Researchfish Award Download for

V4821

Generating human models of acrodysostosis for testing mechanisms and pharmacological intervention

Prof Patrizia Ferretti

Award Title	Generating human models of acrodysostosis for testing mechanisms and pharmacological intervention
Award Reference	V4821
Research Organisation	Institute of Child Health
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Award Categories	Research Field, Research Theme, Grade of submission, AMRC Test Category, Award Type, New Grant Code, AMRC Grant Type, AMRC Animals, AMRC Animals Species, AMRC Animals Modified, AMRC Funder Comments, AMRC Year Awarded

Award Abstract

Lay Summary

Acrodysostosis is a rare birth defect characterized by abnormal skeletal growth particularly affecting the facial bones, hands and feet. In addition to these defects, these patients can display resistance to hormonal stimulation of cells in the developing bone growth area and short stature, develop obesity, and present different degrees of mental retardation. Hence, they face several problems throughout life. The predominance of different defects largely depends on which gene is affected. The two genes known to be mutated in acrodysostosis are PRKAR1A and PDE4D. They are part of an important chemical system that is activated in response to certain hormones including the parathyroid hormone, PTH, and the parathyroid hormone-related protein (PTHrP), which regulates cells in the bony growth plates, by binding to the PTH-receptor. Some patients with PTHR gene abnormalities have bony changes similar to acrodysostosis. There is currently no human model of acrodysostosis, and the few animal models do not fully replicate the human disease features. Furthermore, disease severity varies among patients. In order to develop treatments for children with acrodysostosis that may improve their health and quality of life, we need to understand the pathways through which their genetic changes are impacting on bone growth, hormonal responses, learning, appetite and behavioural

regulation. We plan to establish human models of acrodysostosis in a dish that will allow us to elucidate mechanisms underlying different forms of acrodysostosis, uncover strategies for intervention, and provide a platform for testing new potential therapies. Differences in genetic background, that are differences in genes that give each individual their unique identity, can results in different presentation and severity of a disease. For this reason, first we propose to study the effect of different mutations in the same genetic background. To this purpose we will introduce PRKAR1A or PDE4D mutations found in patients in normal human induced pluripotent stem cells (iPSCs) we have already generated in our laboratories. These cells can be differentiated in all different tissues of the body, including skeletal tissues (cartilage and bone) and neural tissue and studied at the cellular and molecular level. Second, we will generate iPSCs from patients and study them for comparison following differentiation into skeletal and neural tissues. Studying cell differentiation into cartilage will allow us to elucidate how acrodysostosis mutations lead to skeletal defects and which molecule(s) may represent potential therapeutic targets, whilst analysis of neural differentiation may provide information on the basis of cognitive impairment and also guide development of new therapies. Together, generation of the culture models proposed here and their characterization will allow us not only to shed some light on disease mechanism in different forms of acrodysostosis, but also to establish valuable human platforms for drug screening.

Sections with Information

Awards and Recognition

Award Type	Personal invitation as keynote or other named speaker to a conference
Award Name	I was invited to talk about current challenges in the generation and production of therapeutic stem cells but was unable to attend due to other previous commitments.
Individual	
Award Level	Continental/International
Year Awarded	2023
Award Description	Standing in the field.
Impact of Award	N/A
URL	
Digital ID	
Source	Manual
Publication ID	65eaee773b88a2.49933423

Use of Facilities and Resources

Facility or Work Name	Gene expression analysis of Duchenne Muscular Dystrophy astrocytes
Facility Name	UCL Genomics
Provided Service/Resource	RNA-seq
Subsequent Impacts	Paper published in 2022
URL	https://onlinelibrary.wiley.com/doi/10.1002/glia.2 4116
Digital ID	10.1002/glia.24116
Source	Manual
Publication ID	621693f4cd9b63.49156296

Facility or Work Name	Human brain development - dystrophin and other gene expression
Facility Name	MRC/Wellcome Trust Human Developmental Biology Resource
Provided Service/Resource	human developing brain tissue
Subsequent Impacts	Some of this work has been published in 2022.
URL	https://onlinelibrary.wiley.com/doi/10.1002/glia.2 4116
Digital ID	10.1002/glia.24116
Source	Manual
Publication ID	621692c8503254.71725401

GOSH Further Information

Grant Progress

Together, we have been able to collect cells from 5 patients (two ACRDYS1 and three ACRDYS2 patients); we have reprogrammed to induced pluripotent stem cells (iPSC) and characterized cells from 3 of the patients (we did not have sufficient capacity and time for reprogramming them all). One of these lines has been characterized in particular depth, as part of a collaboration with Cellected. The 3 new iPSC lines generated will provide a valuable resource to study disease mechanisms and test therapies; there are PBMCs (peripheral blood mononuclear cells) already isolated from 2 patients for generating additional iPSCs pending funding. Furthermore, additional patients interested in donating cells for studying acrodysostosis have been identified. Confirmatory sequencing of acrodysostosis causing mutations was performed on cells from 3 patients and healthy cell lines to allow for the introduction of the identical mutations in gene edited iPSCs to generate isogenic lines to model the disease. IPSC lines due to be gene edited have been tested for the ability to differentiate along the neural lineage. We have made good progress with differentiation along the chondrogenic lineage of the IPSCs to be used for gene editing, but had to go for a longer than we hoped differentiation protocol, and we are running a final validation. As one of our colleagues/potential collaborators had been unable to generate isogenic lines for acrodysostosis, we have modified the guides previously designed and revised our experimental plan to test efficacy and select edited lines following discussion with Dr Shalini K Reddy, who is running a CRISPR Facility at UCL GOS-ICH supported by the NIHR GOSH BRC. We are now ready to proceed with the new strategy devised. While reprogramming and differentiating cells, both involving very long protocols, we have continued to characterise patient fibroblasts, showing that both acrodysostosis type 1 (ACRDYS1) and 2 patient cells have reduced Sox9 by immunostaining, but no obvious change in PDE4D labelling. We have also shown that patient cells may

have lower PDE4 activity than wild type cells, using a commercial kit. As we did not find the kit satisfactory, we developed a method for quantifying cAMP in cells using HPLC normalised by protein content. Analysis of changes in expression of the isoforms of the gene mutated in acrodysostosis type 2 (PDE4D) and members of this gene family has been carried out in healthy cells and ACRDYS cells. Interesting regulation of PDE4D isoforms has been observed. This work is being finalized for publication. Request for a collaboration agreement to be able to send cells to our collaborator in Glasgow submitted almost 2 years ago never materialized; hence after waiting for well over 1 year we hosted one of Prof. Baillie's PhD students to carry out the work in our lab in order for the collaboration with Glasgow to progress. The visit was successful and demonstrated activity of a PROTAC (proteolysis targeting chimera) on PDE4D, the enzyme mutated in ACRDYS2, on cells from one of the patients. A paper including the data generated from one of the patient's cells is now in preparation. Together, the work carried out so far has generated new tools for the study of acrodysostosis and useful information on possible disease mechanisms that we are summarizing in a review ready for submission, and that will guide development of therapeutic interventions.

Outline

Over the remining time of the award we plan to: - Gene-edit iPSCs to generate isogenic lines using CRISPR-Cas9 and carry. - Carry out RNAseq analysis and validation of results

Achievements

Acrodysostosis is a rare birth defect characterized by abnormal skeletal growth particularly affecting facial bones, hands and feet. Furthermore, patients can display hormone stimulation resistance in the developing bone growth area, hence short stature, obesity, and mental retardation presenting with variable severity. Therefore, they face several problems throughout life. Predominance of different defects depends on which gene is affected, PRKAR1A or PDE4D. Both are part of an important chemical system that is activated in response to certain hormones that regulates growth of the bone. There is currently no human model of acrodysostosis; the few animal models do not fully replicate the human disease features. To

develop treatments, we need to understand pathways through which genetic changes impact bone growth, hormonal responses, learning, appetite regulation. Hence, human disease models are needed. We can reprogramme cells in a dish to generate stem cells (iPSCs) that can be differentiated in all different body tissues, including skeletal tissues (cartilage and bone) and neural tissue. This will allow us to elucidate disease mechanisms underlying defects associated with different tissues, uncover strategies for intervention, and provide platforms for testing new potential therapies. Differences in "genetic background", that are differences in genes that give each individual their unique identity, can results in different disease presentation and severity. Therefore, we will use two approaches: 1) generate iPSCs from patients. iPSC differentiation into cartilage will allow us to elucidate how acrodysostosis mutations lead to skeletal defects and identify potential therapeutic targets, whilst analysis of neural differentiation may provide information on the basis of cognitive impairment. 2) study the effect of mutations in the same genetic background by introducing PRKAR1A or PDE4D mutations found in patients in healthy iPSCs. These models will help to understand disease mechanisms in different forms of acrodysostosis and establish valuable human platforms for drug screening.

Difficulties

Yes

Further information on difficulties

Collection of cells from patients took longer than anticipated: issues with ethics collections were resolved and we have been able to collect cells from 5 patients, more than we could possibly handle as part of this award. Verification of the mutations in two patients generated additional unanticipated work, as there was a lack of clarity from the genomic analysis provided to us on the exact nature of the mutations, and the sequencing provided some challenges. We successfully resolved the issue which is crucial to assessing of the outcome of gene editing. We had issues with a differentiation protocol. Extensive troubleshooting revealed that a key growth factor used for differentiation did not behave any more as expected. This was tracked down to the manufacturer and we are currently using the growth factor

from a different supplier (unfortunately several folds more expensive). The co-I, Dr Oliver Gardner, is going to go in paternity leave, and we anticipate this will slow down progression. In addition, we are trying to resolve issues concerning his salary and discrepancies in the duration of the award.

Influence on Policy, Practice, Patients and the Public

Influence Name	Discussion on acrodisostosis survey and possible improvements to it
Influence Type	Participation in a guidance/advisory committee
Healthcare Area	
Title	
Issuing Organisation	
Publication citing your work	
Contribution description	Adviced on additional questions to be included in the questionnaire to be distributed as widely as possible to collect more granular information on symptoms/tissue types affected in patients affected by acrodysostosis.
Cited Publication	
Year First Realised	2022
Geographic Influence	Multiple continents/international
Country	
Area of policy influence.	Healthcare,Pharmaceuticals and Medical Biotechnology
Describe Other	
Specific Impacts	Not known
Impact Description	
URL	
Digital ID	
Source	Manual
Publication ID	63f267f105d721.18986140

Engagement Activities

Activity Title	Acrodysostosis Patient Group Round Table
Activity Type	A formal working group, expert panel or dialogue
How many people?	11 - 50
Geographical Reach	International
Primary Audience	Patients, carers and/or patient groups
Other Audience	Professional Practitioners,Supporters,Patients, carers and/or patient groups
Activity Years	2022
Result Description	The outcome of an initial survey to provide the opportunity for patients and/or carers to share their experience of acrodysostosis was discussed. The design and content of the survey to collect data for the patient registry was discussed and improvements proposed. Strategies to reach a wider cohort internationally was also discussed (e.g. translating the questionnaire and the background to it into different languages). Short scientific presentations (inlcuding mine) to discuss research progress/plans to foster new collaborations.
Most important impact?	Decision made or influenced
URL	
Digital ID	
Source	Manual
Publication ID	63ef6ef7d563e7.63132223

Activity Title	Challenges In Generating Mature Human Tissue For Regenerative Medicine Applications
Activity Type	A talk or presentation or debate
How many people?	51 - 100

Geographical Reach	International
Primary Audience	Industry/Business
Other Audience	Professional Practitioners,Postgraduate students,Other audiences
Activity Years	2023
Result Description	Invited lecture with discussion and networking
Most important impact?	Requests for further information
URL	
Digital ID	
Source	Manual
Publication ID	65ea0900657da4.65920444

Activity Title	Modelling rare human diseases using iPSCs - potentials and challenges
Activity Type	A talk or presentation or debate
How many people?	11 - 50
Geographical Reach	National
Primary Audience	Professional Practitioners
Other Audience	Industry/Business,Postgraduate students
Activity Years	2023
Result Description	Workshop on the use of stem cells for disease modelling and drug discovery (Bit.Bio x LSCN - IPSC Workshop)
Most important impact?	Plans made for future related activity
URL	
Digital ID	
Source	Manual
Publication ID	65ea09ba7cb343.21032084

Activity Title	Patient Group
Activity Type	Engagement focused website, blog or social media channel
How many people?	101 - 500
Geographical Reach	International
Primary Audience	Patients, carers and/or patient groups
Other Audience	Public/other audiences,Supporters,Other audiences
Activity Years	2021
Result Description	A video discussing the disease was prepared for the acrodysostosis patient group who uploaded it on their site and used it for fundraising. Another video about our planned work/interest in the disease has been recently provided to the acrodysostosis patient group and will soon be live.
Most important impact?	Requests about (further) participation or involvement
URL	
Digital ID	
Source	Manual
Publication ID	6218c720a90bd6.43724697

Activity Title	patients' group representative visit to UCL GOS-ICH
Activity Type	Participation in an open day or visit at my research institution/facility
How many people?	1 - 10
Geographical Reach	National
Primary Audience	Patients, carers and/or patient groups
Other Audience	Patients, carers and/or patient groups
Activity Years	2023

Result Description	Discussion on progression of work with Nina Knight, the Chair & Founder of the Acrodysostosis Support & Research, on beahalf of the parents group and visit of our Institute. This was set to coincide with the visit of our potential collaborator Prof. S. Kimber from Manchester University.
Most important impact?	Requests about (further) participation or involvement
URL	
Digital ID	
Source	Manual
Publication ID	65e7665da09668.25503576

Research Tools and Methods

Material Type	Cell line
Material Name	iPSC generation from patients with acrodysostosis
Description	We have successfully reprogrammed cells from of three patient acrodysostosis (1 pateint with type 1 and 2 patients with type 2). One of the iPSC lines has been fully characterized.
Provided to Others	No
Year First Provided	
Year First Provided	2023
Impact Description	We will share the cells with collaborators and once the work is published they will make broadly available upon setting appropriate MTAs.
URL	
Digital ID	
Source	Manual
Publication ID	65e77849b660b5.66160700

Material Type	Technology assay or reagent
Material Name	cAMP assay
Description	We were not satisfied with the kit commercially availablt to indirectly measure cAMP, hence we established a method for quantifying cAMP in cells using HPLC normalised by protein content in collaboration with Prof. S. Eaton and are refining it.
Provided to Others	No
Year First Provided	
Year First Provided	2023
Impact Description	This is a new method that we will have a significant impact once

	published. We will either publish it on its own or as part of a larger study.
URL	
Digital ID	
Source	Manual
Publication ID	65e776cfb79ed9.02862237

Collaborations and Partnerships

Collaboration Title	Development of biochemical test for direct measurement of cAMP
Partner	
Organisation Name	University College London
Department	Institute of Child Health
Contributed Financially	No
In-kind contribution	No
Contributions Made	Established a protein quantification approach compatible with samples preparation for cAMP measurement, hence allowing for normalization of cAMP levels.
Partner Contributions	Expertise in setting up biochemical measurements / metabolic assays by HPLC
Year Commenced	2023
Year Ended	Still Active
URL	
Resultant Outcomes	In progress.
Formally Governed	No

Collaboration Title	iPSC Quality Control
Partner	
Organisation Name	Cellected
Contributed Financially	No
In-kind contribution	Yes
In-kind contribution currency	GBP British Pound Sterling

Give an estimate of the in-kind value.	4000
Contributions Made	We have generated iPSC lines.
Partner Contributions	Cellected has provided free quality control and banking of the some lines as part of a collaboration to test their system (equivalent value approx £ 4000).
Year Commenced	2023
Year Ended	Still Active
URL	
Resultant Outcomes	Validation of the suitability of the lines generated for further studies and gene editing.
Categorisation of impact	No impact yet
Formally Governed	No

Collaboration Title	Prof. George Baillie
Partner	
Organisation Name	University of Glasgow
Department	College of Medical, Veterinary and Life Sciences
Contributed Financially	No
In-kind contribution	No
Contributions Made	Prepared joint grant application. Set in motion getting an contract agreement to be able to send patient cells available to us to our collaborator, who has complementary expertise.
Partner Contributions	Prepared joint grant application. Discussed experiemntal plans and results.
Year Commenced	2022
Year Ended	Still Active

URL

Resultant Outcomes	The collaboration has just started and we are
Categorisation of impact	No impact yet
Formally Governed	Yes