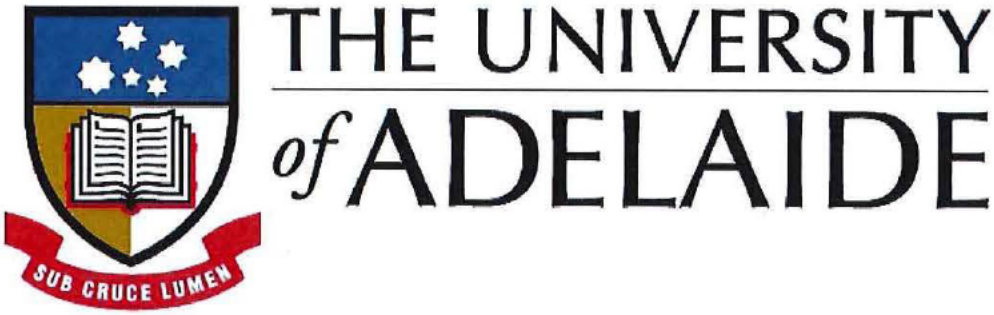
Studies in Craniofacial Development and Disease

A thesis submitted to the University of Adelaide in fulfillment of the requirements of the Degree of Doctor of Dental Science

Peter John Anderson

School of Dentistry, Faculty of Health Sciences,

University of Adelaide



2017

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Abstract

There have been great advances in the understanding of the disease processes that result in craniofacial anomalies in the last thirty years. However, clinical practice with the observation of existing treatments and their outcomes also reveals to the discerning that there remains a significant clinical need for yet further improvements in both.

This body of work encompasses a range of both laboratory based and clinical research which have been undertaken to try to meet address some of these issues.

The clinical research has enhanced the knowledge of the natural history of disease pathology in patients and the long-term impact of specific surgical interventions on growth development and outcomes, and is a vital part of audit of existing treatment protocols.

The laboratory-based basic science has impacted on both a broadening of underlying understanding of craniofacial biology, with correlation of gene anomalies and resulting morphological and functional changes. It also has in some examples raised the possibility of translating these results to develop novel innovative treatments that could lead to improved clinical outcomes.

Combined these two approaches to study have identified new findings and have increased the understanding of underlying mechanisms of craniofacial anomalies and raise the possibility of new treatments and enhanced clinical outcomes for affected individuals.

Declaration

I Peter John Anderson certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Signed

Peter John Anderson

Acknowledgments

I would like to thank and acknowledge the co-operation and patience displayed towards me by staff of the Adelaide Dental School, particularly Professor Grant Townsend and Dr Sarbin Ranjikar. Their unwavering belief in my abilities, have expanded my horizons and skills far beyond what I expected.

I wish to acknowledge the collaborative support from Associate Professor Barry Powell for his encouragement and expertise in molecular biology and introducing me to murine husbandry.

I wish to acknowledge the collaborative support from Professor Stan Gronthos and Professor Andrew Zannetino with their internationally recognized expertise in stem cell biology and bone biology respectively, and for their encouragement to actively experiment.

To all my family but especially my wife for tolerating me and making all the sacrifices that are required to complete another thesis. None of this could have happened without your continued support.

Lastly thank you to the Australian Craniomaxillofacial Foundation (ACMFF) and the Australian Dental Research Foundation for their continued financial support of some of these research projects.

Dedication

This thesis is dedicated to my life partner

Amanda Jane Anderson (nee Hakes)

This is to acknowledge her amazing tolerance of the turbulent lifestyle that she has had to endure, and for the many sacrifices she's made on my behalf while simultaneously indulging my many flights of fancy.

"The Electric Light did not come about by the continuous improvement of Candles"

Oren Harari

Chapter One – Introduction

There have been remarkable advances in the diagnosis and management of children with craniofacial anomalies since the specialty of Craniofacial Surgery emerged after the impetus given to this endeavor by the founding pioneer, Dr Paul Tessier.

Now that he and the other founders of this new branch of surgery have retired, review of the current status of this fledgling specialty reveals that the outcomes of treatment at skeletal maturity have improved, but it also clear that there remains the desire for enhanced social, functional, psychological and aesthetic outcomes, both by patients and their treating surgeons and physicians. It is also apparent that because surgery is often required in infancy, (and it may need to be repeated during childhood), it remains at the forefront of management for many affected children. Clearly, it would be highly desirable to develop (medical) non-surgical treatments to improve outcomes and to reduce the number of surgical interventions. This remains true for almost all of the different craniofacial conditions that are treated in specialized units and result in craniofacial deformity as a stigma of their underlying disease process.

Coincidentally, during this same period of time when craniofacial surgery has been evolving, in parallel to this there has been an explosion in the development of scientific investigative techniques, particularly in biotechnology, often driven by advances in computer technology. This computer enhancement has also allowed the storage of vast amounts of data, which has led to the development of larger, much more refined and sophisticated clinical audit studies, bio-informatics and leading to the initiation and subsequently the increased use of meta-data analysis of treatment outcomes and trials.

The application of these new investigative techniques to study craniofacial biology and the anomalies that cause disease and stigmata is the foundation that could lead to the possibility of new knowledge translated into developing improved, non-surgical adjuvant medical treatments. This can take two forms: to develop radically new treatments using enhanced knowledge of molecular biology or to improve existing treatment modalities by reviewing outcome studies. However, it is important to recognize that the process of translation of either of these approaches resulting from a significant experimental finding into a new treatment, is also becoming an increasingly difficult challenge for individual clinicians and scientists acting alone. As clinicians and scientists become ever more highly specialized within ever narrower fields of (often increasingly uncoordinated) endeavor. To succeed in research, in achieve the goal of medical treatment, coordinated multidisciplinary research teams of clinicians and scientists are required, to

overcome the many significant hurdles: cultural, financial and administrative to their successful clinical initiation and implementation.

The collection of papers that follow in this thesis is the result of following the aim to increase knowledge of the mechanisms of craniofacial development and anomalies, with the view to improving patient outcomes. This includes reducing the need for major surgery in children by developing medical or adjuvant medical treatments, as well is optimizing existing treatment technologies.

These attempts include forming multidisciplinary teams working across different sites to improve study numbers and hence robustness of any results. This is particularly important in studying the rare clinical conditions to produce valid results. Further, the establishment of collaborations across the Continents has the additional benefit in that they enable study of anomalies in different human ethnic groups, which may imply that a study may produce results that have truly worldwide significance and ultimately impact on patient care.

Chapter 7: Discussion and Conclusion

Earlier in Chapter One of this thesis it was highlighted that there is a need to improve existing treatment modalities for those affected with craniofacial anomalies, and in particular there is an obvious desire to reduce the number of surgical interventions in childhood. In attempting to fulfill this desire, which has been the motivation for this series of research projects, extending knowledge of both craniofacial biology and clinical outcomes in response to existing treatment modalities, has been attempted. This has been undertaken with the view to both optimize the use of existing technologies, as well as to develop novel medical treatments to reduce or replace surgery

This body of work encompasses a range of both laboratory based and clinical research. The basic science has impacted on both a broadening of understanding of the cellular mechanisms underlying craniofacial biology and pathology. The findings of these investigations raise the possibility of translating some of these results to develop novel medical treatments that may reduce the need for complex, life-threatening surgery, and could also lead to improved clinical outcomes using existing technology and refining treatment practices.

More specifically, this body of work advances the knowledge with the view to developing new treatments for the following craniofacial conditions: Facial clefting, Hemi-facial microsomia and Craniosynostosis, both non-syndromic and the commonest syndromes. These anomalies are the common craniofacial conditions, which collectively make up a large part of the cohort of patients who are treated at many of the specialized craniofacial centers around the globe.

However, some of the investigations that form part of this thesis have findings that have particular impact and significance and will be highlighted further.

The unexpected identification of novel suture anomalies associated with the transcription factor *Grhl3* in *Grhl3*$^{-/-}$ murine embryos, as part of study of genetic control of facial clefting, and the results of its expression in the suture site or brain, provides evidence that the current philosophy of investigating cell intrinsic defects within cranial osteoblasts may be mis-guided or incomplete (study 4.8). Further, these results suggest that our fundamental approach guiding Craniosynostosis research may need to be challenged and more directed to investigating neural crest patterning, and further investigation of this is clearly warranted.

The importance of the glypican family of cell receptors and more specifically glypicans 1 & 3 with their interactions with BMP2 and profound effects on osteoneogenesis within cranial sutures is a highly significant finding (study 4.4). These molecules are potentially therapeutic agents that could be used to arrest early Craniosynostosis. Further investigations to test this in murine models of Craniosynostosis with knock-in gene mutations associated with craniosynostosis

are currently undergoing evaluation. This requires developing a reliable mechanism for delivering the active agent locally in a sustained manner over a prolonged period of time to maintain suture patency while the underlying brain undergoes rapid growth. If successful this could be a foundation to establish for a phase one human trial.

The important interaction between vascular structures and their vascular derived VEGF and neural crest cells on chondrocyte proliferation in Meckel's cartilage formation have been demonstrated (study 4.9). These results raise to possibility of translation of these findings to clinical practice in that locally delivered VEGF could possibly be used as a therapeutic agent for infants affected with severe forms of hemi-facial microsomia or the hypoplastic mandible in the facial cleft condition of Pierre Robin sequence or even Treacher-Collins syndrome, with their severe respiratory compromise, to promote local chondrocyte proliferation to produce a larger mandible and improve a child's airway, and avoid the need for life-saving tracheostomy. Further innovative studies are currently being undertaken in murine models to investigate if this is practicable.

The significance of the role of cranial and facial suture stem (and pluri-potential) cells and the controls that impact on their different potential differentiation pathways are fundamental to understanding craniofacial biology. The identification that the c-ros-oncogene 1 mediates the gene *TWIST1,* which codes for the transcription factor TWIST, which is critical to bone formation by osteoblasts, is a novel and a highly significant finding (study 4.5). This is because this cellular control mechanism can be manipulated therapeutically, and further studies, funded by the NHMRC are investigating its potential use in patients with Saethre-Chotzen Craniosynostosis to prevent the excessive bone formation associated with the disease pathology, thereby reducing the need for surgical excision of affected cranial sutures.

While the significance of these laboratory based studies and their potential benefit to children is clear there are also benefits from the clinical studies and the systematic reviews in a broader medical context. Examples include, the long-term outcome studies, particularly the one regarding the rare Apert patient cohort managed by a treatment protocol at skeletal maturity (study 2.5). This has significance internationally and important in determining the benefit of a treatment protocol and form a vital part of the audit process. The morphology study of intrauterine cranial growth of sagittal synostosis is novel work, and now permits an informed decision by mothers and their treating clinicians regarding elective caesarian section, thereby altering current Obstetric Practice, with the aim improving outcomes for both Mothers and their babies (study 6.2). The systematic review regarding mandibular distraction has had a profound impact on how it is

used in contemporary surgical practice, since it provides a rational (rather than "ad-hoc") basis for its clinical use and implementation (study 3.3).

However, within the body of work that constitutes this thesis exist specific publications that impact on a dental practitioners.

Of major significance is the invited review, which was peer-reviewed, and published by the Australian Dental Journal on the use of different modalities of radiological investigations for both clinical and laboratory investigations and research (study 6.1). The impact of this paper (which was for a special edition of the journal), has been the most cited paper published by that journal since the time of its publication in 2014.

In important study is the cross sectional radiological study of the dentitions of a population of contemporary Australian children, and compares with previous published Australian studies that study mostly children of Anglo-Saxon ethnicity. This is of importance to those engaged in contemporary clinical practice to understand the commonly occurring variations in congenitally missing and duplicated teeth in the increasingly ethnically diverse Australian children's dentition. Indeed, the impact of ethnic diversity on the morphology of dentition has been studied in detail in ethnic Malays, where clinically significant differences with Anglo-Saxon derived "normal" values have been identified (studies 6.5 & 6.6).

Cumulatively, many congenital craniofacial conditions are ubiquitous, a vital consideration which underlies the potential world-wide impact of this collective body of work. This potential has already been demonstrated by the collaborative study undertaken by Research Centers on four continents, to identify the role of the *FREM1* gene in metopic Craniosynostosis (study 4.7).