

UNDESCENDED TESTES;

the Danish experience

A International Specialised Skills Institute Fellowship.

DR JAYA VIKRAMAN

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i. Executive Summary

The knowledge that undescended testes have increased rates of infertility is known, but not as well managed in Australia as it is in other countries. Improved scientific methods used overseas has changed this area, challenging current practice in Australia.

This ISS Institute Fellowship has enabled Dr. Jaya Vikraman to continue making a high-level contribution to the field of paediatric urology research in Australia on an ongoing basis.

This report summarises the teaching and new knowledge gained by the Fellow during the overseas applied research component of her Fellowship. Dr. Vikraman attended The Children's Hospital (Rigshospitalet) in Copenhagen, Denmark, which is the largest pediatric surgery group in Denmark. Along the journey, the Fellow visited the pathology and research laboratories, inpatient units and the operating theater.

The Fellow compiled a questionnaire of relevant questions for each group of surgeons, medical pathologists and scientists. During her visits and international experience, the Fellow was overwhelmed with the openness to sharing knowledge and the teaching of practical skills offered by all those she met. Other highlights of the international research included that cryopreservation is being offered to children with undescended testes (UDT), and costs subsidised in cases of bilateral UDT.

This Fellowship Report concludes with a number of practical findings for presentations and to report to paediatric surgeons, with suggestions regarding ways of helping to grow a healthy paediatric population with improved outcomes for future fertility of males born with undescended testes in Australia.

ii. Abbreviations & Definitions

Abbreviations

- **GAF** George Alexander Foundation
- **GP** General Practitioner
- **IVF** In-vitro Fertilisation
- **ISS** International Specialised Skills
- MCHN Maternal Child Health Nurse
- PhD Doctor of Philosophy
- **UDT** Undescended Testes

Definitions

Bilateral Occurring on both sides of the body

Cd 117 Immunohistochemical stain

Ckit Immunohistochemical stain

Cryotherapy Refers to the practice of freezing ovarian or testicular tissue, with an aim of restoring fertility (either in the body or invitro) in the future.

D2-40 Immunohistochemical stain of D2-40 within cells

Immunohistochemical The process of selectively imaging antigens (such as protiens) in cells.

Invitro A scientific process performed or taking place in a test tube, culture dish, or elsewhere outside a living organism.

Medicare Publicly funded health care scheme for Australia.

Oct 3/4 Immunohistochemical stain of the transcription factor Oct 3/4 within cells

PLAP An immunohistochemical stain of cellular placental-like alkaline phosphatase

Undescended testis/ testes Where one (testis) or two (testes) are not located in the scrotum.

Unilateral Occurring on one side of the body

1. About the Fellow

Name: Jaya Lakshmi Vikraman

Employment: PhD student, Melbourne University & The Royal Children's Hospital

Qualifications: Bachelor of Medicine & Surgery, The University of Adelaide, 2008

Diploma of Surgical Anatomy, Melbourne University, 2012

Diploma of Child & Adolescent Health, The Children's Hospital at Westmead & The University of Sydney, 2014

Professional memberships:

- » Member Australian Medical Association
- » Associate Member Royal Australian College of General Practitioners

Publications:

JM Hutson, R Li, J Vikraman, M Loebenstein, (2016), What animal models of testicular descent and germ cell maturation tell us about the mechanism in humans. European Journal Pediatric Surgery. Oct; 26(5):390-398.

J Vikraman, JM Hutson, R Li, J Thorup, (2016), The Undescended Testis: clinical management and scientific advances. Seminars in Pediatric Surgery. Aug; 25(4): 241-8.

M Cousinery, R Li, A Vannitamby, J Vikraman, B Southwell, JM Hutson, (2016), Neurotrohpin signaling in a genitofemoral nerve target organ during testicular descent in mice. Journal of Paedatric Surgery. Aug; 51(8): 1321-6.

The International Encyclopedia of Human Sexuality, Female Genital Anatomy Chapter. Co-author with Professor Helen O'Connell, 2015.

Jaya Vikraman, S Donath, JM Hutson, (2017), Undescended testes: Diagnosis and timely treatment in Australia (1995–2014). Australian Family Physician. Mar; 46(3): 152-158.

Presentations/ Posters:

Poster titled "Should prepubertal hormone testing and testicular biopsy be routine at orchidopexy? at the 2017 Pacific Association of Paediatric Urology, May 28th-June 1st.

Oral presentation titled "Incidence of revision orchidopexy in Australia 1995-2014" at the 2017 Pacific Association of Paediatric Urology, May 28th- June 1st.

Oral presentation titled "Testicular descent is associated with the Mammary Bud in Rodents" at the 2014 Pacific Association of Paediatric Urology, May 24-29.

Oral presentation titled "Molecular mechanisms in testicular descent and the effect of androgen blockade" at the 2014 Urological Society of Australia and New Zealand, March 16-19.

Oral presentation titled "Molecular signals regulating cremaster muscle formation" at the 2013 4th World Congress of Paediatric Surgery, October 13-16.

Biography:

Dr Vikraman has always been interested in science, health and people, so it was natural she studied to become a medical doctor at The University of Adelaide. Dr Vikraman's interest in paediatric surgery began during an overseas elective. Her first years working were at The Austin Hospital, and during this time she was rotated to The Royal Children's Hospital for a paedatric urology position. Following this Dr Vikraman worked at Sunshine Hospital, Monash Medical Centre and again at The Royal Children's Hospital before commencing her PhD through the same hospital and Melbourne University.

While undertaking her PhD Dr Vikraman has completed two Diplomas, coauthored an encylopedia chapter (Female Genital Anatomy), and has had a review article in Seminars of Pediatric Surgery (The Undescended Testis) published. Dr Vikraman is currently focusing on completing her doctoral thesis, and publishing the results which will arise.

2. Aims of the Fellowship Program

The Fellowship provided Dr. Vikraman with an opportunity to examine the attitudes towards undescended testis in Denmark and determine why routine treatment includes biopsy with surgical correction, which is not performed in Australia, in particular:

- » To observe how consent is obtained for biopsy at the time of surgery for undescended testis
- » To comparing biopsy techniques to existing techniques in Australia
- » To learn techniques to process biopsy tissue
- » To understand how to interpret the results of hormonal testing before and after surgical correction of undescended testes
- » To understanding how abnormal results are followed in the Denmark.

3. The Australian Context

Undescended testis is very common in Australia, and affects 3 - 5% of newborn boys. Surgical correction is necessary and aims to preserve fertility and prevent cancer. Australian best practice is to operate under the age of 12 months to preserve fertility. This is in line with Nordic, American and European guidelines. However, in Australia testicular biopsies and hormonal testing have never been routinely performed as, up to now, there has not been a clear benefit to patients.

Scientific advances in medical biology have linked germ cell development to the prognosis for fertility and cancer. Northern European surgeons routinely perform testicular biopsies at the time of surgical correction for undescended testis, and in the cases when both testes are affected all parents are offered testicular tissue freezing to aid fertility later in life.

At the Children's Hospital (Rigshospitalet) in Copenhagen paediatric surgeons routinely use hormonal blood testing and testicular biopsies to improve ways of predicting fertility and cancer risk. This approach would revolutionise accepted practice in Australia, as there is now a predictive benefit of performing both biopsies and hormonal testing on fertility risk 20 years in the future, and cancer risk in 30 - 40 years' time.

SWOT Analysis Strengths:

- » Currently there are consistent guidelines for management throughout Australia, both with General Practitioners (GP) and Maternal Child Health Nurses (MCHN)
- » Recommended health checks are in place nationwide, aiming for early detection of UDT with MCHN and GPs
- » Treatment paths are well known and accepted amongst surgeons and tertiary caregivers
- » Paediatric surgeons are available in every state and territory in Australia
- » Surgical approaches are consistent in their aim, despite varying between clinical presentation and surgical preferences
- » All data is collected by Medicare and de-identified
- » Current treatment aims for fertility preservation and early detection of cancer
- » Cryopreservation is currently used in children who are undergoing treatment for cancer.

Weaknesses:

- » Geographically isolated places may require patient travel for specialised services
- » Recommended health checks may not always be completed, especially a genital exam in children who are no longer in nappies
- » Data collection does not include non-Medicare card holders
- » Data collection does not provide background to determine if cases have been missed, or immigration has occurred
- » Current system relies on parental co-operation with health checks, and would benefit from increased parental awareness / knowledge
- » No government incentive to ensure health checks are completed
- » Cryopreservation is not offered to children with UDT, even in bilateral cases.

Opportunities:

- » Medicare data, for the previous 20 years, is accessible
- » Potential to access data before and after the removal of the school entry nursing health check in Victoria
- » Social acceptance towards in-vitro fertilisation techniques (IVF) in Australia will allow uptake of cryopreservation
- » Experimentation on consenting adult tissue will improve male tissue cryotherapy techniques.

Threats:

- » Cost potentially prohibitive for tissue cryopreservation
- » Current technology remains unproven in male cryopreservation
- » Strict ethical regulations regarding use of children's reproductive tissue must be adhered to.

4. Identifying the Knowledge and Skills Enhancement Areas

There are examples of areas in Australian industries where there are weaknesses in innovation, skills, knowledge, experience, policies and/or formal organisational structures to support the ongoing successful development and recognition of individuals and the particular sector.

The focus of all ISS Institute Fellowships is on applied research and investigation overseas by Australians. The main objective is to enable enhancement and improvement in skills and practice not currently available or implemented in Australia and the subsequent dissemination and sharing of those skills and recommendations throughout the relevant Australian industry, education, government bodies and the community.

Specific skill enhancement areas addressed through Dr Vikraman's Fellowship were:

Consent Observation

Consent is an integral part of any surgical procedure. The observation of consent being obtained for biopsy at the time of surgery for undescended testis important in order to:

- » Identify the social context and concerns of the family
- » Observe the medical context, particularly the consent regarding testicular biopsy.

Action: document if a specific discussion about testicular biopsy occurs independently to the surgical procedure.

Action: based on this investigation, develop a specific consent form that could be used for surgical correction and testicular biopsy in Australia.

Surgical Technique

Observe the surgical technique employed during surgical correction and biopsy, with a particular focus on observing:

- » The surgical technique over multiple paediatric surgeons in the same institution
- » Where the biopsy is taken
- » If both testes are biopsied
- » If specific considerations change biopsy technique
- » Common adverse events following surgery.

Action: document the type(s) of biopsy - location, side, surgical technique used.

Action: based on this investigation, develop a suggested approach for biopsy in Australia.

Biopsy Processing

Observe and learn techniques employed to process biopsies - such as:

- » How germ cells are located in biopsies
- » The particular antibodies that are being researched and the long-term impact of cancer and fertility.

Action: document the laboratory techniques used, and the protocols employed.

Action: based on this investigation, develop a group of antibodies and protocols to use on testicular biopsies.

Hormonal Testing

Observe the timing of hormonal blood testing and the:

- » Exact hormones examined
- » The timing of the hormonal testing
- » Interpretation of these results.
- » The collaboration between endocrinologists and surgeons in another paediatric hospital.

Action: document hormonal testing being employed in Denmark.

Action: based on this investigation, develop a suggested plan, in liaison with Royal Children's Hospital Endocrinology department, to include hormonal testing in Australia.

Abnormal Results

Observe and document what happens when unexpected results occur and:

- » Identify what has happened in individual cases with high probabilities of cancer
- » Determine the surgical intervention in these cases
- » Determine the follow up which should be employed in these cases.

Action: document outcomes in cases with unexpected results.

Action: based on this investigation, develop an understanding of what to do when unexpected results occur and how they may be approached in Australian locations.

5. The International Experience

Rigshopitalet is a world-leading centre for testicular research. Testicular biopsy has been in place since 1972, and is a routine part or surgery for UDT. Hormonal testing was introduced in 2010, and has become a routine part of the investigations for UDT since.

The following section is an overview of the applied international research component of Dr Vikraman's Fellowship and is presented according to the key subject areas.



Image 1. Rigshospitalet, Copenhagen, Denmark.

5.1 Testicular biopsy

Destination: Department of Paediatric Surgery, Rigshospitalet, Copenhagen, Denmark

Contact: Jørgen Thorup, Professor and Consultant Paediatric Surgeon, Copenhagen, Denmark

Objectives:

- 1. To observe consent for testicular biopsy with surgery for UDT
- 2. To see, first-hand, the technique for surgical biopsy across all surgeons in the department

Outcomes:

Consent for testicular biopsies is implied at the time of consent for surgical correction of the UDT. Two out of eight surgeons specifically mentioned the process of taking tissue from the testis to examine under the microscope. The Fellow was able to observe the consent process in English by sitting in with English speaking immigrant parents. All surgeons mentioned that other tests (blood and to testicular tissue) would be performed at the time of surgery. All surgeons specifically consented the child's parents for cryopreservation, with all cases of bilateral UDT offered cryopreservation.

The surgical technique for biopsy had minor variations amongst surgeons. Once the testis had been mobilised to reach the scrotum, the thick outer layer of the testis was opened with a 15 blade. This opening was made between 12-3 O'Clock (facing the patient) on the left, or between 9-12 O'Clock on the right side. Gentle pressure was applied to the testis to cause tissue to protrude through this opening, and a strip of tissue was taken with scissors or a blade. If not enough tissue was taken, a second biopsy was taken. The outerlayer of testis was closed (various suture materials between surgeons) with a running stitch before the surgical correction was completed. In cases of unilateral UDT, only the operative side was biopsied. No post-operative complications occurred throughout the Fellow's period of observation at this hospital. Previous complications of stitch reaction had occurred.



Image 2. Operative photo with Professor Thorup and Dr Vikraman.

5.2 Testicular tissue processing and staining

Destinations: Department of Pathology & Laboratory of Reproductive Biology, Rigshospitalet, Copenhagen, Denmark

Contacts: Dr Erik Clasen-Linde and Professor Claus Yding Andersen

Objectives:

- 1. To observe and learn techniques employed to process biopsies.
- 2. To see the staining of particular antibodies.

Outcomes:

All biopsies for routine pathology, and cryotherapy, are sent to the Department of Pathology in Rigshospitalet. Here the specimens are processed into paraffin blocks, and cut at 5µm thickness and placed onto slides. Routine haematoxylin and eosin staining is performed. Immunohistochemistry of multiple stains is performed via an automated system (BenchMark Ultra), with 30 separate chambers with each having a different stain. This allows multiple stains to be performed on up to 90 slides per machine per day, with 12 machines in use.



Image 3. Pathology Department machinery for processing at Rigshospitalet.

5.3 Testicular tissue interpretation

- **Destination:** Department of Paediatrics, Department of Endocrinology Hvidovre Hospital & Department of Paediatric Surgery, Rigshospitalet, Copenhagen, Denmark
- **Contacts:** Professor Dina Cortes and Professor Jørgen Thorup

Objectives:

- 1. Learn how to identify germ cells.
- 2. Learn how to reliably count germ cells per tubule.
- 3. Discuss and observe slides of multiple immunohistochemistry stains.
- 4. Reproduce germ cell counts.
- 5. Reproduce spermatagonia counts, including the subtypes of adult dark and adult pale spermatagonia.

Outcomes:

Germ cells were identified initially on haematoxilin and eosin staining. If there was any question of the presence of a germ cell, the appearance of slides stained by Oct 3/4 or Cd 117 were used to confirm germ cell presence. Only tubules which were cut in a cross section were counted. The number of adult spermatagonia was also examined, as to the type (light or dark).

Image 4. Histological slide from the testes of a 3-year old boy with a normal germ cell number. (A&B) High magnitude haematoxylin and eosin staining with germ cells circled in red, and one tubule cross section circled in black. The tubule in the bottom left of image B is not a cross section and would not be counted. (C) PLAP staining. (D) Oct3/4 (E) C-kit/ CD117 and (F) D2-40 (Clasen-Linde et al, 2016, Figure 1)



Particular stains had individual benefits. Cd 99 and Oct 3 & 4 reliably stains germ cells at all ages, and is particularly useful when counting germ cells. Cd 117 is good at identifying immature germ cells. PLAP also has a strong staining of germ cells, but also tends to have a weak background stain. D240 is particularly useful in examining the tissue in young children, but does fade with age, particularly as gonocytes migrate towards the basement membrane with the loss of embryonic stem cell pleotype. The staining in neoplastic change was also discussed.

5.4 Hormonal testing

- **Destination:** Department of Paediatric Surgery Rigshospitalet (The Children's Hospital), Copenhagen, Denmark
- **Contact:** Professor Jørgen Thorup, Paediatric Surgeon, Copenhagen, Denmark

Objectives:

- 1. To observe and understand the interpretation of hormonal testing results.
- 2. To understand the follow up process employed with results are abnormal.

Outcomes:

The hormonal tests have shown that ~20% of boys with UDT have hypogonadism, where the primary anomaly is in the hypothalamic pituitary axis and the poorly functioning gonad is only secondary. This group is much more common in bilateral UDT. The remaining boys (~80%) have hypergonadotrophic hypogonadism, where there is primary deficiency of testicular function (which may be primary genetic or secondary to high temperature) and the hypothalamic-pituitary axis is responding to the low testis function.

The former group in the future are likely to be treated by gonadoptrophin analogues, to compensate for the deficiency, which is likely to improve the prognosis for fertility.

Concluding Remarks

Advances in medical science have increased the benefit of hormonal testing, as well as biopsy results, to benefit children with UDT. Copenhagen is a research rich city, which specialises in testicular tissue and UDT. Research in this area has benefited from routine biopsy, hormonal testing as well as universal identification numbers for residents allowing follow up within the same database across the country.

Testicular biopsy and hormone measurements are not routine in Australia, but the evidence from Copenhagen suggests that their introduction into the Australian management of UDT would lead to improved treatment and better prognosis for prevention of infertility and malignancy.

6. Knowledge Transfer: Applying the Outcomes

Attending the research Children's Hospital Cophenhagen and other site visits, gave the Fellow unique opportunities to network professionally with leaders in testicular research in both a laboratory and clinical setting, as well as discover innovative projects, ideas and training programs. It allowed the Fellow to become inspired by professionals from a range of professions all working on the common task of improving outcomes for children affected by UDT.

As a result of this Fellowship, the Fellow will be disseminating the information and knowledge she has acquired to strengthen the skills and capabilities of those involved in Australia.

The Fellow is scheduled to present her findings locally, at both surgical research in Murdoch Children's Research Institute and The Royal Children's Hospital (Melbourne). These presentations will include a summary and overview of the information gleaned from the Fellows visit.

The Fellow will also be submitting an abstract to present these findings at the Royal Australasian College of Surgeons annual meeting in Adelaide in early 2017, and at the Pacific Association of Paediatric Surgeons annual meeting in Seattle in May 2017. Both of these meetings are the professional associations for paediatric surgeons, which focus on presenting research to improve care in children in both Australasia and the wider world.

Following oral presentations, the Fellow will submit the information in a written publication in a medical journal to distribute the findings gathered throughout this Fellowship to a broader audience, including paediatricians.

7. Recommendations

The Fellowship has illuminated multiple opportunities for the Australian medical industry and governments to build on the experience and practices of Denmark. Key recommendations are outlined below.

Medical Industry

- » The progression of scientific technique in the areas studied merits open discussion in Australia, and a revisiting of the medical protocols for undescended testes.
- » Hospital groups should consider including biopsy in their standard adjunct to surgical correction of undescended testes, as an approach to cancer risk. The biopsy results may indicate prognosis and present no additional risk to the patient.
- » Consider cryopreservation of biopsied material as a standard adjunct to surgical correction of undescended testes, as this may improve fertility outcomes for UDT patients.
- » Hospital groups should consider including hormonal testing in their standard adjunct to surgical correction of undescended testes, in order to monitor and/ or correct hormonal imbalance.

Government

- » Investigate funding assistance for cryopreservation in cases of bilateral undescended testes (Medicare)
- » Funding grants for further research and studies into this area

- » Further research into stem cells and spermatogenesis from cryopreservation specimens
- » Further research into biopsy characteristics and relationship to testicular cancers
- » Assistance with lobbying to Private Health Insurance companies for cryopreservation and storage rebates
- » In collaboration with the medical industry, further research into the efficacy of hormonal testing for monitoring and correcting hormonal imbalance

8. References

The value of positive Oct3/4 and D2-40 immunohistochemical expression in prediction of germ cell neoplasia in prepubertal boys with cryptorchidism. Erik Clasen-Linde, Kolja Kvist, Dina Cortes and Jorgen Thorup. Scandinavian Journal of Urology. 2016 March; 50(1):74-9.

Testicular biopsy in prepubertal boys: a worthwhile minor surgical procedure? Faure A, Bouty A, O'Briend M, Thorup J, Hutson J, Heloury Y. Nature Review Urology. 2016 March; 13(3):141-50.

9. Acknowledgements

The Fellow would like to thank the following individuals and organisations who generously gave their time and their expertise to assist, advise and guide them throughout the Fellowship program.

Awarding Body – International Specialised Skills Institute (ISS Institute)

The ISS Institute exists to foster an aspirational, skilled and smart Australia by cultivating the mastery and knowledge of talented Australians through international research Fellowships.

The International Specialised Skills Institute (ISS Institute) is proud of its heritage. The organisation was founded over 25 years ago by Sir James Gobbo AC CVO QC, former Governor of Victoria, to encourage investment in the development of Australia's specialised skills. Its international Fellowship program supports a large number of Australians and international leaders across a broad cross-section of industries to undertake applied research that will benefit economic development through vocational training, industry innovation and advancement. To date, over 350 Australian and international Fellows have undertaken Fellowships facilitated through ISS Institute. The program encourages mutual and shared learning, leadership and communities of practice.

At the heart of the ISS Institute are our individual Fellows. Under the International Applied Research Fellowship Program the Fellows travel overseas and upon their return, they are required to pass on what they have learnt by:

- » Preparing a detailed report for distribution to government departments, industry and educational institutions
- » Recommending improvements to accredited educational courses
- » Delivering training activities including workshops, conferences and forums.

The organisation plays a pivotal role in creating value and opportunity, encouraging new thinking and early adoption of ideas and practice. By working with others, ISS Institute invests in individuals who wish to create an aspirational, skilled and smart Australia through innovation, mastery and knowledge cultivation.

For further information on ISS Institute Fellows, refer to www.issinstitute.org.au

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Fellowship Sponsor — The George Alexander Foundation

Dr Vikraman sincerely thanks The George Alexander Foundation for providing funding support this ISS Institute Fellowship.

In 1972, George Alexander AM (1910 - 2008) set up an independent philanthropic Foundation as a way of sharing his wealth and giving back to the community. Today, the main focus of The George Alexander Foundation is access to education for promising young people, particularly students with financial need and those from rural and remote areas.

The George Alexander Foundation (GAF) Scholarship and Fellowship Programs form the core of the foundation's work, operating in partnership with major tertiary institutions, while our Fellowships and other Education grants provide a variety of other unique and challenging educational experiences. George Alexander believed in the notion of 'planting seeds and hoping they grow into pretty big trees'. The programs supported by the Foundation endeavour to support this ideal and as GAF students graduate and go on to contribute to the community, George's legacy and spirit lives on through their achievements. George Alexander came to Australia as a child migrant, and went on to become a mechanic, an entrepreneur and a businessman and later, a generous philanthropist, who held that you do not own the possessions you have, 'you're just minding them'. This philosophy guided him to give during his lifetime and to hope that through his example, he might inspire others to do the same.

Supporters

Dr Vikraman would like to thank the following individuals and organisations who gave generously of their time and their expertise to assist, advise and guide her throughout the Fellowship program.

The Fellow received support from medical practitioners and scientists that she represents through her work.

In Australia:

- » Professor John Hutson, Royal Children's Hospital and Melbourne University
- » Bridged Southwell, Surgical Research, Murdoch Children's Research Institute
- » Treve Menhenniott, Gastrointestinal Research in Inflammation and Pathology, Murdoch Children's Research Institute

In Denmark:

- » Professor Jørgen Thorup, Rigshospitalet
- » Dina Cortes, Hvidovre University Hospital
- » Susanne Reinhard, Rigshospitalet
- » Kolja Kvist, Rigshospitalet
- » Professor Claus Yding Andersen, Laboratory of Reproductive Biology, Rigshospitalet
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- » Stine Kristensen, Post doc, The Department of Reproductive Biology, Rigshospitalet

- » Rós Kjartansdóttir, Post doc, The Department of Reproductive Biology, Rigshospitalet
- » Marjo Westerdahl, Bioanalyst, Laboratory of Reproductive Biology, Rigshopsitalet

Organisations Impacted by this Fellowship

Government:

- » Royal Children's Hospital
- » The Royal Women's Hospital
- » Murdoch Childrens Research Institute
- » Monash Medical Centre

Professional Associations:

- » Royal Australian College of Paediatric Surgeons
- » Royal Australasia College of Surgeons
- » Australian Medical Association
- » Royal Australian College of General Practitioners



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