# Investigation of the Role of the X-Linked Opitz Syndrome Gene, *MID1*, in Craniofacial Development

**By**

**Saidi Jaafar**
BSc.Ed (Hons) (Malaya), MSc (Malaya)


**A thesis submitted in total fulfilment of the requirements of the degree of Doctor of Philosophy (PhD)**

Dental School
Faculty of Health Sciences

&

School of Molecular & Biomedical Science
Faculty of Sciences
The University of Adelaide
Adelaide 5005
Australia

**July, 2005**

# THESIS SUMMARY

Normal formation of the vertebrate face requires appropriate growth, contact and fusion of craniofacial primordia in the ventral midline. Perturbations in these steps in facial development can result in an array of facial defects, the most common of which are cleft lip with or without cleft palate (CLP). Mutations in the *MID1* gene result in the X-linked form of Opitz GBBB syndrome (OS) in which CLP is a prominent feature. In fact, *MID1* represents one of only a few genes causally linked to CLP. The human *MID1* gene encodes a 667 amino acid microtubule-associated RING finger (RBCC) protein that functions as part of a large multi-protein complex. *In situ* hybridisation studies carried out in chick, mouse and human have indicated the highly conserved *MID1/Mid1* is expressed widely throughout embryogenesis although at varying levels depending on the tissue and cell type. However, the specific role of the MID1 protein in the development of these structures/tissues and how mutations of this gene gives rise to the various features seen among OS patients remains to be elucidated. As the various affected systems in OS patients appear to arise as a result of defective tissue fusion or remodelling during embryogenesis, elucidation of the molecular and cellular mechanisms by which the primordia of the face grow and then fuse to form the lip and primary palate will therefore shed light not only on our understanding of the developmental basis of CLP but also the processes leading to other common malformations (such as hypospadias and cardiac septal defects that also characterise OS patients).

A standard knockout of the murine *Mid1* gene was developed as part of a larger project to delineate the functional roles of *Mid1* in a mouse model. The generation involved replacement of the first coding exon of *Mid1* in ES cells with a *LacZ* reporter gene such that the reporter would be under the control of the endogenous *Mid1* cis-regulatory elements. Although the *Mid1* null mice do not display any gross external malformation in the current 129SvJ/MF1 genetic background, use of a *LacZ* reporter gene in the targeted DNA constructs enabled study of the expression pattern of *Mid1* by staining for β-galactosidase activity during early embryogenesis. Data presented in this thesis have revealed that *Mid1* is expressed in specific cell types within the craniofacial complex, the urogenital organ and surprisingly in the developing heart consistent with the defects seen in OS patients.

Detection of *Mid1* expression in the specific tissues during outgrowth and fusion of facial primordia suggests an important function of this gene in regulating these complex morphogenetic events. However, overlapping expression with the highly homologous *Mid2* gene suggests perhaps a level of functional redundancy between MID1 and its protein homologue, MID2. This would be consistent with: (1) the marked clinical variability in the presentation of OS, even among male patients from the same family and thus share identical *MID1* mutations, (2) the failure of the *Mid1* targeted knockout lines to display any gross facial malformation, at least in the current genetic background. To assist in addressing the functional redundancy between MID1 and MID2, specific antibodies recognised each protein were developed. Taken with recent evidence from early chick studies and the results presented in this thesis are consistent with this notion of redundancy also during later embryological stages.

In order to understand the cellular and developmental functions of MID1, inducible Madin-Darby canine kidney (MDCK) (epithelial) and Cos-1 (mesenchymal) cell lines that stably express either wild-type GFP-MID1 or one of a number of different mutant GFP-MID1 fusion proteins were then developed. These cells were used in preliminary investigations to address the role of MID1 in cellular processes such as cell migration, proliferation, cell death and the ability of cells to undergo epithelial-mesenchymal transitions (EMT), a key event in the fusion of epithelial-lined tissue such as in the facial prominences. These preliminary results showed that both epithelial and mesenchymal cell lines stably overexpressing wild-type or mutant MID1 did not effect either proliferation or apoptosis levels. However, in wound healing assays, MDCK cells stably overexpressing wild-type MID1 displayed delayed closure of the wounding area, in contrast to that seen with both MID1ΔCTD expressing cell lines where the rate of wound closure was notably more rapid than control cells. These early observations provide evidence that MID1 regulates the activation of epithelia, an early step in both EMT and cell migration.

This study has demonstrated that *Mid1* expression is expressed in all tissues normally affected in OS patients and, in particular, characterised in detail the expression of *Mid1* during the fusion of the facial prominences. This, together with an increasing knowledge about the cellular role of MID1, will greatly facilitate our understanding of the developmental processes controlled by the MID proteins and how their disruption

contributes to the clinical presentation of OS, and more specifically at least one of the pathways that lead to the susceptibility to CLP.

# TABLE OF CONTENTS