epithelial thickening. The VL then undergoes complex morphogenetic changes during development, forming a branched structure that separates to create the vestibule. Changing expression of Keratins highlight the differentiation patterns in the VL, with fissure formation linked to the onset of Filaggrin. Apoptosis is involved in removal of the central portion of the VL to create a broad furrow between the future cheek and gum. This research forms an essential base to further explore developmental defects in this part of the oral cavity.

2.

Plzf regulates the temporal pattern of Fgf signalling in the developing hindbrain and inner ear

Sami Leino

Kings College London and The Crick Institute, London

Precise regulation of proliferation and differentiation of progenitor cells is essential for embryonic development and tissue homeostasis. These processes are regulated by both cell-extrinsic signals and cell-intrinsic transcription factor networks. During the development of the vertebrate nervous system, the correct pattern of differentiation requires mechanisms which ensure that the expression of signalling molecules is switched on and off in the right place and at the right time. Here, I present evidence suggesting that the promyelocytic leukaemia zinc finger (Plzf) transcription factor modulates the stage-specific levels of fibroblast growth factor (Fgf) signalling in the embryonic hindbrain. In the zebrafish hindbrain, *fgf3* and *fgf8* are expressed at early stages of development and downregulated before the onset of *fgf20* expression. Zebrafish embryos lacking *plzf* function have increased levels of Fgf signalling in the hindbrain at a later stage, likely as a consequence of a failure to downregulate *fgf3*. Preliminary data suggest that rhombomere-specific onset of *plzf* expression controls the switching off of *fgf3*. In addition, ectopic Fgf signalling in the absence of PLZF leads to transient ectopic expression of anterior markers in the otic vesicle, a tissue adjacent to and patterned by signals from the hindbrain. These results indicate that transcriptional regulation by PLZF is a key mechanism that controls the temporal pattern of Fgf signalling in the hindbrain.

3.

Mechanobiology of craniosynostosis

Mahbubeh Hejazi[1], Dawn Savery[2], Ali Alazmani[3], Erwin Pauws[2], Mehran Moazen[1]

Department of Mechanical Engineering, University College London, UK
Institute of Child Health, University College London, UK
Department of Mechanical Engineering, University of Leeds, UK

Crouzon mouse model, type *Fgfr2*^{C342Y}, was developed following the discovery of the genes responsible for early fusion of the coronal suture (joining the parietal and frontal bones) in humans. In this well-established mouse model, the coronal sutures are primarily affected, causing a short and domed head shape. The coronal suture starts to deteriorate at embryonic stages (E18.5) with partial closure at about P10 and full closure at P20 but never fuses in the wild type mouse.

The specific aim of this study was to test the hypothesis that applying tension to the coronal suture can delay early fusion of the coronal suture and restore the calvarial morphology in the Crouzon mouse.

Using a custom built loading set up, two sets of *in vivo* experiments were carried out. External cyclic loading of 0.1 N (at 1 Hz) was applied for 10 days from postnatal day 7 (P7) to P21 to: (1) the frontal bone close to the coronal suture i.e. applying tension to the coronal suture; (2) the parietal bone close to the coronal suture i.e. applying compression to the coronal. In the last day of treatment animals were sacrificed, genotyped and microCT scanned. Results were compared against a control group.

Results highlighted that loading the Coronal suture under tension in the Crouzon mouse can delay early fusion of this suture and restore the normal skull morphology. This was not the case when the coronal suture was loaded under compression. Further investigations are ongoing to understand the minimum level of loading that is required to delay suture fusion in the Crouzon mouse.

4.

Cardiac competence of the head mesoderm fades concomitant with a shift towards the head skeletal muscle programme

Afnan Alzamrooni,

University of Portsmouth, Portsmouth, UK

The vertebrate head mesoderm provides the heart, the great vessels, smooth and most head skeletal muscle, and parts of the skull base. The ability to generate cardiac and smooth muscle is thought to be the evolutionary ground-state of the tissue, and initially the head mesoderm has cardiac competence throughout, even in the paraxial region that normally does not engage in cardiogenesis. How long this competence lasts, and what happens as cardiac competence fades, is not clear.

Using a wide palette of marker genes in the chicken embryo, we show that the paraxial head mesoderm has the ability to respond to Bmp, a known cardiac inducer, for a long time. However, Bmp signals are interpreted differently at different time points. Bmp triggers cardiogenesis up to early head fold stages; the ability to upregulate smooth muscle markers is retained slightly longer. Notably, as cardiac competence fades, Bmp activates the head skeletal muscle programme instead.

5.

Overexpression of *Grhl2* causes midline craniofacial defects

Zoe Crane-Smith, Z; Mather, E; Santos, C; Copp, A; Greene, NDE

GOS Institute of Child Health, UCL, London, UK

Craniofacial malformations represent the most common birth defects in humans, and include defects such as microsomia and cleft lip/palate. The grainyhead-like (*Grhl*) family of transcription factors has been implicated in the development of craniofacial defects, with mutations in *Grhl3* causing Van der Woude syndrome, a syndrome where facial development is affected, and mutations in *Grhl2* leading to cranioschisis and cleft palate. Overexpression of *Grhl2*, in the *Axial defects* (*Axd*) mutant mouse line, has been shown to lead to neural tube defects due to reduced hindgut proliferation. However, whether increased *Grhl2* expression, like decreased expression, also leads to craniofacial defects remains to be established. We have used the *Axd* mouse line to determine the presence of defects within the facial structures. We have found that overexpression of *Grhl2*, leads to delayed palatal fusion due to broadening of the frontonasal processes (FNP), which in turn also leads to nasal septum defects. Additionally, FNP broadening lead to cleft lip/palate in around 33% of mutants and frontoethmoidal encephalocele (FEE) in around 48% of mutants at E17.5. Interestingly, the severity of defects observed in mutants correlated with the degree of broadening observed, with mutants presenting with the most diverged FNPs also presenting with FEE and cleft palate, indicating that many of the defects observed may occur secondarily as a result of the physical effects of FNP broadening.

6.

DISC1 Regulates Neurogenesis via Modulating Kinetochore Attachment of Ndel1/Nde1 during Mitosis

Eunchai Kang

University of Aberdeen, Aberdeen, UK

DISC1 (disrupted-in-schizophrenia 1), originally identified in a large Scottish family suffering from multiple psychiatric disorders due to a chromosomal translocation-induced disruption, has been established as a genetic risk factor for a wide array of psychiatric disorders, including schizophrenia, bipolar disorder, major depression, and autism spectrum disorders .Over 200 different proteins with very diverse functions have been reported to interact with DISC1, although the physiological relevance of most of these protein interactions remains to be verified. Moreover, little is known about biochemical and structural characterizations of DISC1 and its interactions with target proteins.

Ndel1/Nde1, a modulatory component of the dynein complex, is one of numerous reported DISC1-binding targets. Mutations of Nde1 are known to cause microcephaly both in mice and in humans. Ndel1 has been shown to epistatically associate with DISC1 in psychiatric disorders Elucidation of cellular functions of the interaction between DISC1 and Ndel1/Nde1 in brain development has been difficult, as DISC1 may interact with numerous target proteins other than Ndel1/Nde1. Similarly, Ndel1 and Nde1 are also scaffold proteins that can interact with several subunits of the cytoplasmic dynein complex, including the dynein heavy chain and Lis1. Thus, results derived from loss-of-function approaches on either of DISC1 or Ndel1/Nde1 can be difficult to interpret due to potential compound effects.

Here we demonstrate that Ndel1/Nde1 binds to a short, extreme C-terminal fragment of DISC1 with very high specificity and affinity. We reveal the molecular basis governing the specific interaction via solving the atomic structure of this DISC1 C-terminal fragment in complex with its binding sequence of Ndel1/Nde1. The high-resolution structure of the complex between DISC1 and Ndel1/Nde1 solved by NMR spectroscopy allowed us to design a method to specifically investigate functions of the interaction between DISC1 and Ndel1/Nde1 in vivo without interfering with Ndel1/Nde1-mediated dynein complex functions and with minimal obstructing of DISC1 binding to other partners.

Using this new method, we identified that DISC1/Ndel1 interaction regulates Ndel1's kinetochore localization and control mitosis of neural stem cells in radial glial neural stem cells in the embryonic mouse cortex in vivo, and in human forebrain organoids. In addition, we explored the potential role of this interaction in the context of human psychiatric disorders using patient-derived brain organoids with a specific DISC1 mutation and observed mitosis delay in the patient forebrain organoid with a DISC1 mutation. Together, these multifaceted approaches unravel a novel mechanism of action by DISC1, and they provide insight into the pathogenesis of psychiatric disorders.

7.

Neural stem cells alter nucleocytoplasmic partitioning and accumulate nuclear polyadenylated transcripts during quiescence

Rita Sousa-Nunes [1*], Rossi, A.[1,2†], Coum, A.[1†], Madelenat, M.[1], Harris, L.[2], Miedzik A.[1], Strohbuecker, S.[2], Chai, A.[1], Fiaz, H.[1], Chaouni, R.[1], Faull, P.[2], Grey, W.[2], Bonnet, D.[2], Makeyev, E. V.[1], Snijders, A. P.[2], Kelly, G.[2], Guillemot, F.[2]

[1]Centre for Developmental Neurobiology, Institute of Psychiatry, Psychology and Neuroscience, King's College London, Newcomen Street, London SE1 1UL, United Kingdom
[2]The Francis Crick Institute, 1 Midland Road, London NW1 1AT, United Kingdom
[†]Equal contribution

Quiescence is a cellular state characterised by reversible cell-cycle arrest and diminished biosynthetic activity, providing protection against environmental insults, replicative exhaustion and proliferation-induced mutations. Entry into and exit from this state controls development, maintenance and repair of tissues, and - in the adult central nervous system - generation of new neurons and thus cognition and mood. Cancer stem cells too can undergo quiescence, which confers them resistance to current therapies. Despite clinical relevance, quiescence is poorly understood and is defined functionally given lack of molecular markers. Downregulation of the protein synthesis, the most resource-intensive cellular process, is a feature of quiescence, controlled across species and cell types by inhibition of the Target of Rapamycin pathway. Here, we combine *Drosophila* genetics and a mammalian cell model to show that altered nucleocytoplasmic partitioning and nuclear accumulation of polyadenylated RNAs are novel evolutionarily conserved hallmarks of quiescence regulation. These mechanisms provide a previously unappreciated regulatory layer to reducing protein synthesis in quiescent cells, whilst priming them for reactivation in response to appropriate cues.

8.

Genome-wide CRISPR screen for genes regulating human cortical progenitor proliferation and differentiation

Lewis Evans, Alessio Strano, Francesco Paonessa & Rick Livesey,

GOS Institute of Child Health, UCL, London, UK

The cerebral cortex is the integrative and executive centre of the CNS, making up over three-quarters of the human brain. During embryonic development, excitatory cortical projection neurons are generated from multipotent neural progenitor cells (NPCs) that produce clones composed of different types of neurons, generated in a fixed temporal order. The balance between proliferation and terminal differentiation of NPCs is regulated by an extended network of intracellular factors and extracellular cues. The genes regulating this balance are only beginning to be understood. Aberrant gene expression of critical regulators can severely alter the number of neurons generated during corticogenesis, resulting in microcephalic or macrocephalic and megalencephalic syndromes and associated neurodevelopmental disorders. To identify genes that control human NPC proliferation, survival and differentiation, we performed genome-wide CRISPR knockout screens using a human iPSC directed differentiation model of cortical development. We broadly classified two groups of genes, where loss-of-function resulted in either suppression or promotion of NPC output, read out as the numbers of cells generated by NPCs. Among the gene knockouts that suppressed NPC output, we identified a number of genes encoding ribosomal proteins and genes in which missense mutations cause microcephaly. Conversely, gene knockouts that promoted NPC output included tumour suppressor and macrocephaly associated genes. We a currently following up these findings with functional studies on individual gene knockouts evaluating NPC output and associated transcription changes. In summary, these genetic screens highlight genes that have key roles in regulating human cortical development and brain tumour biology.

9.

Generation of non-ventricular neural stem cells in the developing brain

Daniel Berg

University of Aberdeen, Aberdeen, UK

The hippocampus is a part of the brain that plays an important role in learning, memory, and emotional processing. Unlike other areas of the brain, such as the cortex, in which neurons are generated embryonically, most neurogenesis in the dentate gyrus of the hippocampus (DG) occurs postnatally and the generation of functional neurons continues into adulthood. The new neurons in the DG of the adult brain are generated by neural stem cells (NSCs) that are situated in the subgranular zone. These cells differ from NSCs in other areas of the developing or adult vertebrate brain in that they are situated away from the ventricular system and can remain there for long time periods in a state of quiescence. The NSCs of the DG have radial glial like morphology and can generate both neurons and astrocytes. Little is known about the embryonic development of the NSCs in the DG and how NSCs are established away from the ventricular system.

Using immunohistochemistry and genetic clonal lineage tracing, we identified the embryonic origin of the NSCs and neurons in the mouse DG. We found that the DG NSCs are generated by radial glial NSCs in the dentate neuroepithelium, an area in the medial pallium of the developing brain. We show that both developmental and adult neurogenesis in the DG is a continuous process fueled by a common progenitor that shares molecular signatures through development. Quiescence is a hallmark of adult NSCs in the DG. By combining immunohistochemical analysis of cell cycle markers with EdU pulse-chase experiments, we established that majority of the DG NSCs are actively proliferating during early development but enter quiescence during the first week after birth.

The NSCs of the DG is not unique in the vertebrate brain with their capacity to retain stem cell while being situated away from the ventricle, outer radial glial cells (oRGs) in the developing human cortex have also been shown to proliferate and generate neurons in an abventricular position. oRGs generate the majority of the neurons in the human cortex and are in turn generated by ventricular NSCs (vRGs). By comparing morphological features, protein expression and transcriptional data, we find that NSCs of the developing DG share key features with cortical oRGs. DG NSCs express markers that were previously identified to be highly enriched in the oRGs, such as Hopx. Both mouse DG NSCs and human oRGs lack contact with the ventricles, while retaining a radial process and are able to proliferate and generate neurons in an abventricular position. Finally, by analyzing the angle of the division plane in relationship to the ventricular wall, we find that DG NSCs and oRGs are both generated by horizontal divisions, in comparison with the vRGs, that mostly undergo vertical divisions.

In summary, we have identified the embryonic origin of the NSCs in the mouse DG and found that the NSCs are established during the first week after birth. Additionally, we have found remarkable similarities in molecular signatures and cellular behaviors between the mouse DG NSCs and human cortical oRGs, suggesting a common evolutionary origin of these two neural stem cell types.

10.

Studies in CNTNAP2 mutant mice uncover sex differences in cortex development – Implications for autism research

Matt Dawson,[1,2] Kevin Gordon-Fleet,[1] and Uwe Drescher[1]

[1]Centre for Developmental Neurobiology (CDN), King's College London

[2]Forensic and Neurodevelopmental Sciences (FANS), King's College London

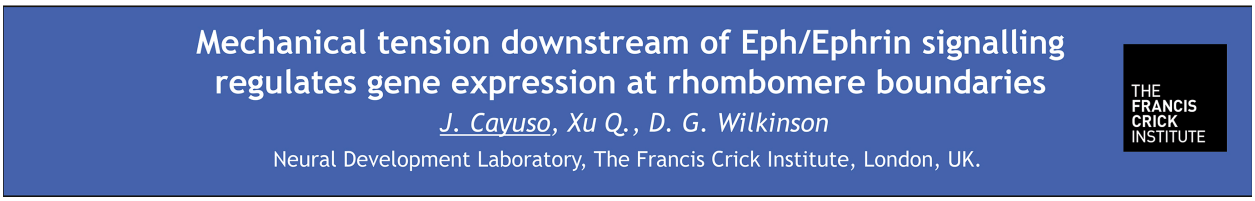
Like many neurodevelopmental conditions, Autism Spectrum Disorder (ASD) differentially affects one sex more than the other, with 4 in every 5 diagnoses being in males.[1] Variation in the gene CNTNAP2 is linked to development of ASD. Knock out (KO) of CNTNAP2 in mice has been shown to cause a delay in myelination, decreased stability in dendritic spines and deficits in the three core ASD behavioural domains.[2],[3] However, almost all studies in this mouse model have been performed in male only populations, and the effect of CNTNAP2 KO on the development of synapses and glial cells remains poorly understood.
Here, we investigated the development of dendritic spines in layer 1 of the mouse anterior cingulate cortex (a hub for social interaction) at three timepoints (P14, P28 and P56) and in both sexes. In males, knock out of CNTNAP2 lead to a transient reduction in spine density versus wild types, with significant reductions at P14 and P28, but no difference at P56. However, in females, no transient reduction in spine density was observed between CNTNAP2 KOs and wild types.

Given microglia are known to undertake some synapse pruning, while also displaying morphological and behavioural differences in males and females, we hypothesised that microglia may play a role in our spine density results. At P8, male microglia in CNTNAP2 KOs displayed a more activated state, with significant reductions in number of primary processes, total process length, and number of branch points versus wild types. There were no differences between the microglia of female CNTNAP2 KOs and wild types.

These initial findings suggest a possible link between the brain's resident immune cells, microglia, and the differential spine densities in males and females found in this mouse model of ASD. In the future, collecting data from both sexes when studying neurodevelopmental disorders may help shed light on their sex-dependent incidence rates.

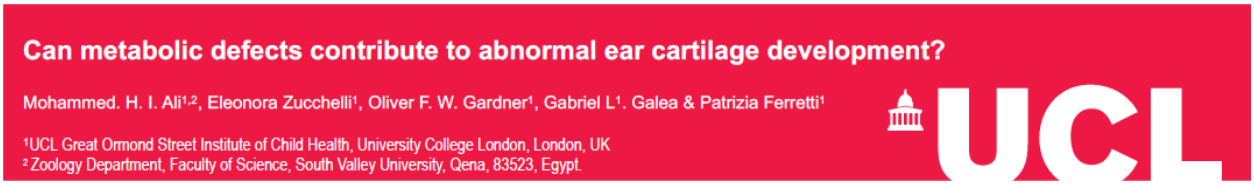
POSTERS

Poster 1.



Poster 2.

Poster 3.



Mechanical tension downstream of Eph/Ephrin signalling regulates gene expression at rhombomere boundaries

J. Cayuso, Xu Q., D. G. Wilkinson

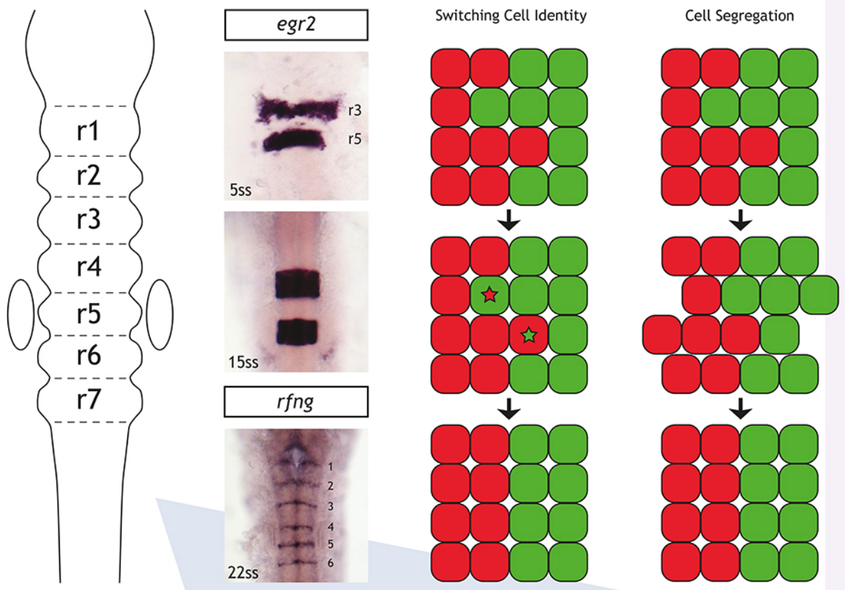
Neural Development Laboratory, The Francis Crick Institute, London, UK.

THE FRANCIS CRICK INSTITUTE

INTRODUCTION

Hindbrain Segmentation

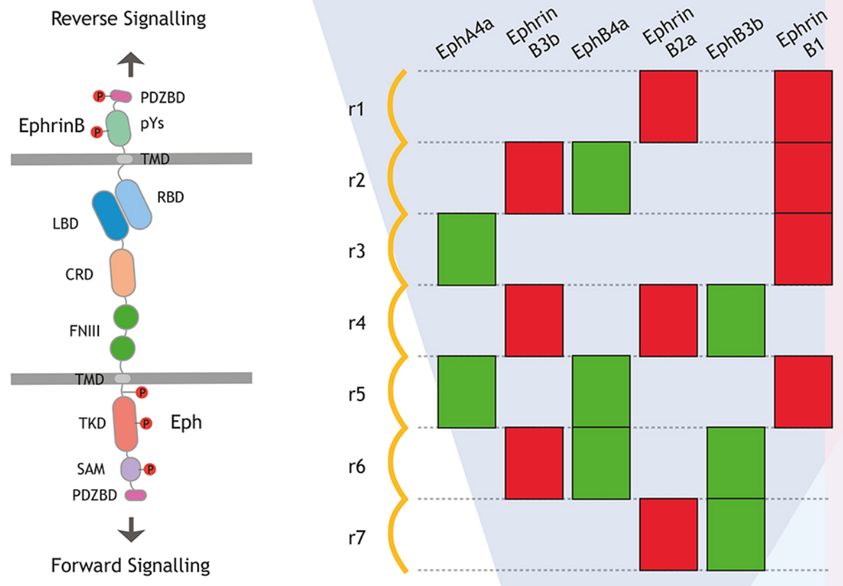
The establishment of sharp borders between tissue interfaces is critical for the correct organization of tissues and organs. The hindbrain patterning along the anterior-posterior axis leads to the segmental expression of several transcription factors such as *egr2*, which is initially fuzzy and becomes refined. Subsequently, boundary cells expressing *radical fringe* (*rfng*) are specified at segment borders where they act as signalling centres. Border sharpening between different segments or rhombomeres encompasses changes in cell fate and Eph/Ephrin-mediated cell sorting.



Eph/Ephrin signalling

Ephs and Ephrins are transmembrane proteins mediating bidirectional cell-to-cell communication: forward signalling downstream of the receptor and reverse signalling in the ligand-expressing cells.
Forward signalling is mediated either by the intracellular TKD (Tyrosine kinase domain) or the C-terminal PDZBD (PSD95, Dlg1, ZO-1 Binding Domain), while reverse signalling uses the cytosolic phospho-tyrosines (pYs) or a C-terminal PDZBD.

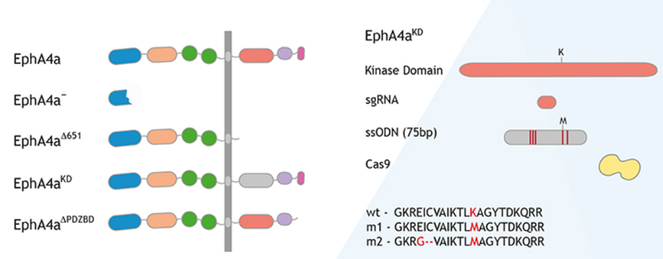
Multiple Eph/Ephrin pairs are complementarily expressed in the hindbrain. Ephs and Ephrins initate heterotypic signalling at segment borders, essential for sharpening and boundary cell formation. Cell repulsion and mechanical tension are required for border sharpening, however the mechanism regulating boundary cell formation remains elusive.



RESULTS

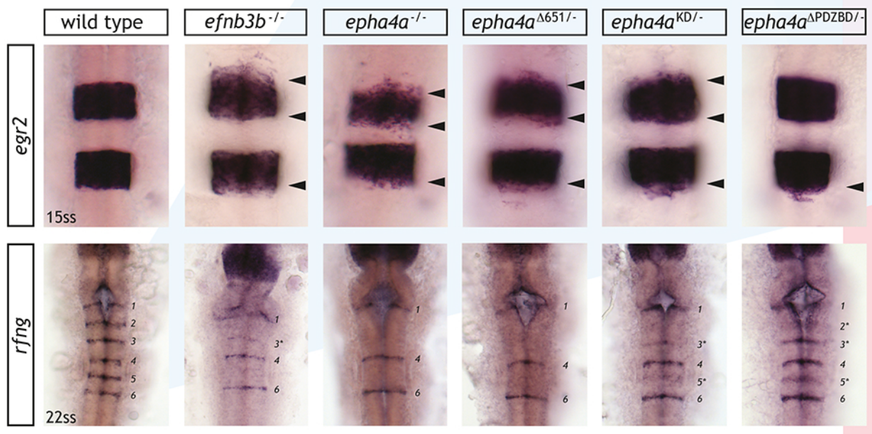
Generation of mutant lines for EphA4a and EphrinB3b

We generated null and domain-specific mutants for EphA4 and EphrinB3b using knock-out and knock-in CRISPR/Cas9 approaches. Specific sgRNAs were used to obtain the *epha4aΔ*^{651} and *epha4aΔ*^{PDZBD} truncated alleles. A kinase dead version of EphA4a (*epha4a*^{KD}) was obtained by coinjection with a single stranded oligonucleotide (ssODN) containing a mutation in a conserved lysine.



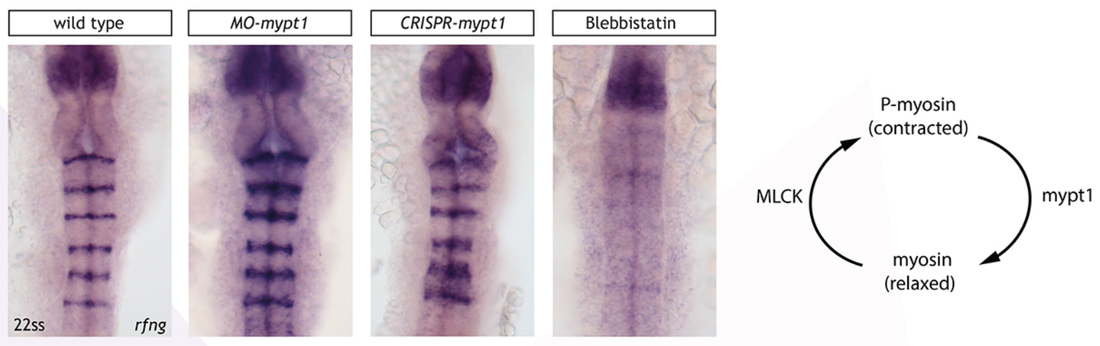
EphA4a kinase-dependent signalling mediates boundary cell formation

Sharpening and boundary cell specification are impaired at specific borders in *epha4a* and *efnb3b* mutants. EphA4a forward-signalling-deficient (*epha4aΔ*^{651}) and the kinase dead (*epha4a*^{KD}) mutants have similar sharpening defects but loss of boundary cell is more prominent in the first. Loss of the PDZBD causes milder phenotypes, suggesting that forward signalling is mainly mediated by the kinase domain during hindbrain segmentation.



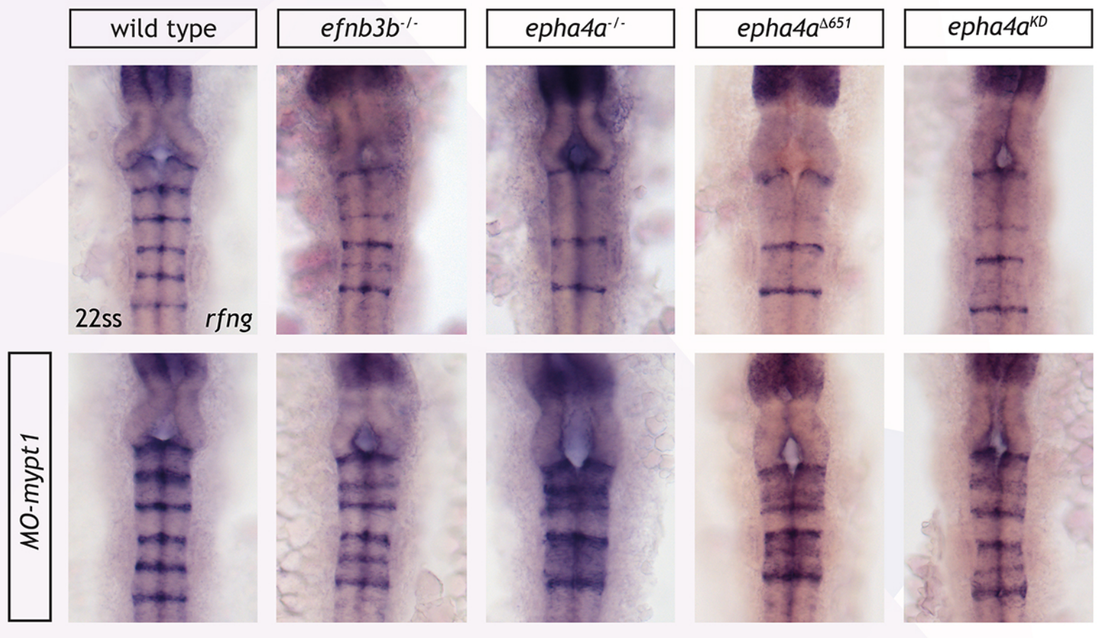
Actomyosin tension regulates *rfng* expression at boundaries

In the hindbrain, Eph/Ephrin signalling maintains border sharpness by increasing actomyosin contraction. To assess whether a similar mechanism controls the expression of boundary markers we interfered with myosin function. A morpholino or transient CRISPR against *myosin phosphatase target subunit 1* (*mypt1*) caused increased *rfng* expression at boundaries while the myosin inhibitor Blebbistatin had the opposite effect.



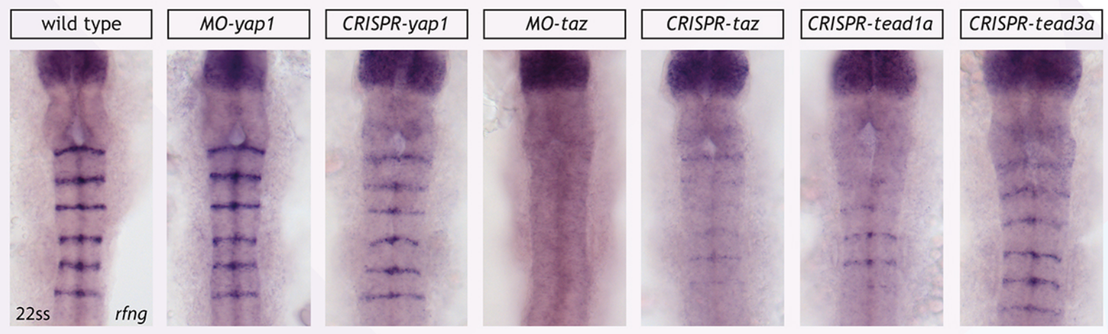
Eph/Ephrin signalling activates *rfng* by increasing mechanical tension at boundaries

Increased actomyosin contraction restores the expression of *rfng* in EphrinB3b and EphA4a mutants. This suggests that Eph/Ephrin signalling activates boundary-marker expression by increasing actomyosin contraction at segment borders.



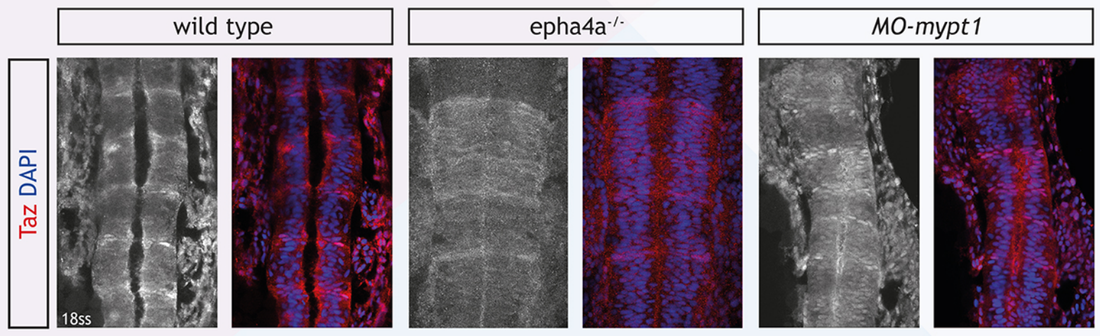
Taz activates boundary expression of *rfng*

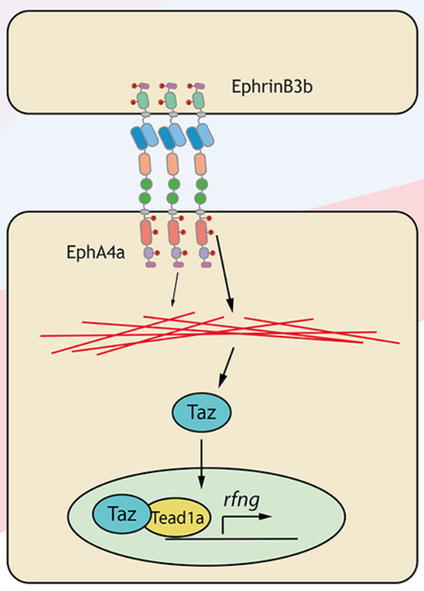
The Yap/Taz transcription factors, in conjunction with Tead co-factors, are known to activate transcription in response to increased mechanical tension. In the hindbrain, Taz and Tead1a, but not Yap and Tead3a, are required for boundary expression of *rfng*.



Taz activity is regulated by Eph/Ephrin signalling in the hindbrain

Cells at segment boundaries display increased nuclear Taz localisation, indicative of increased Taz transcriptional activity in these cells.
Nuclear Taz is reduced at boundaries in EphA4 mutants while enhanced actomyosin contraction leads to ectopic cells with elevated nuclear Taz. This indicates that Taz acts downstream Eph/Ephrin signalling and mechanical tension at segment boundaries to regulate boundary-gene expression.





Model

Heterotypic interactions at segment borders activate EphA4a forward signalling. Mainly via the kinase domain but with contribution of the PDZBD, EphA4a increases actomyosin contraction. Increased tension promotes the translocation of Taz to the nucleus where, together with Tead1a, activates *rfng* expression.

CONTACT
Email: jordi.cayuso@crick.ac.uk
Tw: @jordicayuso
Web: www.crick.ac.uk

Brain Dystrophin: Challenges in detection of different isoforms in developing human brain

Reem Alkharji [1,2], Jenny Lange [1], Patrizia Ferretti [1]

1 Stem Cell and Regenerative Medicine Section, UCL Great Ormond Street Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK

2 Research Department, Health Science Research Centre, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Introduction

- Dystrophin is an actin binding protein differentially spliced to produce several isoforms with preferential tissue distribution (Fig. 1) .
- Dystrophin mutations cause Duchenne muscular dystrophy (DMD), a muscle degenerative disease [1].
- One-third of DMD patients present cognitive and behavioural problems.
- There is limited knowledge on dystrophin isoform expression and function in developing and adult human brains.

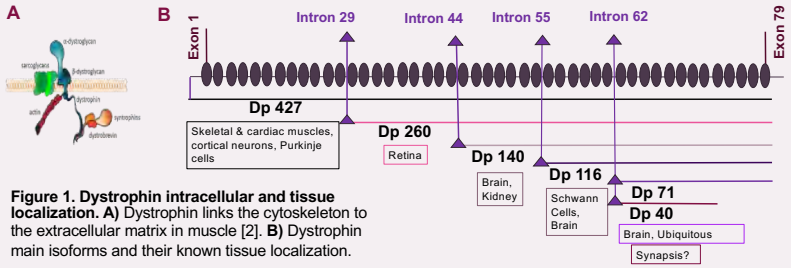


Figure 1. Dystrophin intracellular and tissue localization. A) Dystrophin links the cytoskeleton to the extracellular matrix in muscle [2]. **B)** Dystrophin main isoforms and their known tissue localization.

Aims

1. Identify expression and localization of dystrophin isoforms during human brain development.
2. Use induced pluripotent stem cells (IPSCs) from healthy and DMD patients to study changes in isoform expression with neural differentiation and their role in neurones and astrocytes.

Challenges

1. Sequences of different isoforms largely overlap, hence there are a very limited number of unique sites for isoform cellular localization and there is a lack reagents targeting unique sequences.
2. Many available antibody have not been properly characterized particularly in human brain and protocols established for paraffin section staining to maintain good morphology for fine localization.

Objectives

Optimize Immunohistochemistry and Immunocytochemistry protocols for the detection of different dystrophin isoforms in developing human brains and for monitoring neural differentiation in vitro

Results

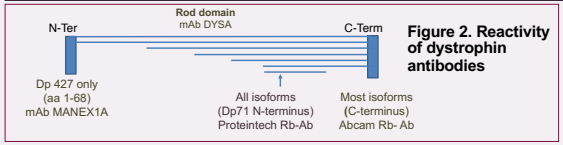


Figure 2. Reactivity of dystrophin antibodies

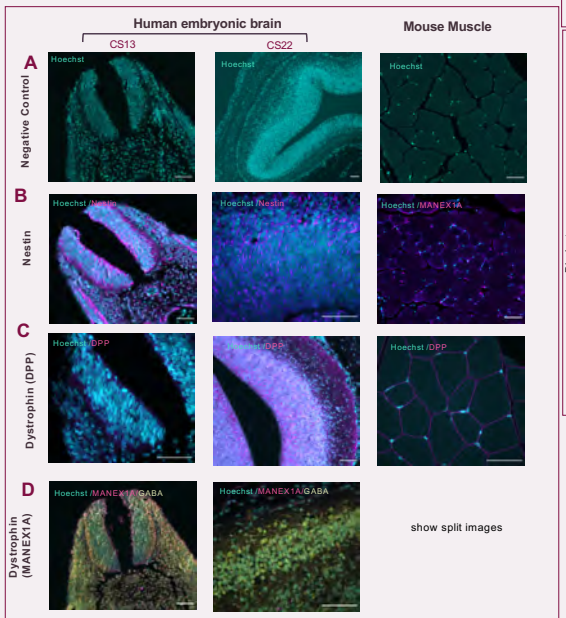


Figure 3. Immunofluorescence staining for dystrophin in human developing brains (CS13, CS22) and mouse muscle. A) Negative control with secondary antibody only. **B)** Nestin staining. **C) Staining for d**ystrophin with the Proteintech **(**DPP) antibody. **D)** Double-staining for full length dystrophin (MANEX1A) and GABAergic neurones. Nuclei are stained with Hoechst dye (blue). Scale bars= 50 µm.

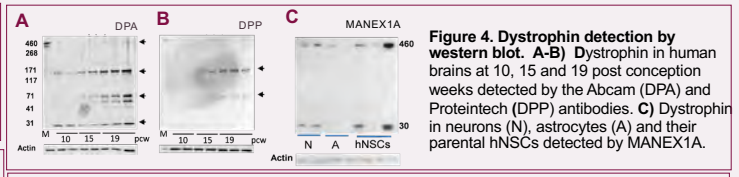


Figure 4. Dystrophin detection by western blot. A-B) Dystrophin in human brains at 10, 15 and 19 post conception weeks detected by the Abcam (DPA) and Proteintech **(**DPP) antibodies. **C)** Dystrophin in neurons (N), astrocytes (A) and their parental hNSCs detected by MANEX1A.

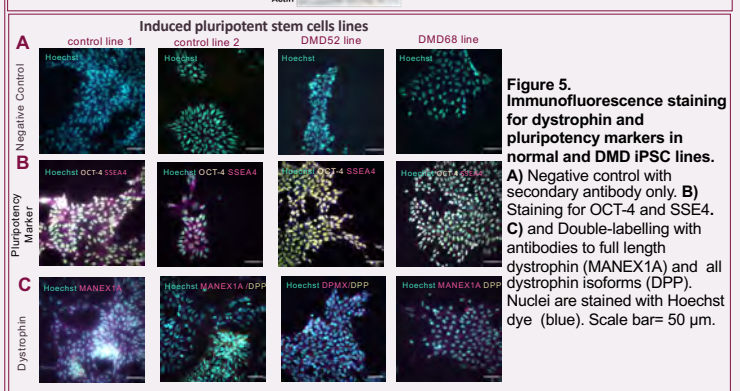


Figure 5. Immunofluorescence staining for dystrophin and pluripotency markers in normal and DMD iPSC lines. A) Negative control with secondary antibody only. **B)** Staining for OCT-4 and SSE4. **C)** and Double-labelling with antibodies to full length dystrophin (MANEX1A) and all dystrophin isoforms (DPP). Nuclei are stained with Hoechst dye (blue). Scale bar= 50 µm.

Ab ***Reactivity***	MANEXA1 (DPMX) *N-terminus*	Proteintech (DPP) *Dp71 N-terminus*	Abcam (DPA) *C-terminus*	NCL-DYSA *Rod domain*
Paraffin sections	√	√	-	√ (high background)
Western blot	√	√	√	not reported

Conclusions

- A set of antibodies that detects dystrophin isoforms by western blot and in paraffin sections of human developing brains and iPSCs by immunofluorescence has been identified.
- Sensitivity is lost when using immunofluorescence as compared to chromogenic detection because of autofluorescence / high background particularly with DYSA and DPP, hence further staining optimization is needed.
- RNA localization using unique sequences will be required for accurate localization of different dystrophin isoforms in neural cells.

References

1. Hoogland et al., (2019) *Hippocampus*, 29, 102-110
2. Muntoni et al., (2003) Neurology 2, 731-740
3. Doorenweerd et al., (2017) *Sci Rep*, 7, 12575.
4. Leyva-Leyva et al., (2018), *J Membr Biol*, 251, 535-550.

Acknowledgment: Support from Saudi Arabian Cultural Bureau and Ministry of Education to RA is gratefully acknowledged

Can metabolic defects contribute to abnormal ear cartilage development?

Mohammed. H. I. Ali[1,2], Eleonora Zucchelli[1], Oliver F. W. Gardner[1], Gabriel L[1]. Galea & Patrizia Ferretti[1]

[1]UCL Great Ormond Street Institute of Child Health, University College London, London, UK
[2] Zoology Department, Faculty of Science, South Valley University, Qena, 83523, Egypt.

Background

- Microtia is a congenital malformation of the external and middle ear. It is caused by abnormal development of the first and second zygomatic arch and the first sulcus (Chen and Zhang, 2019), (Fig. 1).
- The mechanisms underlying abnormal auricle development are not well understood.
- Our previous study has shown presence of blood vessels in microtic cartilage, whereas healthy cartilage is normally avascular, and disorganized extracellular matrix (ECM) (Fig. 2), .
- Our initial proteomic analysis has indicated up-regulation of proteins involved in metabolic processes in the microtic tissue compared with healthy donors.
- Two of the proteins found to be differentially expressed are superoxide dismutase 3 (SOD3) and peroxiredoxin VI, both involved in the detoxification of reactive oxygen species (ROS). SOD3 is also important for maintaining ECM homeostasis.

Figure 1. Classification of microtia severity (Meurman, 1957). Microtic ears are given different scores according to shape and size deficiencies. Images from https://www.earwells.com/.

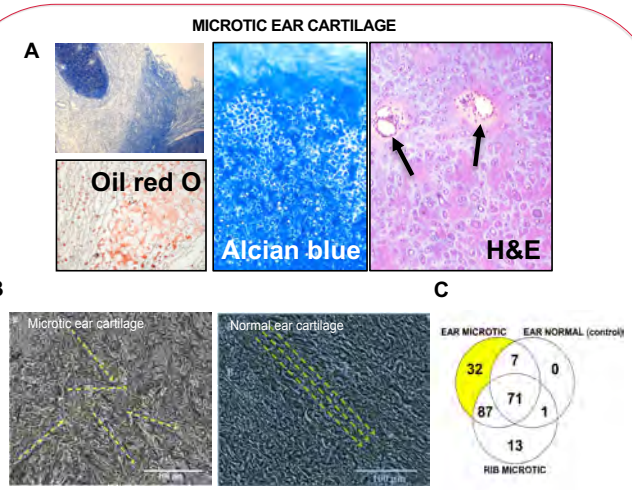


Figure 2. Features of microtic cartilage. A) microtic cartilage; note cartilage remnants surrounded by mesenchyme and adipose (oil red-positive), poorly defined perichondrium more akin to the developing ear, and presence of blood vessels within the cartilage. Sections are stained with H&E or alcian blue; From Zucchelli et. al, 2020. **B)** Random orientation of fibres is observed in the microtic ear perichondrium (highlighted by the yellow dashed lines). **C)** Venn diagram from proteomic analysis.

Hypothesis

- Metabolic imbalances may contribute to defective development of the auricle.
- SOD3 might increase in microtic ears to counteract defective oxidative stress responses.

Aims

- Assess whether normal & microtic chondrocytes respond differently to stress.
- Assess whether SOD3 may regulate this response.

Results

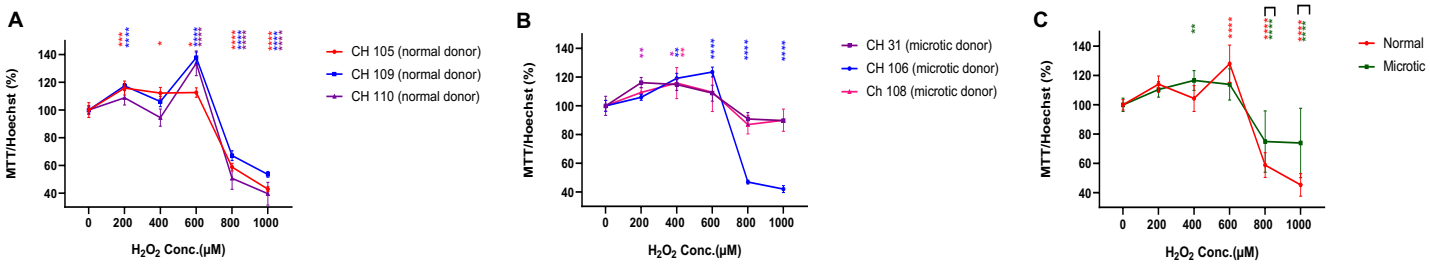


Figure 3. Effect of one-hour treatment with different H_2O_2 concentrations on the metabolic activity of healthy and microtic chondrocytes. A) Chondrocytes derived from normal ear cartilage show very similar behaviour. **B)** Chondrocytes derived from microtic ear cartilage. **C)** Combined data from (A) and (B). Two patients show increased tolerance to oxidative stress than controls with 800 and 1000 μM H_2O_2. MTT activity is normalized to cell number as assessed by DNA content. Data are presented as mean ± SD (n=5 /donor).

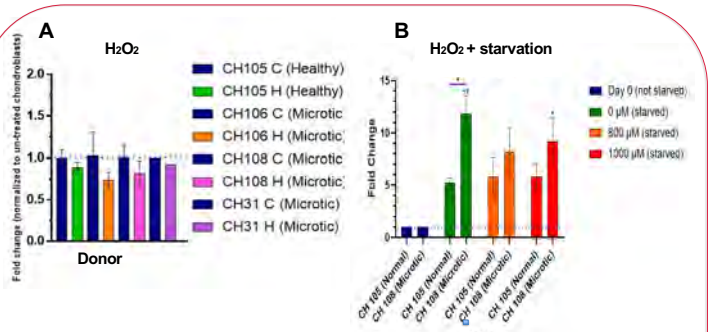


Figure 4. Effect of H_2O_2 alone and in combination with starvation on SOD3 gene expression in normal and microtic chondrocytes. A) SOD3 transcript levels assessed by RT-qPCR in chondrocytes from 3 microtic donors and 1 normal donor treated for one-hour with 1000 μM H_2O_2 (H) or untreated (C). **B)** SOD3 expression in chondrocytes from 1 microtic and 1 normal donor maintained for 24 hours in serum-free medium, followed by one-hour treatment with 800 μM or 1000 μM H_2O_2. SOD3 expression is significantly increased following starvation alone and 1000 μM H_2O_2 following starvation, but not by H_2O_2 treatment alone. Data are presented as mean ± SD (n=3/donor).

Conclusions

- Microtic chondrocytes from 2/3 patients are less sensitive to H_2O_2 –induced oxidative stress than healthy ones. This may reflect difference in the causes of microtia and is in contrast to the presence of blood in all microtic cartilages studied (5/5).
- Starvation alone reveals differences in SOD3 expression between microtic and normal chondrocytes in all microtic lines studied.
- No significant changes in SOD3 transcript expression are detected following H_2O_2 treatment, whereas after starvation, the fold increase in SOD3 is significantly higher in microtic chondrocytes than in controls.

Future work

- Investigate a larger cohort of patients to establish whether metabolic changes are involved in defective cartilage development in most microtic patients.
- Assess SOD3 and PRDX6 protein expression and enzymatic activity, as well as other metabolic enzymes, in addition to transcript analysis.

References

Chen X. and Zhang R. (2019). Microtia epigenetics, an overview of review and new veiwpoint. Medicine; 98:41.

Meurman Y (1957) Congenital microtia and meatal atresia. Arch Otolaryngol; 66: 443–449.

Zucchelli, E, Birchall, M, Bulstrode, NW & Ferretti, P. (2020). Modeling Normal and Pathological Ear Cartilage in vitro Using Somatic Stem Cells in Three-Dimensional *Culture. Front Cell Dev Biol,* 8.

Newton-Mosharafa Fund