TAY SACHS DISEASE: ANALYSIS OF AUSTRALIAN SCREENING STRATEGIES (1995-2013)

A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy
The University of Sydney

Dr Raelia Lew
MBBS (Hons) Monash University 2004
MMed(Reproductive Health Sciences and Human Genetics) The University of Sydney 2010
FRANZCOG

Thesis supervisor: Professor Leslie Burnett
Thesis co-supervisors: Dr Robert Markham, Professor Lucy Raymond
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statements of contribution

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Statement of Authentication

This thesis is submitted in fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in the Department of Obstetrics, Gynaecology and Neonatology, Faculty of Medicine, The University of Sydney, Sydney, New South Wales, Australia.

The work presented within this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

I certify my understanding that, if my candidature is successful, this thesis will be lodged with the University Librarian and made available for immediate public use.

Signature ___________________________ Date: 17/03/2016

Dr Raelia Lew
Contribution Statement and Co-Authors

The roles played by the co-authors in chapters 3, 4, 5, 6 and 8 were as follows:

1. Professor Leslie Burnett was my primary research supervisor. He provided intellectual input into the review of chapters 3, 4, 5, 6 and 8.
2. Professor Lucy Raymond was an associate supervisor. She provided support during my period of study in Cambridge, UK.
3. Dr Robert Markham was an associate supervisor. He provided intellectual input into review of this thesis.
4. Ms Anné Proos was a co-investigator. She provided intellectual input into the review of chapters 3, 4, 5, 6 and 8.
5. Professor Martin Delatycki was a co-investigator. He provided intellectual input into the review of chapters 3, 5 and 6.
6. Professor Agnes Bankier was a co-investigator. She provided intellectual input into the review of chapters 5 and 6.
7. Dr Michael Fietz was a co-investigator. He provided intellectual input into the review of chapters 5 and 6.
8. Dr Doug Chesher was a co-investigator. He provided intellectual input into the review of chapter 8.
9. Dr Lucy Ding was a co-investigator. She provided intellectual input into the review of chapter 8.
10. Dr Lan Nguyen was a co-investigator. She provided intellectual input into the review of chapter 8.
11. Dr Kristine Barlow-Stewart was a co-investigator. She provided intellectual input into the review of chapter 6.
12. Dr Yemima Berman was a co-investigator. She provided intellectual input into the review of chapter 6.
13. Mr Ron Fleischer was a co-investigator. He provided intellectual input into the review of chapter 6.
14. Mr Harry Aizenberg was a co-investigator. He provided intellectual input into the review of chapter 6.
15. Dr Michael Field was a co-investigator. He provided intellectual input into the review of chapter 6.

The final editorial authority remained my own. With the exception of contributions specifically detailed above, all work contributing to publications included in this thesis was my own. Individually signed statements of co-author contribution are included as Appendix 7.

Declared by: Raelia Lew  Signature:  Date: 24/01/2015
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Abstract

This thesis evaluates 20 years of Tay Sachs disease (TSD) preconception genetic screening conducted in Jewish communities of Sydney and Melbourne.

TSD is an inherited neurodegenerative disease, usually lethal in infancy or early childhood. More common in individuals with Ashkenazi Jewish (AJ) heritage, TSD was the first Mendelian condition to be targeted by community-based preconception genetic screening. Internationally AJ TSD screening programs have been operating since the 1970’s and have reduced the incidence of TSD in AJ populations in Canada, USA and Israel by 90%. The aim of such programs is to identify at-risk individuals and couples and to offer access to antenatal genetic counselling and reproductive options. In Sydney, following a successful pilot program (1993-1995), a TSD screening program was launched in 1995. In Melbourne a TSD screening program commenced in 1998. TSD screening programs in Australia are an archetypal model for preconception genetic screening of inherited conditions. The success of TSD screening programs in targeting individuals at risk (Chapter 4), reducing Tay Sachs disease incidence (Chapter 5), and translating screening program principles to a primary care setting (Chapter 6) has not been evaluated previously.

Jewish community genetic screening for TSD now occurs in the context of a panel of 26 genetic conditions common in individuals of Jewish descent.
The advent of emerging DNA sequencing technologies and their incorporation into standard laboratory assays for screening purposes introduces the facility to expand the spectrum of screening programs to include a broader range of conditions without significantly increasing laboratory costs.

The repercussions of an expanded AJ screening panel on the identification of carriers for 1 or more conditions has not been evaluated previously. The clinical impact of expanded screening on genetic counselling service referral and need for partner testing was explored in this thesis (**Chapter 7**).

On the basis of evidence from my original publications and my extensive systematic review of the international literature, I developed the first Clinical position paper to guide Ashkenazi Jewish genetic screening in the Australasian context. This work was ratified and published by the Human Genetics Society of Australasia (**Chapter 8**).

Work exploring issues of acceptability and cost effectiveness of broader screening panels in a preconception context in Jewish and other Australians, the validity of informed consent, and issues related to privacy and storage of genetic information obtained via DNA sequencing technologies is ongoing.

**Key words:** Tay Sachs disease, Ashkenazi Jewish, genetic screening, Australia, preconception, health promotion
List of publications and presentations arising from this thesis

This is a thesis by publication. Much of the work presented in this thesis has been published in the listed peer review academic journals and or presented at peer review meetings during the course of PhD candidature. The convention for author placement in the list of contributing authors within the medical discipline is by degree of contribution to the authorship (corresponding author first).

Published works

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Oral Presentations:


2. **Lew, RM**, Preconception Genetic Screening for Tay Sachs disease: An Australian Perspective Royal Prince Alfred Hospital Department of Women and Babies Annual Research Meeting, Royal Prince Alfred Hospital, Sydney, NSW, November 23rd 2012

3. Burnett, L, Ding, L, **Lew RM,** Chesher D, Proos, A. The impact of reporting exome and whole genome sequencing: Predicted frequencies of primary, secondary and incidental findings based on modelling, European Society of Human Genetics, Milan Italy, June 2014

Poster Presentations


Human Research Ethics Committee (HREC) Approvals

1. Hawkesbury HREC of the Northern Sydney Central Coast Area Health Service of the New South Wales Government Department of Health, (Reference 0810229M) 2008

2. Royal Children’s Hospital HREC (Reference 32016A) 2011

3. Northern Sydney Local Health District HREC (Reference 1201 – 034M) 2012

All HREC approvals relating to all elements of research conducted in the course of this PhD assessed work conducted to be of negligible/low risk.
Certificate of Satisfaction

Co-ordinating supervisor: Professor Leslie Burnett

In my opinion as the coordinating supervisor of this thesis, the form of presentation is satisfactory, complying with the requirements of the University of Sydney Guidelines.

Leslie Burnett

LESLIE BURNETT | MBBS PhD DBA FRCPA FHGSA FFS(RCPA) FCAP
Genetic Pathologist | Honorary Professor in Pathology and Genetic Medicine
THE UNIVERSITY OF SYDNEY | Discipline of Genetic Medicine, E25 | School of Information Technologies, J12
Sydney Medical School - Northern, Faculty of Medicine, Royal North Shore Hospital campus, E25
E: leslie.burnett@sydney.edu.au ; W: http://sydney.edu.au/medicine/people/academics/profiles/burnett.php

NSW HEALTH PATHOLOGY | SEALS | South Eastern Area Laboratory Services | Genetics
Prince of Wales Hospital, Level 4 Campus Centre Building, Barker St, Randwick | Sydney NSW 2031 Australia
T: +61-2-9382-9164 ; E: leslie.burnett@sesiahs.health.nsw.gov.au ; W: www.seals.health.nsw.gov.au
List of Abbreviations

TSD               Tay Sachs Disease
HREC             Human Research Ethics Committee
AJ               Ashkenazi Jewish
NJ               Non- Jewish
AR               Autosomal Recessive
AD               Autosomal Dominant
MPS              Massively Parallel Sequencing
Chapter 1: Introduction

1.1 The structure of this thesis

This thesis contains 5 publications, consisting of four peer-reviewed papers and an original position paper and clinical practice guideline, ratified and published by the Human Genetics and Genomics Society of Australasia (Chapter 8). Included in Appendix 1 are 4 posters, which have been presented at international meetings and published in the proceedings of the relevant society. The posters were submitted to further document the course of my research journey.

Collectively these publications describe my doctoral studies, including the generation of study hypotheses, the design of research protocols, the assessment of outcomes and their clinical translation through the formation of clinical practice guidelines. A copy of each paper is inserted into the body of the thesis.

The literature review contained in this chapter provides the research background to the practice of preconception TSD screening. Chapter 3 is a published literature review, detailing the Australian experience in Tay Sachs disease (TSD) preconception screening. Chapters 4, 5 and 6 consist of published papers, each reporting its aims, methodology and containing a referenced literature review specific to the body of work contained in that chapter. Some repetition within the literature reviews within published papers was unavoidable and mandatory, providing relevant background and contextualising my research within the highly
subspecialised area of Ashkenazi Jewish preconception conception screening in
the Australian primary health promotion clinical environment.
1.2 Research Background and Literature Review

Tay-Sachs disease (TSD)

TSD is a fatal neurodegenerative lysosomal sphingolipid storage disorder, caused by mutations of HEXA MIM *606869 (gene map locus 15q23-q24). Identified in 1969, the HEXA gene product is the α-subunit of β-hexosaminidase, a dimeric enzyme involved in the lysosomal degradation of GM2 gangliosides.\(^1\)\(^2\) TSD has autosomal recessive inheritance, where genetic carriers are themselves phenotypically normal. In a couple where both partners are carriers, 25% of successful pregnancies will result in a TSD-affected infant. Most infants with TSD appear healthy at birth. After a relatively short period of normal development, TSD-affected babies experience slow neurological decline, resulting in death in infancy (infantile TSD) or early childhood (intermediate TSD). This occurs secondary to neuronal accumulation of sphingolipid GM2 gangliosides. No cure or effective treatment that slows disease progression is known.\(^3\) A later onset form of TSD also exists. In 1971 an assay for HEXA protein was developed, allowing the possibility of TSD antenatal testing and carrier screening.\(^4\)

Tay-Sachs disease in Ashkenazi Jewish populations

TSD carrier frequency is approximately 1:25 in individuals of Ashkenazi Jewish (AJ) (Eastern and Central European) descent.\(^5\) TSD incidence in Jewish people is one in 3,900 births, compared to one in 320,000 births in other populations.\(^6\)
The HEXA gene

The HEXA gene was first characterised in 1985 and cloned further to identify intron-exon organization in 1987. In AJ populations several pathological variant HEXA alleles have been demonstrated. 96% of Jewish TSD carriers are found to have one of only three common mutations. 10, 11 c.1278insTATC, the most common mutation in AJ populations, is a frame-shift mutation due to a 4 base pair insertion in exon 11, introducing a premature stop codon and causing HEXA protein truncation. 12 c.1421+1G>C, the second most common mutation in AJ populations, features a G→C transversion in the donor splice site of intron 12. 9, 13, 14 The third mutations is p.Gly269Ser, a rarer missense mutation. TSD also occurs in higher frequencies than the general population in some French Canadian, Pennsylvania Dutch, Irish and Cajun communities; the relative prevalence of HEXA mutations found in these populations is different to those found in AJ populations. 16

The most likely explanation for the origin of multiple HEXA mutations in AJ populations is that they arose around 1100 AD by founder effect and genetic drift. 17-22 Four independent sphingolipid storage diseases have arisen in AJ populations (TSD, Niemann-Pick disease, Gaucher disease and mucolipidosis type IV) leading some investigators to hypothesize a possible heterozygote advantage. 23, 24, 25

North American demographic studies of AJ TSD carrier frequency have identified varying carrier frequencies between AJ communities founded by immigrants from different regions of Europe (Toronto 1:14, Baltimore 1:22 and Washington D.C. 1:28, Average USA 1:30). 26 However, these studies were based on results of
enzyme-based carrier testing and therefore made no distinction between individual AJ HEXA allele frequencies among the sub-populations studied.

**History of TSD screening**

The first TSD carrier screening program was introduced in the USA in 1971.\textsuperscript{11} By the year 2000, some 1.4 million individuals had participated in these programs around the world, and over 51,000 TSD carriers, including more than 1,400 at-risk couples (in which both partners are TSD carriers), had been identified via TSD screening programs.\textsuperscript{10} As technology has evolved, many Jewish TSD screening programs have expanded from testing for TSD alone to testing for a panel of recessive genetic conditions common to Jewish populations (Canavan disease, Niemann-Pick disease type A, Bloom syndrome, Fanconi anaemia, familial dysautonomia, mucolipidosis type IV) and cystic fibrosis.\textsuperscript{27} Cystic fibrosis in Jewish communities has a 1:25 carrier frequency, similar to other Caucasian populations, although the prevalence of individual mutations may differ.

**Testing Methodology**

TSD screening was originally conducted by biochemical testing of HEXA enzyme activity and more recently by direct DNA testing of the HEXA gene. Enzyme testing in theory affords the possibility of detecting low prevalence HEXA DNA mutations and is considered to be the gold-standard testing method in populations of mixed or non-AJ ancestry.\textsuperscript{6} DNA testing has been reported as the best procedure for identifying TSD carriers in AJ populations.\textsuperscript{6, 28} In an orthodox AJ cohort of 38,197 individuals in Israel, DNA testing had high sensitivity (93.1-99.1%) and equivalent
specificity compared to biochemical testing (88.1-98.8%). DNA testing of mouthwash/cheek brush samples improves participation rates compared with venepuncture/blood sampling required for biochemical testing. Successful DNA testing of hair root specimens offers a sample amenable to extreme ease of transport, of potential use for outreach screening programs. In the future, Massively Parallel DNA Sequencing technologies (MPS) is likely to replace standard DNA testing for simultaneous identification of carriers of TSD and other genetic errors. This method is explained further in later chapters of this thesis.

The Dor Yeshorim Screening Program

The Dor Yeshorim screening program was established in 1983 to provide access to TSD screening for the ultra-orthodox Jewish community in the USA in a format culturally acceptable to this demographic. For many ultra-orthodox Jewish couples, termination of a TSD-affected pregnancy diagnosed prenatally is not an option they would consider acceptable on religious grounds. In ultra-orthodox Jewish communities, “arranged marriages” are common, in which young couples are introduced with a view to being married. In the Dor Yeshorim model, screening is offered to unmarried individuals and occurs prior to the introduction of young couples. The outcomes of screening remain anonymous – results are not directly disclosed to individuals tested.

In the Dor Yeshorim program, “compatibility” of couples is determined prior to introductions being made. Introductions proceed only if couples are found to be “compatible”, that is, they are not both carriers of mutations for TSD. Alternatively
couples contemplating engagement and found to be “non-compatible” (both carrying a TSD mutation, and thus at high-risk of having TSD-affected children) are simply not introduced to each other, and instead are introduced to different prospective partners.

The Dor Yeshorim screening model has >95% uptake in ultra-orthodox Jewish communities. A branch of this program has been operating in Israel since 1986. Since the Dor Yeshorim program was established, the scope of genetic testing offered to ultra-orthodox Jewish couples has been expanded to test for other significant recessive conditions at higher than background frequencies in Jewish populations in addition to TSD.

**TSD Screening in Israel**

In addition to the Dor Yeshorim screening program, TSD screening is offered free of charge in Israel to at-risk individuals through voluntary community based screening programs. While it is recommended to undertake screening prior to pregnancy, Israeli health care providers are directed to discuss TSD genetic screening with all women of fertile age or in early pregnancy. With the advent of widespread TSD screening in Israel, cases of TSD amongst the Jewish Israeli population are now exceedingly rare. The Israeli system is an example of how clinicians in primary care settings can effectively facilitate preconception genetic screening strategies, which result in the prevention of targeted conditions such as TSD.
The Montreal Experience

The first example of a TSD screening program targeting senior high school students was initiated in Montreal, Quebec, in the setting of general access secular high schools. In this community individuals of either French Canadian origin or AJ origin are at high risk of TSD, and a screening program targeting general access secular high school students was deemed to be viable. During 30 years of TSD screening, TSD incidence in Quebec has been reduced by over 90%.

Pros and cons of TSD genetic screening in an adolescent population

The ideal setting in which to screen for TSD is an informed adult couple planning a future pregnancy. Unfortunately this demographic often do not access preconception testing for many reasons including low levels of knowledge of TSD and carrier risk, low motivation, cost of testing or unplanned pregnancy. The factor that has been the most successful in increasing uptake of pre-conception genetic screening for TSD internationally has been the implementation of screening programs that target an adolescent population. Screening at senior high school level captures a large proportion of the target population in an environment where both pre-test education and testing can be carried out with relative ease. Senior high school students are mature minors, usually able to understand the implications of testing and provide informed consent. Students educated immediately prior to testing and then screened for TSD in a high school setting have also been found to retain more information at the time of testing and recall this information 3-6 years
following testing than do adults screened in other settings\textsuperscript{29, 35, 42, 43} In order to be optimally relevant, TSD carrier screening must occur in a window before conception, with all reproductive options accessible, allowing couples to make informed choices in planning their family.\textsuperscript{34} Carrier testing in an adolescent population addresses the issue of TSD screening prior to pregnancy planning, thus allowing future informed access to all possible reproductive options for at-risk carrier couples and extended families.\textsuperscript{44} Targeting adolescents in a multicultural high school environment for genetic screening has been criticised for the potential to invoke racial/culturally based bullying of carriers,\textsuperscript{39} however this has not been reported in practice.

**International success of TSD screening programs**

In Jewish communities around the world, TSD carrier screening programs have reduced the births of infants with TSD by >90\%.\textsuperscript{11, 35, 45} A review of the first 20 years of the Montreal TSD carrier screening program evaluated in 1996\textsuperscript{35} showed that it had reached 89\% of Montreal Jewish high school students with 67\% of students reached choosing TSD screening. Information regarding the rationale for TSD screening was strongly retained and anxiety levels amongst carriers remained low. Over this period, new TSD diagnoses in the Jewish population of Quebec fell by 90\%, attributable to both the impact of TSD screening and a decline in population birth rate, while TSD frequency in Quebec’s non-Jewish population remained static.\textsuperscript{35} Prior to 1970, 85\% of TSD affected infants were born in Quebec had Jewish ancestry. In contrast, by 1993 an estimated 60-80\% of TSD affected infants born had no Jewish ancestry.\textsuperscript{11}
**Australian Ashkenazi Jewish Communities**

Although a small AJ community has existed in Australia since the time of European colonization of Sydney in 1788, the majority of the Australian AJ population was founded by immigration subsequent to World War II.

The Australian Jewish community (estimated population over 90,000) mainly reside in Melbourne and Sydney. The majority of Australian Jews have AJ heritage. Over 70% of Jewish adolescents in Melbourne and 50% in Sydney attend Jewish High Schools that access TSD screening programs. Studies of the allele frequencies of several genes in the Australian Jewish population in Sydney including HEXA, BRCA1, BRCA2 and APC indicate the Australian AJ population is not statistically different in these frequencies from Jewish populations in Israel, USA and Canada and therefore is comparable to international AJ populations in regards to burden of genetic diseases. The impact of wide reaching TSD screening programs targeting Jewish communities in Australia would therefore be expected to have similar potential for reducing TSD incidence as international programs have demonstrated. A detailed review of the Australian experience of TSD genetic screening appears in Chapter 2 of this thesis. Chapter 5 of this thesis measures for the first time the impact and health outcomes of TSD screening programs in Australia.

**Elements of successful TSD screening programs**

Successful TSD screening programs require much more than simply offering laboratory testing for TSD. In successful International TSD screening programs,
educational campaigns have been conducted, focusing on the medical community, the lay Jewish community and religious leaders. Community leaders and lay representatives should be actively engaged in the planning process to ensure that the eventual TSD screening program model is designed and adapted to be conducted sustainably in line with community wishes, needs, expectations and resources.\textsuperscript{39,53} Screening programs should adhere to international ethical standards with regards to individual privacy and patient autonomy.\textsuperscript{54} Genetic screening should be fully informed and voluntary. Genetic screening programs that address these issues, and which also target senior high school students, have been associated with high uptake, both internationally and in Australia.\textsuperscript{35,42,45}

**Future directions**

My work directly resulted in collaboration between the two independent Australian AJ preconception screening programs. My research and systematic reporting of Australian AJ population data with respect to TSD has provided an evidence base to support a planned campaign for future Medicare funding of AJ preconception genetic screening strategies. My work will ultimately assist in improving preconception genetic screening access to all AJ Australians.

As the routine testing modality for AJ individuals evolves from traditional mutation-based DNA testing to MPS-based testing, screening for a greatly expanded panel of genetic conditions will not only be possible, but also affordable. Broader MPS screening panels have the potential to benefit individuals who choose to access
preconception genetic screening in their future health care. In this scenario, initial results reporting strategies may be limited to targeted pathological variants, with the option of in-silico reanalysis at a future time as clinically appropriate. Maintaining ethical principles without compromising patients access to longitudinal benefits of information derived from genome sequencing is a challenge for our times.55

Measures of the success of a genetic screening program include:

- the level of uptake within the target population,
- the percentage of the target population offered access to screening,
- the level of informed consent among individuals screened,
- the reduction of target disease incidence over time through the uptake of Assisted Reproductive Technologies (ART) and selective pregnancy termination, and
- economic outcomes.56

Studies are underway to audit screened Australian AJ TSD carriers’ access to Pre-implantation Genetic Diagnosis (PGD) and prenatal diagnostic results from amniocentesis and chorionic villus sampling (unpublished data Dr Raelia Lew, Professor Martin Delatycki, Ms Anné Proos).

High school screening programs, although extremely successful, cannot hope to be the sole mode of delivery for the goal of avoidance of TSD-affected births in Australian AJ populations. This is because a minority of Australian Jewish individuals
of reproductive age have attended the schools that offer access to these programs. This majority Australian AJ target population relies on primary health care clinician referral in order to gain access to preconception and antenatal TSD screening, with a small minority also accessing testing via community outreach programs.

Due to expertise I gained via pursuing the research described in earlier chapters of this PhD thesis, I was invited to chair a Human Genetics Society of Australasia (HGSA) committee, and to be the primary author of Australia’s first clinical practice guideline for preconception genetic screening in Ashkenazi Jews. I invited experts and lay representatives with leadership experience in the field of Ashkenazi preconception genetic screening to form the guideline steering committee. We met and collaboratively agreed on a framework for the guideline. I then conducted the research review and wrote the guideline, which was then subjected to committee review. I then re-wrote the guideline to implement collectively suggested changes. The committee then reviewed the guideline for a second time and with unanimous agreement submitted the manuscript to the HGSA and RANZCOG joint screening committee for external peer review. I received and implemented suggested changes from the peer review process, and submitted them to the steering committee for consideration. I the prepared and submitted a final version of the guideline to HGSA, which was ratified and published in 2015. With permission of the HGSA, the position paper has been included in this thesis (Chapter 8). Development of this guideline enables the two Australian Jewish Community genetic screening programs (in Sydney and Melbourne) to align their programs’ operations (personal communication Professors Leslie Burnett and Martin Delatycki) and will lead to
adoption at a national level of a single agreed best-practice framework. This guideline is designed to change clinical practice, resulting in the adoption of the practice of genetic screening in the primary healthcare setting. It provides a valuable resource to Australasian Health care clinicians and will assist in extending access to preconception genetic screening to at-risk AJ individuals outside of the Australian high school screening model.

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Chapter 2: Research protocols, Aims and Hypotheses

2.1. Introduction

The body of work contained in this thesis represents the evolution of my research conducted in association with Australasian TSD screening programs. In this introduction, I will outline the evolution of this project and of the research questions hypothesised and addressed by this thesis. My involvement in TSD preconception screening research began in 2008. While enrolled as a Master of Medicine (Reproductive Health Science and Human Genetics) student at the University of Sydney, I began a research treatise investigating the frequencies of common AJ HEXA mutations among student participants of existing Australasian TSD screening programs, and the correlations between mutation type, grandparent’s country of birth and identification of AJ heritage. A questionnaire designed by the Sydney screening program, completed by all students undertaking preconception screening over time, asked students to document their grandparents’ birthplace. However, this information was never synthesised or analysed by other collaborators in the screening program and so no conclusions had been drawn from this data. I obtained ethics approval to create a secure, de-identified database of screening program participants historical family demographic data from completed questionnaires. I was then able to link demographic information to allelic status and thus create a mutation profile for Ashkenazi and non-Ashkenazi participants by country of grandparents’ origins. The work and conclusions reached proved to be of broad consequence, beyond the scope of the proposed treatise and I decided to continue this work as a Master of Philosophy. This initial work provided evidence that:
a) Australian AJ individuals can correctly self-identify TSD carrier risk to health professionals and that

b) a sustained high TSD carrier frequency exists amongst Australian AJ individuals, based on allele frequencies in individuals now aged 20-40 (senior high school students at the time of screening).

The methodology and results of this early work (Lew et al, 2011 Tay-Sachs disease preconception screening in Australia: self-knowledge of being an Ashkenazi Jew predicts carrier state better than does ancestral origin, although there is an increased risk for c.1421+1G>C mutation in individuals with South African heritage, Journal of Community Genetics) is included as Chapter 4 of this thesis.

In light of these clinically significant findings, I hypothesised that if TSD preconception genetic screening was an effective preventative strategy, then near complete prevention of future Jewish TSD cases in Australasia should be achievable. More exciting still, if a single rare recessive disease could be prevented by preconception screening, why not extend the concept to other recessive diseases with similar carrier frequencies such as cystic fibrosis? However, I identified several impediments to this happening. These included:

1. The lack of awareness of pre-conception genetic screening in the general medical community

2. The lack of public funding for preconception genetic screening
3. In the case of Ashkenazi individuals and TSD, the uneven access to preconception genetic screening programs, based on whether students attended a participating private school.

An obvious barrier to a future application for public health funding of a preconception health promotion and preventative medicine based genetic screening strategies was the lack of current Australasian supporting evidence based medicine publications supporting the relevance and efficacy of this practice.

At the suggestion of my annual progress report panel and with the support of my research supervisors, I decided to extend my research to identify evidence supporting or refuting my hypothesis that TSD preconception screening of AJ individuals in the Australian context was effective, and that extending TSD screening should be supported by an evidence based guideline. I then converted my candidature to this PhD.

Prior to my research into this area, the clinical benefit of existing Australasian TSD screening programs was hypothesised but unproven. Collaborative research had not been previously undertaken by existing Australian AJ genetic screening programs. Pooling and analysis of their combined data had never previously been achieved. To confirm and quantify the benefit of preconception genetic screening for TSD in at-risk AJ populations and from there to argue for extension of TSD preconception screening to all Australasian AJ individuals of reproductive age, it was necessary to investigate the clinical effectiveness of previously undertaken TSD screening in
Australasia. There existed many reasons why this had not been attempted before. TSD cases were not mandatorily reported or collected in any existing database and occurred across different medical and state jurisdictions. Multiple barriers existed to acquiring comprehensive knowledge of cases, amongst them, obtaining the approval of 3 parallel ethics committees.

I proposed to conduct a complete and consecutive retrospective audit of all TSD cases diagnosed in Sydney and Melbourne during the period in which TSD screening programs operated (Chapter 5). A key logistic discovery gave me confidence that this undertaking was possible and achievable - only 3 Australian testing laboratories had functional diagnostic capability for TSD testing during my proposed study period. It was therefore apparent that all TSD cases were referred for diagnostic testing to one of these three laboratories. To discover and synthesise a complete record of TSD cases into a single de-identified database involved collaboration between the two Australasian TSD screening programs and the three national testing laboratories to which diagnostic tests were referred during the time period, located in Sydney, Melbourne and Adelaide. With multiple permissions and trans-institutional collaborations, I created was able to analyse all cases of TSD diagnosed in Sydney and Melbourne during the study period. This not only involved a review of laboratory records, but also a physical review of hospital records for which I travelled interstate and personally reviewed handwritten medical notes relating to cases. From medical and genetic counselling clinical documentation and laboratory records I was able to determine and compare observed TSD cases (Jewish and non-Jewish) with the number of cases predicted for the same period.
based on Australian birth registry and Australian Bureau of Statistics national census data. From TSD screening program database comparison, I determined that no AJ screening program participant found to be a TSD carrier had been the parent of a TSD affected child born in Sydney or Melbourne during the study period. I also demonstrated a reduction in Jewish TSD cases during the study period. The methodology and results of this work (Lew et al, 2012, *Tay Sachs disease in Australia: reduced disease incidence despite stable carrier frequency in Australian Jews, Medical Journal of Australia*) is included as Chapter 5 of this thesis. In two Jewish cases of TSD diagnosed during the study period, none of four AJ parents had TSD preconception screening. Furthermore, no AJ individual who had TSD preconception screening had gone on to have a TSD affected child.

It became evident that TSD preconception screening in Australasia, previously proved to be cost effective (Warren et al.) was also a highly effective preventative strategy. My hypothesis that near-complete prevention of TSD would be possible should the entire AJ population of reproductive age at risk of TSD have access to preconception genetic screening was now supported by evidence derived from Australasian research data. The challenge for the future, would be reaching the residual at-risk Australasian AJ target population who currently have not had access to established TSD genetic screening programs and affiliated outreach screening strategies. Currently, TSD screening programs target mature adolescents and operate in non-government Jewish high schools. Due to these limitations, fewer than 50% of Australian AJ individuals of reproductive age at risk of TSD have accessed these programs. In contrast, Australians may universally access community-based
healthcare provision. A feasible strategy to capture the residual AJ target population is to offer preconception and antenatal TSD screening via primary healthcare providers. Up until now, no Australasian clinical practice guidelines have existed to support primary health care clinicians and publicise this practice.

As a precursor to the development of such a guideline, in collaboration with a team of experts, I undertook to systematically review the Australian and international literature on TSD preconception and antenatal screening practice. This was necessary as no similar contemporary Australian or international publication had been authored by other researchers. Using the Australian National Health and Medical Research Council evidence grading system, we derived the first Australasian evidence based best practice recommendations series for TSD preconception and antenatal screening in AJ individuals of reproductive age. This work, (Lew et al 2014, Ashkenazi Jewish population screening for Tay–Sachs disease: The International and Australian experience, Journal of Paediatric and Child Health) is included as Chapter 6 of this thesis. This paper was intended to aid and encourage extension of the proven benefits of TSD screening programs to all AJ individuals of reproductive age, with the aim to achieve near-complete prevention of TSD. While conducting this work, I was thrilled to be invited by the Human Genetic Society of Australasia (HGSA)/ Royal Australia and New Zealand College of Obstetricians and Gynaecologist Joint Committee for preconception and antenatal screening to chair a multidisciplinary expert panel to derive the first Australasian clinical practice guideline for population genetic screening in Ashkenazi Jews (Chapter 8).
During the course of my involvement of TSD preconception screening research, technological advances have been rapidly occurring. The Sydney TSD program, currently based at the PaLMS Pathology North laboratories of NSW Health Pathology at Royal North Shore Hospital, St Leonards department of Laboratory and Community Genetics (but shortly to transition to the SEALS Genetics laboratories of NSW Health Pathology at Prince of Wales Hospital, Randwick), is in the process of assessing the possible implementation of MPS technology as a diagnostic standard for AJ preconception screening. As part of the process of analytical and clinical validation, testing for the 2013 and 2014 Sydney AJ screening program for TSD was run in parallel using both conventional DNA testing for 5 conditions and a pilot MPS expanded panel of 26 conditions (Leslie Burnett, Personal Communication). Projected clinical impact of this change and ethical issues raised are explored in Chapters 7.

Results from the 26 condition AJ MPS preconception screening pilot study are anticipated to be available for analysis in 2017. Together with the Sydney AJ Community Genetic screening research group, I plan to submit a further manuscript, comparing predicted and observed outcomes for the 26 condition MPS panel pilot study. This work will be completed beyond the end of my PhD candidature.

In Chapter 9, I discuss my results, draw conclusions and also outline the directions my continued research in the area of preconception health promotion will take in the immediate future.
Posters presented at international peer review forums and published in their respective proceedings have been included alongside this thesis (Appendix 1).
2.2 Aims and Hypotheses

2.2.1. Chapter 4

Hypothesis: Ashkenazi Jewish individuals at risk of TSD are able to know and accurately report their heritage, which correlates strongly to their TSD risk profile.

Study Aim: To assess the ability of Australian Jewish individuals screened for TSD to self-report Ashkenazi Jewish heritage. To compare TSD carrier frequency in contemporary Australian AJ populations of reproductive age to historical international cohort data from the 1970’s and 1980’s in order to quantify current reproductive risk. To assess how AJ heritage and grandparental country of origin correlates with TSD risk, both overall and in the case of specific common AJ HEXA gene mutations.

Implications: In order to successfully apply preconception screening for TSD in at risk individuals as a broadly effective health promotion and disease prevention strategy, individuals at risk must be identifiable to clinicians. It has been an unconfirmed assumption that high TSD carrier risk exists in contemporary Australian AJ populations of reproductive age. Demonstration of individual’s accurate self-reporting of Ashkenazi Jewish heritage, and strong correlation with TSD risk supports the possibility of extending TSD screening to Jewish individuals at risk outside of established TSD screening programs through primary care clinician led screening.
2.2.2 Chapter 5

**Hypothesis:** Australian TSD preconception screening programs are a cost-effective and effective primary prevention strategy, which result in a reduced incidence of TSD.

**Aim:** To perform a complete and consecutive audit of all cases of TSD diagnosed in Melbourne and Sydney during the period correlating to Jewish TSD screening programs operation. To identify the Jewish and non-Jewish heritage of TSD cases. To assess for a reduction in observed Vs expected Jewish TSD cases. Predicted TSD cases were determined on the basis of Australian Bureau of Statistics census population data and known TSD carrier frequencies in Jewish and other populations.

**Implications:** Demonstrated effectiveness of Australian TSD screening programs in primary prevention of TSD cases supports:

A) The continuation of existing high-school based TSD screening programs offering preconception genetic screening to a mature adolescent target population.

B) The extension of TSD screening to the Australian Jewish population who 1) are at increased risk of being a TSD carrier and 2) have not accessed existing TSD preconception screening programs.

C) The development of practice guidelines to facilitate clinician-led, community-based TSD screening to complement existing screening programs.

2.2.3 Chapter 6

**Hypothesis:** Near total prevention of TSD in Jewish individuals could be achieved by extending preconception and antenatal genetic screening to all Jewish individuals at
risk. This is cost-effective and achievable via a combination of high-school based genetic screening programs and primary care clinician lead TSD screening. Best practice guidelines for TSD preconception screening are called for to provide an accessible evidence based resource to aid clinicians to extend the benefits of TSD preconception screening to the AJ target population at risk of TSD.

**Aims:** To systematically review the Australian and international literature and develop an Australasian best-practice model for primary care clinician lead AJ TSD screening.

**Implications:** Clinicians may access an Australasian evidence based recommendations for TSD screening in Jewish individuals at increased risk. By facilitating TSD screening in the community setting, equitable access to screening may be achieved for all AJ individuals at risk of TSD. This will predictably result in a net increase in informed reproductive choice and, potentially near total prevention of Jewish TSD cases may be achieved.

### 2.2.4 Chapter 7

**Hypothesis:** Next generation DNA sequencing technologies are changing the way genetic testing will be conducted in the future. This technology will in the future replace conventional DNA testing for preconception genetic screening programs. Workplace and ethical implications of this change require evaluation.
**Aims:** To compare current with projected future genetic counselling referral rates using a conventional DNA mutation testing panel vs a massively parallel sequencing model to conduct Ashkenazi Jewish pre-conception genetic screening.

To mathematically model rates of primary, secondary and unexpected incidental findings (IFs) detected using an MPS screening model for AJ preconception genetic screening.

To evaluate the clinical and workforce implications of adopting modernised DNA sequencing technologies for TSD carrier screening in the context of an expanded Ashkenazi Jewish panel. Findings of early mathematical modelling of expanded screening AJ screening panels projected results are reported in **Appendix 1**.

**Implications:** Preconception genetic screening using next generation DNA sequencing and an expanded panel will result in much higher number of carriers identified requiring genetic counselling. The rate of secondary and unexpected incidental findings in this cohort is unknown. Sequencing derived data may be longitudinally re-interpreted as scientific knowledge evolves over time, resulting in delayed secondary and IFs. The current concepts of informed consent are challenged by this practice. Ethical and legal obligations of laboratories and clinicians are unspecified. Supportive infrastructure is lacking. These issues require discussion and debate.
2.2.5 Chapter 8

**Hypothesis:** Providing a high quality, comprehensive, professionally ratified universally accessible online resource to primary care clinicians will improve access to best practice AJ preconception and antenatal screening and reduce disease incidence.

**Aims:** To synthesise findings from evidence based research conducted in the course of this thesis and from the international literature. To design screening protocols and clinical pathways that are easy to implement in various relevant reproductive scenarios. To support primary healthcare clinicians by documenting current referral pathways and by providing links to patient information and support services.

**Implications:** Use of this guideline will aid in improved access to preconception genetic screening, improve reproductive options open to couples and reduce disease burden in our Australian community.

2.3 Research protocols

Research methodology of each arm of my thesis is described in detail within publications incorporated in Chapters 3, 4, 5, 6, and 8.
2.4 Human Research Ethics Committee (HREC) Approvals

Ethics approval for the conduct of research described in this thesis was obtained prospectively from the following HRECs:

1. Hawkesbury HREC of the Northern Sydney Central Coast Area Health Service of the New South Wales Government Department of Health, (Reference 0810229M) 2008

2. Royal Children’s Hospital HREC (Reference 32016A) 2011

3. Northern Sydney Local Health District HREC (Reference 1201 – 034M) 2012

All HREC approvals relating to all elements of research conducted in the course of this PhD assessed work conducted to be of negligible/low risk.
Chapter 3:

Chapter 3 reviews current Australian practice in screening for TSD.

This paper has been published as:


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Tay-Sachs disease: current perspectives from Australia

Abstract: Tay-Sachs disease (TSD) is a fatal, recessively inherited neurodegenerative condition of infancy and early childhood. Although rare in most other populations, the carrier frequency is one in 25 in Ashkenazi Jews. Australian high-school-based TSD preconception genetic screening programs aim to screen, educate, and optimize reproductive choice for participants. These programs have demonstrated high uptake, low psychological morbidity, and have been shown to result in fewer than expected Jewish TSD-affected births over 18 years of operation. The majority of Jewish individuals of reproductive age outside of the high school screening program setting in Australia have not accessed screening. Recent recommendations advocate supplementing the community high school screening programs with general practitioner- and obstetrician-led genetic screening of Ashkenazi Jewish individuals for TSD and other severe recessive diseases for which this group is at risk. Massively parallel DNA sequencing is expected to become the testing modality of choice over the coming years.

Keywords: Tay-Sachs disease, genetic screening, Australia

Tay-Sachs disease

Tay-Sachs disease (TSD), a fatal condition, is a neurodegenerative lysosomal sphingolipid storage disorder. TSD is caused by mutations of HEXA (MIM *606869, gene map locus 15q23-q24). The HEXA gene product was identified in 1969 as the α-subunit of β-hexosaminidase enzyme (HEXA). The normal function of HEXA is to degrade GM2 gangliosides in central nervous system cell lysosomes. In TSD, neuronal accumulation of sphingolipid GM2 gangliosides results in progressive loss of central nervous system function. Most infants with TSD appear healthy at birth. After a period of normal development, affected individuals experience slow neurological decline and death in infancy (infantile TSD) or early childhood (intermediate TSD). A still milder form of TSD exists where individuals survive into adulthood. No cure or effective treatment to slow the progression of the disease is known. TSD has autosomal recessive inheritance, with TSD carriers being unaffected. In the case of carrier couples, 25% of pregnancies will be affected by TSD. TSD is a rare disease, with an incidence of one in 320,000 births in general populations (carrier frequency one in 250). The specific populations at a higher risk of TSD are: Ashkenazi Jewish (AJ), French Canadian, Irish, Pennsylvania Dutch, and Cajun communities. All populations exhibit specific HEXA founder mutations at high allelic frequencies.

TSD screening initiatives

TSD incidence in unscreened Jewish populations is one in 3,900 births. The first TSD carrier screening program was introduced in the USA in 1971, representing a precedent...
for population genetic screening for inherited diseases. By the year 2000, over 51,000 TSD carriers, including more than 1,400 at-risk couples, had been identified via TSD screening programs around the world. Internationally, screening has reduced the incidence of Ashkenazi Jews with TSD-affected children by more than 90%.

**TSD and the Australian AJ population**

The Australian Jewish community mainly reside in Melbourne and Sydney and was estimated to number 97,300 in the 2011 Australian Bureau of Statistics Census of Population and Housing. The majority of Australian Jews have AJ heritage. TSD carrier frequency is approximately one in 25 in Australian individuals of AJ descent.

**Australian TSD genetic screening programs**

Over 70% of Jewish adolescents in Melbourne and 50% in Sydney attend Jewish high schools that access TSD screening programs (Eckstein, unpublished data, 2006). Although the at-risk population was primarily residing in Melbourne and Sydney, the testing laboratories were located remotely in different states of Australia: at the Chemical Pathology Department, Royal Brisbane Hospital (now part of Pathology Queensland) and at the Chemical Pathology Department, Adelaide Women’s and Children’s Hospital (now part of SA Pathology). All cases of TSD diagnosed in Australia prior to the commencement of TSD carrier screening in the Australian AJ community underwent confirmatory biochemical and/or genetic testing at one or more of these laboratory sites. Prior to 1993, TSD testing and family cascade carrier screening in Australia was available through medical consultation only at these two laboratories. In 1993, a 2-year pilot study commenced in Sydney targeting 15–17-year-old students attending Jewish high schools. Originally based at Westmead Hospital, the community and administrative headquarters of the program were relocated to the Wolper Jewish Hospital (situated geographically close to the heart of Sydney’s Jewish community), and the laboratory service subsequently moved to the Laboratory and Community Genetics Department, Pacific Laboratory Medicine Services [PaLMS, NSW Health Pathology North], Royal North Shore Hospital [RNSH], Sydney) began as the first Australian TSD screening program in 1995, targeting 15–17-year-old students attending Jewish high schools. Originally based at Westmead Hospital, the community and administrative headquarters of the program were relocated to the Wolper Jewish Hospital (situated geographically close to the heart of Sydney’s Jewish community), and the laboratory service subsequently moved to the Laboratory and Community Genetics Department, Pacific Laboratory Medicine Services [PaLMS, NSW Health Pathology North]. A further possible relocation, to be similarly geographically closer to the Jewish community, is currently under discussion. Testing is free of charge to participating students, with costs being funded partially by philanthropic support from the Jewish community, and the balance from NSW Health. Parental written consent for participation in screening is required for students aged less than 16 years. Young adult outreach preconception genetic screening for TSD, and now also other recessive diseases common in the AJ community (cystic fibrosis, mucolipidosis type IV, Fanconi anemia, familial dysautonomia, Canavan disease, Bloom syndrome), is offered annually. As part of the high school program, an initial compulsory education session is facilitated for all students offered testing several days prior to voluntary sample collection, currently led by a qualified genetic counselor.

**Elements of successful international TSD screening programs were adapted for an Australian community**

Educational campaigns focusing on the medical community, Jewish community, and religious leaders, and training of volunteers were undertaken to ensure dialogue occurred and the eventual model of TSD screening designed was adapted to community needs, expectations, and resources. Australian TSD screening programs adhere to international ethical standards with regard to individual privacy and patient autonomy. Genetic screening within programs is fully informed and voluntary. Genetic screening programs targeting senior high school students have been associated with high uptake, both internationally and in Australia.

**TSD screening in Sydney**

Following a 2-year pilot study (1993–1994), the Australasian Community Genetics Program (Laboratory and Community Genetics Department, Pacific Laboratory Medicine Services [PaLMS, NSW Health Pathology North], Royal North Shore Hospital [RNSH], Sydney) began as the first Australian TSD screening program in 1995, targeting 15–17-year-old students attending Jewish high schools. Originally based at Westmead Hospital, the community and administrative headquarters of the program were relocated to the Wolper Jewish Hospital (situated geographically close to the heart of Sydney’s Jewish community), and the laboratory service subsequently moved to the Laboratory and Community Genetics Department, Pacific Laboratory Medicine Services [PaLMS, NSW Health Pathology North]. A further possible relocation, to be similarly geographically closer to the Jewish community, is currently under discussion. Testing is free of charge to participating students, with costs being funded partially by philanthropic support from the Jewish community, and the balance from NSW Health. Parental written consent for participation in screening is required for students aged less than 16 years. Young adult outreach preconception genetic screening for TSD, and now also other recessive diseases common in the AJ community (cystic fibrosis, mucolipidosis type IV, Fanconi anemia, familial dysautonomia, Canavan disease, Bloom syndrome), is offered annually. As part of the high school program, an initial compulsory education session is facilitated for all students offered testing several days prior to voluntary sample collection, currently led by a qualified genetic counselor.
genetic counselor. Students’ retention of knowledge following participation in the pre-test education session has been found to be high.\textsuperscript{13} Uptake of voluntary testing is 94%. Students who decline testing are provided with written information regarding access to testing in the future. Three options (A, B, and D) are offered to students regarding access to their test results. Scheme A is analogous to the Dor Yeshorim model,\textsuperscript{19} where carrier status is withheld by the testing laboratory. Results may, at a nominated later date, be interpreted in the context of a partner’s screening result and reported as a risk or possibility of TSD-affected pregnancy. In scheme B, results of the screening are made available to students immediately with genetic counseling. In Scheme D, students nominate to have a DNA sample taken and analyzed but reporting is deferred until a later time of the student’s choosing.\textsuperscript{20} The vast majority of students currently choose scheme B (direct disclosure). Students who enter in Scheme D can, at a later date, nominate to change to Scheme A or B.

The emotional impact of testing has been shown to be acceptable to students, generating low levels of concern in adolescents found to be carriers.\textsuperscript{13} Specimen collection was initially by venepuncture (1995–2004) and subsequently by mouthwash sampling (2004–2014). Testing was initially by biochemical methods with DNA testing of confirmed carriers (1995–2004). Today DNA-only initial testing is standard, with confirmation of carrier status by a second DNA method (1995–2014).\textsuperscript{21} Genetic counseling is routinely offered to all students found to be genetic carriers, and is also available on request to all students.

**TSD screening in Melbourne**

In 1998, a TSD carrier screening program, based on the Sydney model and conducted by Genetic Health Services Victoria until 2009, and subsequently by Austin Health, was established in Jewish high schools in Melbourne.\textsuperscript{22} Testing incurred a cost of $40 AUD per student tested in 1998, but was fully funded thereafter by Melbourne Jewish Community philanthropic organizations. The program is voluntary and is available to students aged 15–18 years. Initially, screening was for TSD only but has since expanded to include a panel of recessive conditions at increased frequency in Jewish populations. Pre-test education is provided, currently led by a qualified genetic counselor. Uptake increased from 67% (1998–2001) to 96% (2003) following the change of sample collection method from venepuncture to cheek-brush sampling.\textsuperscript{23} Informed consent is obtained by a screening program team member in a one-to-one interview prior to students providing a DNA sample.\textsuperscript{18} Students found to be carriers are contacted by phone by a genetic counselor and offered an outpatient genetic counseling appointment. Additionally, carriers receive a copy of their result and an explanatory letter by mail to facilitate cascade screening of family members. A copy of their result and an explanatory letter is mailed to non-carrier students.\textsuperscript{18,23}

A recent study was undertaken to assess the long-term (5–11-year) outcomes of screening within the Melbourne program.\textsuperscript{24} Validated questionnaires were sent to all carriers and two non-carriers per carrier screened between 1999 and 2005. The questionnaire was completed by 34.8% of carriers and 21.7% of non-carriers. The percentage of participants who retained good knowledge of TSD with no significant difference in knowledge between carriers and non-carriers was 82%, and 83% of respondents were happy with the timing and high school setting of the screening and thought that this approach should continue. There was no significant difference in negative psychological consequences between carriers and non-carriers as assessed by validated anxiety and decision regret scales. Screened individuals were supportive of the program.

**The Dor Yeshorim screening program – an alternative approach in ultraorthodox Jewish communities**

The Dor Yeshorim screening program was established in 1983 to provide access to TSD screening to the ultra-orthodox Jewish community in the USA in a format culturally acceptable to this demographic. A branch of this program has been operating in Israel since 1986.\textsuperscript{19} The program has since been expanded to test for other significant recessive conditions at higher than background frequencies in Jewish populations, and can be accessed by request by ultra-orthodox AJ individuals around the world, including Australians. This program differs from mainstream genetic screening programs in several ways. Individual genetic results are not disclosed; instead, the point of interest is a potential couple’s genetic compatibility. Non-compatible carrier couples not introduced as a suitable match. This mode of screening is designed to prevent disease and to avoid the stigma that may be associated with the knowledge of individual carrier status within relatively small ultra-orthodox communities.
Pros and cons of TSD genetic screening in an adolescent population

The ideal setting in which to screen for TSD is an informed adult couple planning a future pregnancy. Unfortunately, this demographic often do not access preconception testing for many reasons, including low knowledge of carrier risk, low knowledge of screening options, low motivation, cost of testing, or unplanned pregnancy. A factor that has most successfully increased uptake of preconception genetic screening for TSD internationally has been the implementation of screening programs that target an adolescent population. Screening of senior high school students, as occurs in Australian AJ TSD genetic screening programs, captures a large proportion of the target population in an environment where both pre-test education and testing can be facilitated with relative ease. Senior high school students are mature minors, and are generally able to understand the implications of testing and provide informed consent. Students educated prior to testing and then screened for TSD in a high school setting have been found to retain more information at the time of testing and 3–6 years following testing than adults screened in other settings. In order to be optimally relevant, TSD carrier screening must occur in a window before conception, with all reproductive options accessible, allowing couples to make informed choices in planning their family. Carrier testing in an adolescent population addresses the issue of TSD screening prior to pregnancy planning, thus allowing future informed access to a full range of reproductive options for at-risk families.

TSD screening and diagnosis: assays and strategies

HEXA enzyme assay

In 1971 an assay for HEXA protein was developed, allowing the possibility of TSD antenatal testing and carrier screening. HEXA enzyme testing remains the accepted gold standard for TSD screening and diagnosis. HEXA testing has 98% sensitivity, and can be used in pan-ethnic populations and in the presence of rare and/or founder mutations. TSD carriers have enzyme activity <52% compared to non-carriers where enzyme activity is >60%. However, HEXA enzyme testing has several pitfalls. Testing requires venepuncture, which can lead to lower acceptance and participation rates in community screening. Technical limitations in overlapping reference intervals mean that there is an inconclusive range of enzyme activity (52% to 60%) in which it is difficult to distinguish TSD carriers from non-carriers. Rarely, false negative and false positive results can occur (eg, B1 allele and pseudodeficiency alleles, respectively). Medications including oral contraceptives may affect detected enzyme levels.

Traditional HEXA DNA testing

DNA molecular testing for a panel of known mutations is highly sensitive and specific when applied to an appropriately selected population.

In AJ populations, several mutant HEXA alleles have been demonstrated. Ninety-six percent of Jewish TSD carriers have one of three common mutations: c.1278insTATC, the most common mutation in AJ populations, is a frame-shift mutation due to a four-base-pair insertion in exon 11, introducing a premature stop codon and causing protein truncation; c.1421+1G>C, the second most common mutation in AJ populations, is a G→C transversion in the donor splice site of intron 12, and p.Gly269Ser is a rarer missense mutation.

In AJ populations it has been shown that DNA mutation testing is the most accurate and cost-effective assay for TSD population genetic screening. DNA testing of mouthwash/cheek brush samples improves participation rates compared with blood sampling required for biochemical testing. Successful DNA testing of hair root specimens offers a sample amenable to extreme ease of transport, and potential use for outreach screening.

Massively parallel DNA sequencing

Massively parallel DNA sequencing (MPS) has been shown to be superior to HEXA enzyme-based screening and traditional DNA genotyping methodologies in research settings. MPS-based genetic screening can be applied to TSD, but also has the potential to simultaneously offer screening for high numbers of targeted genetic conditions in non-selected populations. The costs of MPS technology are reducing, but remain significant. Another limiting factor in the application of MPS-based genetic screening strategies is the unresolved issue of the interpretation and reporting of incidental findings and variants of unknown significance. The Sydney AJ Community Genetic Screening program is currently conducting a pilot study using an expanded MPS panel for up to 26 recessive conditions as part of AJ preconception genetic screening (L. Burnett and A Proos, unpublished data, 2014). Ioannou et al found that increasing the number of conditions included in the Melbourne AJ Community Genetic Screening program from TSD alone to seven conditions...
resulted in a decrease in knowledge and increase in predicted negative feelings if found to be a carrier of one or more of the conditions. Other studies are underway assessing the psychological impact of an expanded screening menu incorporating seven recessive conditions. Further research will be needed to ensure that MPS screening is conducted in a way that maintains ethical principles of autonomy, beneficence, non-maleficence, and justice. Information derived from MPS sequencing may have additional longitudinal benefits for patients as data collected may be re-interrogated in the future.

Cost of testing
The costs of enzyme-, DNA-, and MPS-based assays are all similar in the range of $100 to $500 AUD (L Burnett and A Proos, unpublished data, 2014).

Following TSD enzyme testing (serum enzyme activity ± leukocyte enzyme activity), DNA testing may also be required to address some of the technical limitations described above.

In addition to reagent costs, MPS sequencing will have supplementary costs for bioinformatics interpretation and reporting, which are yet to be formally assessed.

Models of screening
Screening models to identify carrier couples include one-step models where both partners undertake screening immediately and two-step models where partners are screened sequentially. Under most circumstances, two-step carrier screening is the most cost-effective option for at-risk couples. In the two-step screening model, the second partner is tested only where the first partner was confirmed by screening to be a TSD carrier. One-step carrier screening is appropriate in settings where the results of screening are urgently required (e.g., during a pregnancy). The Dor-Yeshorim model also utilizes one-step carrier screening.

Reproductive options for carrier couples
Options to avoid TSD-affected pregnancies in a couple at risk include using assisted reproductive technologies, including in vitro fertilisation/intracytoplasmic sperm injection with pre-implantation genetic diagnosis or use of a donor gamete to achieve healthy embryos. In spontaneous pregnancies, antenatal diagnosis can be availed with the possibility of selective termination of TSD-affected pregnancies. Invasive methods of fetal diagnostic testing include chorion villus sampling and amniocentesis. Both these methods carry a test-related risk of miscarriage (0.5% to 1%) and are offered from the late first trimester of pregnancy onward. Detection of free fetal DNA in a maternal blood sample offers a promising non-invasive option for future fetal diagnosis that could potentially be offered from the very early first trimester of pregnancy onward. Other options for couples at risk include the use of donor gametes, adoption, making a decision to remain childless, leaving the pregnancy outcome to fate, or (as with the Dor Yeshorim program) avoiding the issue by having chosen a partner who was already known not to be a potential “at-risk” couple partnership.

Success of Australian TSD screening programs
Measures of the success of a genetic screening program include uptake within the target population, percentage of the target population offered access to screening, the level of informed consent among individuals screened, the reduction of target disease incidence over time through the uptake of assisted reproductive technologies, and selective pregnancy termination and economic outcomes. In world Jewish communities, TSD carrier screening programs have reduced the births of infants with TSD by >90%. Prior to 1970, 85% of TSD affected infants were born to Jewish parents. TSD frequency in non-Jewish populations has, during the same time-frame, remained stagnant. Currently the majority of TSD affected infants are born to non-Jewish parents. Studies of the allele frequencies of several genes in the Australian Jewish population in Sydney including HEXA, BRCA1, BRCA2, and APC indicate the Australian Jewish population is not statistically different from Jewish populations in Israel, USA, and Canada and is therefore comparable to international Jewish populations with regard to the burden of genetic diseases. The impact of wide reaching TSD screening programs targeting Jewish communities in Australia can, in the long-term, be expected to have similar implications for reducing TSD incidence as international programs have demonstrated. In 2011, a comprehensive and consecutive audit of all TSD cases diagnosed in Australia from 1995 onward was conducted. Fewer than expected numbers of Jewish TSD cases were noted within the audit, corresponding to the period where Jewish community TSD preconception genetic screening programs were operating in Sydney and Melbourne. The majority of TSD cases were found to have occurred in non-Jewish Australian families. No AJ TSD preconception genetic screening program participant over the past 18 years has gone on to have a TSD-affected child. Two cases of TSD have occurred in...
Australia since 1995 affecting two different AJ families. None of the four AJ parents of the two AJ TSD-affected infants had previously undergone TSD genetic screening.

A major pitfall in Australian AJ genetic screening is low participation rates outside of adolescent-focused high school screening programs, with fewer than 50% of Australian Jewish individuals of reproductive age having accessed these programs (Eckstein, unpublished data, 2006). This residual target population relies on personal request or health care clinician referral for TSD screening. Barriers to clinician referral include costs, time constraints, and availability of supporting services, as well as patient and clinician education. In Israel, clinicians are instructed to offer access to genetic screening for TSD to all AJ women of reproductive age, ideally prior to pregnancy, but also during pregnancy. The Israeli system is an example of how clinicians can effectively facilitate preconception genetic screening strategies to prevent the targeted condition/s. The Human Genetics Society of Australasia is currently formulating guidelines to assist Australian healthcare providers in implementing this practice (I Stechiowski, unpublished data, 2014).

As MPS-facilitated genetic screening technology evolves and costs reduce over time, it is highly likely that genetic screening for a large range of serious rare genetic diseases will become routine in antenatal care in pan-ethnic populations. As has been demonstrated with TSD in AJ communities that have been intensively studied, genetic screening can be expected to have a net health benefit to families and communities. Further research is needed to understand how expanded genetic screening for a greater number of conditions will impact screened individuals psycho-socially to optimize the potential net health benefit of screening.

Disclosure
The authors report no conflicts of interest in this work.

References


Chapter 4:  

Chapter 4 reports allele specific TSD carrier frequencies from longitudinal Australian TSD preconception screening program data (1995 to 2007) and correlates self-reporting of Ashkenazi Jewish heritage with TSD carrier risk within the screened cohort.

This paper has been published as:


Tay-Sachs disease preconception screening in Australia: self-knowledge of being an Ashkenazi Jew predicts carrier state better than does ancestral origin, although there is an increased risk for c.1421+1G>C mutation in individuals with South African heritage

Raelia Lew · Leslie Burnett · Anné Proos

Abstract The Australasian Community Genetics Program provided a preconception screening for Tay-Sachs disease (TSD) to 4,105 Jewish high school students in Sydney and Melbourne over the 12-year period 1995–2007. By correlating the frequencies of mutant HEXA, MIM *606869 (gene map locus 15q23-q24) alleles with subjects’ nominated ethnicity (Ashkenazi/Sephardi/Mixed) and grandparental birthplaces, we established that Ashkenazi ethnicity is a better predictor of TSD carrier status than grandparental ancestral origins. Screening self-identified Ashkenazi subjects detected 95% of TSD carriers (carrier frequency 1:25). Having mixed Ashkenazi and non-Ashkenazi heritage reduced the carrier frequency (1:97). South African heritage conveyed a fourfold risk of c.1421+1G>C mutation compared with other AJ subjects (odds ratio (OR), 4.19; 95% confidence interval (CI), 1.83–9.62, \( p = 0.001 \)). However, heritage from specific European countries investigated did not significantly alter the overall odds of TSD carrier status.

Keywords Tay-Sachs disease · Australia · South Africa · Jewish · Screening

Abbreviations

AJ Ashkenazi Jewish

TSD Tay-Sachs Disease

Introduction

Tay-Sachs disease (TSD) is a fatal neurodegenerative lysosomal sphingolipid storage disorder caused by mutations of HEXA MIM *606869 (gene map locus 15q23-q24). The HEXA gene product is the \( \alpha \)-subunit of \( \beta \)-hexosaminidase, a dimeric enzyme involved in the lysosomal degradation of GM2 gangliosides. TSD carrier frequency is approximately 1:25 in individuals of Ashkenazi Jewish (AJ) descent (Kolodny 2009). TSD incidence in Jewish people is one in 3,900 births, compared to one in 320,000 births in the general population (Triggs-Raine et al. 2001). Several mutant HEXA alleles have been demonstrated in the AJ population (Arpaia et al. 1988).
The Australian Jewish community (estimated population 104,000) mainly reside in Melbourne and Sydney (Rubinstein 1995). The majority of Australian Jews have Ashkenazi heritage (Rutland 2005).

The Australasian Community Genetics Program (Laboratory and Community Genetics Department, Pacific Laboratory Medicine Services [PaLMS, Pathology North], Royal North Shore Hospital, Sydney) facilitates senior high school student and young adult outreach preconception genetic screening for TSD and other recessive diseases common in the AJ community (Gaucher disease, cystic fibrosis, mucolipidosis type IV, Fanconi anemia, familial dysautonomia, Canavan disease, Bloom syndrome) (Barlow-Stewart et al. 2003). Over 50% of Jewish adolescents in Melbourne and Sydney attend Jewish high schools that access TSD screening programs (Australian Bureau of Statistics 2006).

From 1995 to 2007, we conducted in Sydney TSD genetic screening tests for 4,105 Jewish high school students from Sydney and Melbourne. The Melbourne subgroup have previously been reported in part (Gason et al. 2005), and the design of the Sydney program has also been described (Barlow-Stewart et al. 2003). These screening programs are based on established best-practice principles, and the program design draws on the experience of overseas TSD screening programs (Ekstein and Katzenstein 2001; Bach et al. 2007; Lowden and Davidson 1977; Kaback et al. 1977). However, over time, individuals screened are becoming drawn from a population that is demographically different from any preceding generation. Social change such as intermarriage, both within the Jewish community (Ashkenazi/Sephardi) and within the general Australian community (Jewish/non-Jewish) may be changing their risk profiles. It is therefore possible that strategies of offering screening to the entire Jewish community through high school and adult preconception access points may become less effective over time due to lack of identification of subjects as being at risk for TSD carrier status.

Our study has reviewed key demographic and genealogical parameters of subjects and correlated them with the encountered TSD carrier frequencies of different mutant HEXA alleles in the modern Australian AJ community, with respect to self-recognition of being AJ, and with grandparental country of origin. We separately compared findings from Australia’s two largest cities, Sydney and Melbourne, to maximize the likelihood of detecting local community trends or differences. From these studies, we sought to clarify the risk profiles of the current generation of Jewish youth choosing preconception TSD carrier screening in the context of planning screening strategies for the future.

Materials and methods

The study was approved by the Hawkesbury Human Research Ethics Committee of the Northern Sydney Central Coast Area Health Service of the New South Wales Government Department of Health.

Between 1995 and 2007, TSD carrier screening was offered through a community-funded program to 16–18-year-old students attending Jewish high schools in both Sydney and Melbourne. Participation was voluntary and informed through pretest genetic counseling. Participants provided written consent for TSD genetic testing, with optional additional consent for involvement in further research and development. In Sydney, all students also answered a demographic questionnaire at the time of testing. Data collected included nominated ethnicity (Ashkenazi/Sephardi/mixed Jewish/non-Jewish), the country of birth of the participants, of their two parents, and their four grandparents. In Melbourne, this questionnaire was answered only by participants screened between 1997 and 2002. Data from all subjects who answered the questionnaire have been included in the study. Further follow-up contact with individual subjects was limited to only those subjects who had consented to involvement in research and development.

TSD carrier screening was performed using DNA extracted either from venous blood samples (1995–2004) or from buccal cell wash sample (2005–2007). Results of testing were entered into a secure database system and de-identified prior to this study. Our laboratory methods were as described previously (Warren et al. 2005); note that our laboratory protocol requires HEXA mutations identified by enzyme analyses to be confirmed by HEXA DNA analysis before inclusion in our database. HEXA enzyme testing was replaced by HEXA DNA-only testing from 2005 onwards. DNA-only-based testing is designed to detect the three common HEXA mutations in the AJ population (Table 1). DNA-only testing has been shown to be a highly sensitive and cost-effective method at detecting heterozygotes in an orthodox AJ cohort of 38,197 individuals in Israel (Bach et al. 2001), with equivalent specificity compared to enzyme-based testing. In Bach’s cohort, enzyme testing had variable sensitivity (93.1–99.1%) and specificity (88.1–98.8%) and, amongst 151 obligate carriers tested, no low-prevalence mutations were found. Enzyme testing in theory affords the possibility of detecting low-prevalence HEXA DNA mutations (Triggs-Raine et al. 1990). In contrast to the findings of Bach et al., we identified in our cohort two low-prevalence HEXA DNA mutations by enzyme testing and confirmed by DNA sequencing. However, due to the low prevalence of these mutations, our group agrees with the finding of Bach et al.
that DNA-only testing is currently the most cost-effective method in a heterozygote AJ population.

Published analysis of this screening program found it to be effective, with high uptake, low negative perceptions, and high knowledge levels amongst participants. (Ioannou et al. 2010a, b)

Demographic classification as to whether the subjects considered themselves to be AJ, Sephardi, or mixed was by self-declaration from the questionnaire responses provided. Grandparents’ country of birth was also obtained from the subjects’ questionnaires. Responses were stratified into regions (Table 2), based on political geographic boundaries and language to reflect Jewish community life in Europe prior to World War II (WWII), with additional reference to the Australian Bureau of Statistics Standard Australian Classification of Countries (second edition) (Harper 2008).

The resultant geographic groupings we used were comparable to those used in past published papers investigating the frequencies of TSD carriers amongst AJ and non-AJ groups during the premolecular and molecular era (Risch et al. 2003; Peleg et al. 1994; Myrianthopoulos and Melnick 1977).

We also regrouped the data on grandparents’ birthplaces so that individuals were categorized into only two groups: having no grandparent or else having at least one grandparent from any specific region/country. Using this classification schema, note that individuals could belong to more than one ancestral group.

All statistical analyses were conducted using the SPSS v15.0 (IBM SPSS, Inc., Somers, NY, www.spss.com). P values less than 0.05 were considered statistically significant.

Logistic regression analyses were performed to determine if country groups or individual countries were significant predictors of carrier status. In the series of logistic regression analyses, the dependent variables were either c.1278insTATC mutation status (carrier/noncarrier) or c.1421+1G>C mutation status (carrier/noncarrier). The small sample size and number of cases of p.Gly269Ser

### Table 1 Common HEXA mutations and their frequencies in Jewish populations previously studied (National Centre for Biotechnology Information gene and protein sequence reference NM_000520.4: NP_000511.2)

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>c.1278insTATC</td>
<td>4-base pair insertion in exon 11 → frame shift → stop codon (Myerowitz and Costigan 1988)</td>
<td>14 (70%)</td>
<td>125 (82%)</td>
<td>108 (73%)</td>
<td>114 (73%)</td>
<td>361/476 (76%)</td>
</tr>
<tr>
<td>c.1421+1G&gt;C</td>
<td>G→C transversion in the donor splice site of intron 12 (Arpaia et al. 1988; Ohno and Suzuki 1988)</td>
<td>–</td>
<td>15 (10%)</td>
<td>26 (18%)</td>
<td>24 (15%)</td>
<td>65/456 (14%)</td>
</tr>
<tr>
<td>p.Gly269Ser</td>
<td>Missense mutation (Ohno et al. 1988)</td>
<td>–</td>
<td>6 (4%)</td>
<td>5 (3%)</td>
<td>6 (4%)</td>
<td>17/456 (4%)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>–</td>
<td>6 (4%)</td>
<td>9 (6%)</td>
<td>12 (8%)</td>
<td>27/456 (6%)</td>
</tr>
</tbody>
</table>

### Table 2 Geographic country groupings (constructed to study differential HEXA allele carrier frequencies by region of grandparents’ birthplace)

<table>
<thead>
<tr>
<th>Country groupings</th>
<th>Member countries (for purposes of this table)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Europe</td>
<td>Austria, Belgium, Denmark, Finland, France, Germany, Holland/Netherlands, Italy, Norway, Portugal, Spain, Sweden, Switzerland</td>
</tr>
<tr>
<td>North Eastern Europe</td>
<td>Belarus, Estonia, Latvia, Lithuania, Moldova, Poland, Russia, Ukraine, Siberia</td>
</tr>
<tr>
<td>South Eastern Europe</td>
<td>Bulgaria, Croatia, Czechoslovakia, Hungary, Romania, Serbia, Slovakia, Yugoslavia</td>
</tr>
<tr>
<td>UK</td>
<td>England, Ireland, Scotland, Wales, UK (unspecified)</td>
</tr>
<tr>
<td>Southern Africa</td>
<td>Mozambique, Namibia, South Africa, Zambia, Zimbabwe</td>
</tr>
<tr>
<td>North Africa and the Middle East</td>
<td>Egypt, Iran, Iraq, Lebanon, Libya, Morocco, Syria, Tunisia, Turkey, Yemen, Middle East (unspecified)</td>
</tr>
<tr>
<td>Australia/New Zealand</td>
<td>Australia, New Zealand</td>
</tr>
<tr>
<td>Israel/Palestine</td>
<td>Israel, Palestine</td>
</tr>
<tr>
<td>Other countries</td>
<td>Afghanistan, Argentina, Armenia, Azerbaijan, Barbados, Bolivia, Brazil, Burma, Canada, Chile, China, Cyprus, Fiji, Greece, India, Indonesia, Japan, Kazakhstan, Malaysia, Mexico, Mongolia, Pakistan, Peru, Philippines, Singapore, Sri Lanka, Trinidad and Tobago, USA, Uzbekistan, West Indies</td>
</tr>
<tr>
<td>Unknown</td>
<td>Unknown, entry blank</td>
</tr>
</tbody>
</table>
mutations prevented a similar analysis for p.Gly269Ser mutation status. Either a country group or else an individual country was entered as a covariate using a forced method of entry. The logistic regression analyses generated odds ratios with 95% confidence intervals, and absence of a grandparent from the major country group or individual country was considered the reference category. Data from the Sydney and Melbourne groups were initially analyzed separately and, where no statistically significant difference of results was found, results were then pooled and reanalyzed.

Results

Nominated ethnicity amongst AJ subjects studied is expressed in Table 3. Table 4 describes the proportion of subjects studied with at least one grandparent from each predefined geographical region (Table 2) and from the nine most common countries of origin. Table 5 describes the relative proportions of common and de novo HEXA mutations found in our study population. The two most prevalent mutations seen, c.1278insTATC and c.1421+1G>C, were further classified into ancestral regional groupings (Table 6).

AJ ethnicity is a good predictor of being a TSD mutation carrier ($X^2=69.07$, $df=1$, $p<0.001$). Students with European ancestry were more likely to be TSD carriers ($X^2=2247.24$, $df=9$, $p<0.001$); however, further analysis by individual European country of origin did not increase the predictive power. Melbourne had a significantly higher proportion of AJ subjects, compared with Sydney ($X^2=10.48$, $df=1$, $p=0.001$) (Table 3), and the two cities also had differences in the relative proportions of subjects whose ancestors were from different European geographic origins (Table 4). South African ancestry conveyed a fourfold increased likelihood of carrying the mutation c.1421+1G>C (OR, 4.19 (95% CI, 1.83–9.62), $p=0.001$) compared with other AJ subjects. Odds generated from logistic regression analysis comparing c.1278insTATC and c.1421+1G>C carrier status and grandparents’ birthplace for AJ subjects are summarized in Table 7.

Discussion

The most likely explanation for the origin of multiple HEXA mutations in AJ populations is that they arose around 1100 AD by founder effect and genetic drift (Slatkin 2004; Durst et al. 2001; Risch et al. 1995; Goldstein et al. 1999; Niell et al. 2003; Frisch et al. 2004). Four independent sphingolipid storage diseases have arisen in the AJ population (TSD, Niemann-Pick disease, Gaucher disease, and mucolipidosis type IV) leading some investigators to hypothesize a heterozygote advantage (Zlotogora et al. 1988; Motulsky 1995; Myrianthopoulos and Melnick 1977).

The geographic ancestral origins of the Jewish population screened for TSD carrier status in our study were

<table>
<thead>
<tr>
<th>Region</th>
<th>Sydney, $N=2,846% (n)$</th>
<th>Melbourne, $N=1,259% (n)$</th>
<th>Country</th>
<th>Sydney, $N=2,846% (n)$</th>
<th>Melbourne, $N=1,259% (n)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe (unspecified)</td>
<td>68.6 (1,953)</td>
<td>87.2 (1,098)</td>
<td>Ukraine</td>
<td>3.8 (107)</td>
<td>4.7 (59)</td>
</tr>
<tr>
<td>Western Europe</td>
<td>19.5 (554)</td>
<td>19.8 (249)</td>
<td>Lithuania</td>
<td>9.7 (276)</td>
<td>4.4 (56)</td>
</tr>
<tr>
<td>North Eastern Europe</td>
<td>47.0 (1,337)</td>
<td>71.2 (897)</td>
<td>Czechoslovakia</td>
<td>8.0 (227)</td>
<td>10.0 (126)</td>
</tr>
<tr>
<td>South Eastern Europe</td>
<td>21.8 (620)</td>
<td>22.0 (277)</td>
<td>Germany</td>
<td>11.7 (332)</td>
<td>12.6 (159)</td>
</tr>
<tr>
<td>South Africa</td>
<td>32.2 (916)</td>
<td>11.0 (138)</td>
<td>England</td>
<td>16.3 (464)</td>
<td>9.8 (124)</td>
</tr>
<tr>
<td>UK</td>
<td>18.3 (522)</td>
<td>11.0 (139)</td>
<td>Hungary</td>
<td>11.8 (337)</td>
<td>10.1 (127)</td>
</tr>
<tr>
<td>North Africa+Middle East</td>
<td>7.8 (221)</td>
<td>5.6 (71)</td>
<td>Russia</td>
<td>12.9 (366)</td>
<td>17.2 (217)</td>
</tr>
<tr>
<td>Australia/New Zealand</td>
<td>22.7 (646)</td>
<td>23.6 (297)</td>
<td>Poland</td>
<td>26.2 (747)</td>
<td>54.6 (687)</td>
</tr>
<tr>
<td>Israel/Palestine</td>
<td>4.7 (133)</td>
<td>6.8 (86)</td>
<td>South Africa</td>
<td>32.0 (910)</td>
<td>11.0 (138)</td>
</tr>
<tr>
<td>Other</td>
<td>9.8 (279)</td>
<td>7.0 (88)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
extremely diverse. When grandparents’ country of birth was examined, 86 countries and five continents were represented. North American demographic studies of AJ TSD carrier frequency have identified varying carrier frequencies between AJ communities founded by immigrants from different regions of Europe (Toronto, 1:14; Baltimore, 1:22; and Washington D.C., 1:28; Average USA, 1:30) (Lowden and Davidson 1977). However, these studies were based on results of enzyme-based carrier testing and therefore made no distinction between individual AJ HEXA allele frequencies among the subpopulations studied.

Although a small AJ community has existed in Australia since the time of European colonization of Sydney in 1788, the majority of the Australian AJ population was founded by immigration subsequent to WWII. Similar to American AJ immigration patterns, focussed communities of immigrants with shared recent language and heritage settled separately in both Melbourne and Sydney, resulting in slightly different subpopulation profiles. Significantly, more subjects from Melbourne identified as AJ than from Sydney (81.3% vs. 76.8%), correlating to reports of more grandparents born in Russia (17.2% vs. 12.9%) and Poland.

Table 5  Australian AJ mutation profile; HEXA mutations identified in our study population

<table>
<thead>
<tr>
<th></th>
<th>Sydney</th>
<th>Melbourne</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>2,186</td>
<td>1,024</td>
<td>3,210</td>
</tr>
<tr>
<td>Total TSD carriers</td>
<td>95</td>
<td>42</td>
<td>137</td>
</tr>
<tr>
<td>Noncarriers</td>
<td>2,091</td>
<td>982</td>
<td>3,073</td>
</tr>
</tbody>
</table>

Mutation-specific carrier absolute number/frequencies:

<table>
<thead>
<tr>
<th></th>
<th>Sydney</th>
<th>Melbourne</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total TSD carriers</td>
<td>95 (43:1,000)</td>
<td>42 (41:1,000)</td>
<td>137 (43:1,000)</td>
</tr>
<tr>
<td>c.1278insTATC</td>
<td>67 (31:1,000)</td>
<td>34 (33:1,000)</td>
<td>101 (32:1,000)</td>
</tr>
<tr>
<td>c.1421+1G&gt;C</td>
<td>20 (9:1,000)</td>
<td>5 (5:1,000)</td>
<td>25 (8:1,000)</td>
</tr>
<tr>
<td>p.Gly269Ser</td>
<td>4 (2:1,000)</td>
<td>2 (2:1,000)</td>
<td>6 (2:1,000)</td>
</tr>
<tr>
<td>p.Arg24Tryp</td>
<td>2 (1:1,000)</td>
<td>0</td>
<td>2 (1:1,000)</td>
</tr>
<tr>
<td>p.Phe304del</td>
<td>0</td>
<td>1 (1:1,000)</td>
<td>1 (&lt;1:1,000)</td>
</tr>
<tr>
<td>Private mutations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p.Arg499Cys</td>
<td>1 (1:2,000)</td>
<td>0</td>
<td>1 (&lt;1:1,000)</td>
</tr>
<tr>
<td>p.His204Pro</td>
<td>1 (1:2,000)</td>
<td>0</td>
<td>1 (&lt;1:1,000)</td>
</tr>
</tbody>
</table>

Table 6  AJ origin of grandparents by region, carrier status, and allele frequency of two common HEXA mutations (N=12,840)

<table>
<thead>
<tr>
<th>Regional groups</th>
<th>Total (N=12,840), n (% of N)</th>
<th>Non-carrier (N1=12,322), n (% of N1)</th>
<th>Carrier (N2=508), n (% of N2)</th>
<th>Absolute carrier frequency ratio (%)</th>
<th>c.1278insTATC carrier frequency ratio (%)</th>
<th>c.1278insTATC carrier frequency ratio (%)</th>
<th>c.1421+1G&gt;C carrier frequency ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe (all)</td>
<td>7,005 (54.5)</td>
<td>6,740 (54.7)</td>
<td>265 (52.1)</td>
<td>1:26 (3.8)</td>
<td>217 (57.8)</td>
<td>1:32 (3.1)</td>
<td>22 (22.9)</td>
</tr>
<tr>
<td>North Eastern Europe</td>
<td>4,310 (33.6)</td>
<td>4,166 (33.8)</td>
<td>144 (28.3)</td>
<td>1:30 (3.3)</td>
<td>113 (30.1)</td>
<td>1:39 (2.6)</td>
<td>20 (20.8)</td>
</tr>
<tr>
<td>Southern Africa</td>
<td>2,672 (20.8)</td>
<td>2,534 (20.5)</td>
<td>138 (27.2)</td>
<td>1:19 (5.2)</td>
<td>75 (19.9)</td>
<td>1:36 (2.8)</td>
<td>55 (57.3)</td>
</tr>
<tr>
<td>South Eastern Europe</td>
<td>1,489 (11.6)</td>
<td>1,428 (11.6)</td>
<td>61 (12.0)</td>
<td>1:24 (4.1)</td>
<td>59 (15.7)</td>
<td>1:25 (4.0)</td>
<td>2 (2.1)</td>
</tr>
<tr>
<td>Western Europe</td>
<td>969 (7.5)</td>
<td>919 (7.5)</td>
<td>50 (9.8)</td>
<td>1:19 (5.2)</td>
<td>38 (10.1)</td>
<td>1:26 (3.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Australia/New Zealand</td>
<td>1,172 (9.1)</td>
<td>1,134 (9.2)</td>
<td>38 (7.5)</td>
<td>1:31 (3.2)</td>
<td>34 (9.0)</td>
<td>1:34 (2.9)</td>
<td>4 (4.2)</td>
</tr>
<tr>
<td>UK</td>
<td>785 (6.1)</td>
<td>762 (6.2)</td>
<td>23 (4.5)</td>
<td>1:34 (2.9)</td>
<td>17 (4.5)</td>
<td>1:46 (2.2)</td>
<td>4 (4.2)</td>
</tr>
<tr>
<td>Uncertain</td>
<td>606 (4.7)</td>
<td>590 (4.8)</td>
<td>16 (3.1)</td>
<td>1:38 (2.6)</td>
<td>9 (2.4)</td>
<td>1:67 (1.5)</td>
<td>7 (7.3)</td>
</tr>
<tr>
<td>Other</td>
<td>350 (2.7)</td>
<td>335 (2.7)</td>
<td>15 (3.0)</td>
<td>1:23 (4.3)</td>
<td>13 (3.5)</td>
<td>1:27 (3.7)</td>
<td>2 (2.1)</td>
</tr>
<tr>
<td>Unspecified Europe</td>
<td>237 (1.8)</td>
<td>227 (1.8)</td>
<td>10 (2.0)</td>
<td>1:24 (4.2)</td>
<td>7 (1.9)</td>
<td>1:34 (3.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>North Africa/ Middle East</td>
<td>53 (0.4)</td>
<td>45 (0.4)</td>
<td>8 (1.6)</td>
<td>1:7 (15.1)</td>
<td>8 (2.1)</td>
<td>1:7 (15.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Israel/Palestine</td>
<td>197 (1.5)</td>
<td>192 (1.6)</td>
<td>5 (1.0)</td>
<td>1:39 (2.5)</td>
<td>3 (0.8)</td>
<td>1:66 (1.5)</td>
<td>2 (2.1)</td>
</tr>
</tbody>
</table>

N.B. The carrier category contains n=376 grandparents of a c.1278insTATC carrier, 96 grandparents of a c.1421+1G>C carrier and 40 grandparents of subjects with other mutations
(54.6% vs. 24.2%). A larger proportion of Sydney compared with Melbourne AJ subjects had grandparents from South Africa (32.0% vs. 11.0%).

Demographic differences within the Sydney and Melbourne communities did not affect overall TSD carrier frequency in our study, which was 3.3% in both cities with no significant difference between the cities (Table 5). AJ TSD carrier frequencies were also comparable (Melbourne, 4.1%; Sydney, 4.3%) and similar to other AJ populations worldwide (Kaback et al. 1977), suggesting participants correctly identified their AJ origins. Self-identification of AJ ethnicity correlated statistically with a higher proportion of grandparents from North Eastern Europe, South Africa, and South Eastern Europe (all) compared with Melbourne AJ subjects who had grandparents from South Africa (32.0% vs. 11.0%).

Table 7 Odds of being a c.1278insTATC/c.1421+1G>C carrier considering grandparents’ birthplace for Ashkenazi subjects: none versus at least one

<table>
<thead>
<tr>
<th>Regional groups</th>
<th>Zero grandparents</th>
<th>≥1 Grandparent</th>
<th>P value</th>
<th>Zero grandparents</th>
<th>≥1 Grandparent</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>c.1278insTATC carrier</td>
<td>Total</td>
<td>c.1278insTATC carrier</td>
<td>c.1278insTATC carrier OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Europe (all)</td>
<td>732</td>
<td>17</td>
<td>14</td>
<td>2,478</td>
<td>77</td>
<td>1.35 (0.79–2.30)</td>
</tr>
<tr>
<td>North Eastern Europe</td>
<td>1,327</td>
<td>40</td>
<td>14</td>
<td>1,883</td>
<td>54</td>
<td>0.95 (0.63–1.44)</td>
</tr>
<tr>
<td>Southern Africa</td>
<td>2,286</td>
<td>70</td>
<td>9</td>
<td>924</td>
<td>24</td>
<td>0.84 (0.53–1.35)</td>
</tr>
<tr>
<td>South Eastern Europe</td>
<td>2,473</td>
<td>62</td>
<td>22</td>
<td>737</td>
<td>32</td>
<td>1.77 (1.14–2.73)</td>
</tr>
<tr>
<td>Western Europe</td>
<td>2,570</td>
<td>67</td>
<td>24</td>
<td>640</td>
<td>27</td>
<td>1.65 (1.04–2.60)</td>
</tr>
<tr>
<td>Australia/New Zealand</td>
<td>2,511</td>
<td>70</td>
<td>22</td>
<td>699</td>
<td>24</td>
<td>1.24 (0.77–1.99)</td>
</tr>
<tr>
<td>UK</td>
<td>2,698</td>
<td>82</td>
<td>21</td>
<td>512</td>
<td>12</td>
<td>0.77 (0.42–1.41)</td>
</tr>
<tr>
<td>North Africa/Middle East</td>
<td>3,172</td>
<td>89</td>
<td>24</td>
<td>38</td>
<td>5</td>
<td>5.24 (2.00–13.77)</td>
</tr>
<tr>
<td>Israel/Palestine</td>
<td>3,068</td>
<td>92</td>
<td>23</td>
<td>142</td>
<td>2</td>
<td>0.46(0.11–1.90)</td>
</tr>
</tbody>
</table>

Frish et al. (2004) identified a conserved c.1278insTATC haplotype in 55 unrelated AJ individuals, suggesting the occurrence of a common founder in Central Europe. The c.1278insTATC mutation was diagnosed in 73.2% of Australian carriers (Sydney, 69.8%; Melbourne, 81.0%), comparable to the figure of 70–82% reported in other AJ populations (Peleg et al. 1994; Grebner and Tomczak 1991; Paw et al. 1990; Myerowitz and Costigan 1988). However, grandparents’ birthplace in specific European countries or regions showed no significant relationship with grandchildren’s risk of c.1278insTATC carrier status (Table 7).

The c.1421+1G>C mutation was diagnosed in 18.9% of Australian TSD carriers (Sydney, 21.1%; Melbourne, 11.9%), compared with 13% of American AJ TSD carriers (Arpaia et al. 1988). The c.1421+1G>C mutation carrier frequency was 1:49 in subjects with grandparents from South Africa (OR, 4.19 (95% CI, 1.83–9.62), p=0.001), compared to 1:129 in all Australian AJ subjects. The increased proportion of Sydney AJ subjects with grandparents from South Africa (32.0% vs. 11.0%) mirrored the c.1421+1G>C increased frequency in Sydney vs. Melbourne subpopulations (0.00915, 1:109 vs. 0.00488, 1:204).

The Jewish community in South Africa is of Eastern European AJ origin (Meiner et al. 1991; Levin 2001), overwhelmingly originally from Lithuania (Tatz et al. 2007). The AJ population in Lithuania plummeted from the 755,000 recorded in Lithuania’s 1897 census to 153,743
in its 1923 census (Lane et al. 1985), caused by emigration to South Africa, USA, and Canada. From 1941, Nazi genocide achieved near-complete annihilation of the Jews of Kaunas and Vilnius provinces in Lithuania, the relatively small area from which more than half of the South African AJ population trace their ancestry (Lane et al. 1985). As a result, there is now no surviving European reference population. In a published survey conducted between 2003 and 2004, 608 Australian and New Zealand recent South African AJ immigrants made 697 mentions of ancestral homes in Kaunas (Kovno) province and 65 in the Vilnius (Vilna) province (Tatz et al. 2007). The South African AJ community expanded from 10,000 members in 1890 (Jenkins et al. 1977a, b; Lane et al. 1985) to 120,000 in the early 1980s. By 2007, an estimated 40% of South Africa’s AJ population (47,000) had emigrated for social and political reasons (Tatz et al. 2007).

It has been postulated that c.1421+1G>C mutations may have existed at a higher allele frequency in a relatively small area of Lithuania by founder effect and might be preserved in individuals with grandparents from South Africa. In data analyzed from the Dor Yeshorim TSD screening program in New York and Jerusalem; Risch et al. (2003), demonstrated a higher c.1421+1G>C allele frequency in subjects with at least one grandparent from Lithuania (0.0113) compared with mixed AJ group (0.0041), where 3,718 of 249,372 grandparents with Lithuanian origins. Risch et al. (2003) included an unspecified number of subjects with South African ancestry in the Lithuanian group but did not report of a significant result in the subjects with South African heritage as a subgroup. In our study, c.1421+1G>C mutation carrier frequency was not increased among the small sample of Australian subjects identifying at least one grandparent from Lithuania (1:300).

In 1985, the TSD carrier frequency among the Jews of South Africa was estimated to be 1:23 (Lane et al. 1985). The National Health Laboratory service (previously the South African Institute for Medical Research) has offered a genetic screening service for TSD since the 1970s which can be availed by individuals at risk (personal communication Dr. Amanda Krause and Ms. Fahmida Essop 2009). Six of 43 AJ individuals screened by them for TSD were c.1421+1G>C carriers (14% of individuals tested, 18% of 33 TSD carriers identified) a proportion greater than the 13% of TSD carriers expected for American AJ populations. This result is not directly comparable to our data, given the different mode of patient selection and small sample size. Further evaluation is undoubtedly required; however, the trend is in the same direction as our Australian data. AJ ancestry in our cohort was a risk factor for TSD inheritance (X²=69.07, df=1, p<0.001). Mixed heritage (AJ/Sephardi/non-Jewish) was shown to dilute this risk.

One hundred thirty out of 137 of the HEXA mutation carriers detected through screening in our study were in AJ self-identifying individuals. A policy of screening individuals with AJ ethnicity would detect the majority of HEXA mutation carriers with sensitivity of 95%. Amongst 895 individuals tested who did not self-identify as AJ, but as “Sephardi,” “uncertain,” “Jewish,” or “mixed” ethnicity, seven carriers were identified. The majority of these non-AJ carriers (five of seven) were found in individuals with “mixed” ethnicity (carrier frequency, 1:97; 1%) representing 3.6% of all carriers. All five mixed ethnicity carriers were found to express the c.1278insTATC mutation. One carrier of the c.1278insTATC mutation identified origins as uncertain, and one carrier of the c.1421+1G>C identified as Jewish.

Two AJ individuals screened by enzyme analysis prior to 2005 were found to carry private mutations. One individual carried the p.Arg499Cys mutation with grandparents from Poland. The p.Arg499Cys mutation has been previously seen in several ethnic groups including Polish (Mules et al. 1992).

The second mutation p.His204Pro has not been previously described, and we are in the process of characterizing this (unpublished data). Grandparental ancestry of this subject was Dutch and English.

Our research strategy involved the use of a de-identified database of results. De-identification of data resulted in the possibility of non-acknowledgment of familial relationships within the database for both carriers and noncarriers. c.1278insTATC carrier status was observed in five individuals with at least one grandparent from the North African/Middle Eastern region (OR, 5.25; 95% CI, 2.00–13.76, p=0.001). These individuals all nominated their ethnicity as AJ, suggesting European ancestry. The five subjects, all of whom consented for their results to contribute to research and development, were re-identified at arm’s length by a secondary investigator post-analysis, and a familial relationship was confirmed in two of the five cases.

A strategy of investigating the relationship between grandparent’s birthplace and TSD carrier status has some inherent weaknesses. Without family pedigree testing, the grandparent from whom an identified mutation was inherited remains uncertain. Grandparent and parent carriers with noncarrier offspring are undetected.

In countries like Australia which have become adopted homes to immigrant Jewish communities, new generations of AJ descent are experiencing continuing evolution of cultural history and identity. Demography of the generation we studied revealed the effect of the Australian “melting pot,” with a large proportion of Jewish subjects identifying as having mixed ethnicity. Our program was successful in identifying its target population as 78.6% of subjects screened identified as AJ. However, students attending
non-Jewish high schools were not able to be offered cohort TSD screening, representing a significant proportion of young people remaining without access to screening and thus at risk for being TSD carriers with decreased opportunity to gain that knowledge.

Despite postulated demographic changes that have been occurring since earlier studies of HEXA mutation frequencies of AJ populations were undertaken (Lowden and Davidson 1977; Kaback et al. 1977), the risk profile of AJ individuals in our study regarding TSD carrier status remains undiluted (carrier frequency, 1/25).

Conclusions

Nominated AJ ethnicity was the single best predictor of TSD carrier risk in our study. Screening only those individuals identified as having AJ heritage would identify 95% of all Australian Jewish TSD carriers.

Individuals of mixed AJ and non-AJ heritage have reduced risk of being TSD carriers (1:97). Individuals with one or more grandparents from South Africa had a fourfold greater risk of being a carrier of c.1421+1G>C mutation, compared with other AJ subjects.

The TSD carrier frequency for all mutations in Australian Jewish subjects is 1:30. The TSD carrier frequency for all mutations in Australian AJ subjects is 1:25. This proportion of AJ subjects who are TSD carriers is unchanged from previous international studies despite widespread demographic change and social influences such as intermarriage in the wider community.

These findings suggest that the policy approach remains sound in encouraging access to high school and preconception TSD carrier testing for all members of the Jewish community. However, should funding or resources limit the ability to undertake full community screening, then the alternative of screening only those subjects who identified themselves as being AJ would identify 95% of carriers.

Acknowledgments

We thank Professor Martin Delatycki (Director of the Bruce Lefroy Centre for Genetic Heath Research) and the Melbourne TSD screening program for prospectively allowing the inclusion in our database of results from Melbourne-based students screened for TSD where testing was conducted at PaLMS Pathology North in Sydney (1997–2002). We thank Dr. Amanda Krause and Ms. Fahmida Essop, Division of Human Genetics, National Health Laboratory Service, and School of Pathology, the University of the Witwatersrand, Johannesburg, South Africa, for their contribution of unpublished results. We thank Dr. Georgina M. Luscombe, adjunct lecturer, Department of Obstetrics and Gynaecology, University of Sydney for aiding in the statistical analysis of the results. We thank Dr. Robert Markham, senior lecturer of the Department of Obstetrics and Gynaecology, postgraduate coordinator of the Reproductive Health Sciences and Human Genetics, Department of Obstetrics and Gynaecology, Queen Elizabeth II Research Institute for Mothers and Infants, The University of Sydney. We also thank Dr. Gary Eckstein who made available his unpublished report on Demography of the Sydney Jewish Community 2006, (prepared from the Australian National Census for the Jewish Communal Appeal 2008).

Conflict of interest

Authors have no competing interests to declare. This study was not funded.

References


Chapter 5:

Chapter 5 reports the result of a complete and consecutive audit of all diagnosed cases of TSD in Australia and longitudinal TSD carrier frequencies in Australian AJ communities in the era of TSD preconception genetic screening program operation. It examines Australian Jewish community demographic data from Australian Bureau of Statistics assessments and reflects that fewer than expected Jewish TSD cases have occurred in the genetic screening era.

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Tay Sachs disease (TSD) is an autosomal recessive lysosomal storage disorder caused by mutations of the *HEXA* gene (Mendelian Inheritance in Man [MIM] number,*606869; gene map locus, 15q23-q24) that cause hexosaminidase A enzyme deficiency. It is 100 times more common in Ashkenazi (European) Jews (carrier frequency, 1 in 27) than in general populations (carrier frequency, 1 in 250). Affected babies appear normal at birth, then experience slow neurological decline and death in infancy (infantile TSD) or early childhood (intermediate TSD). No cure or effective treatment exists.

Preconception genetic screening programs for TSD have been introduced in Jewish communities worldwide to determine individuals’ carrier status. Ninety-nine per cent of TSD cases among Jewish people are caused by three known *HEXA* mutations. Prospective identification of risk allows individuals and couples to make informed decisions about reproduction.

Based on international best-practice principles, Australian genetic screening programs for TSD have targeted senior Jewish high school students in Sydney (from 1995 to 2012) through the Australasian Community Genetics Program, Laboratory and Community Genetics Department, Pacilory Medicine Services (PaLMS, Pathology North) and in Melbourne (from 1998 to 2011 through Victorian Clinical Genetics Services [VCGS]; and in 2012 through Austin Health). Sydney and Melbourne are home to Australia’s two largest Jewish communities, where 50%–70% of Jewish high school students attend schools that access screening (Eckstein G. Demography of the Sydney Jewish community: an overview of information from the 2006 Census. Unpublished report commissioned by the Jewish Community Appeal [JCA]; copies available on request from http://www.jca.org.au). The design of the Sydney program, on which the Melbourne program is based, has previously been described. Jewish–community-funded screening is free to students and uptake is high (99.6%). Screening programs offer testing for additional conditions relevant to the target community, including Fanconi anaemia, familial dysautonomia, Canavan disease, Bloom syndrome, glycogen storage disease type 1a, mucolipidosis type IV, Niemann–Pick disease type A and cystic fibrosis.

An important measure of the success of a genetic screening program is the reduction of target disease incidence over time. Other measures of success include access to and uptake of screening in the target population. Here, we report the outcomes of Jewish screening programs for TSD on the incidence of TSD-affected births in Sydney and Melbourne from 1995 to 2011, inclusive.

**Abstract**

**Objectives**: To evaluate the outcomes of preconception screening of Jewish Australians for Tay Sachs disease (TSD) carrier status on Jewish TSD-affected births.

**Design, participants and setting**: Epidemiological observational study involving a complete retrospective audit of infantile and intermediate TSD cases diagnosed in Sydney and Melbourne between 1 January 1995 and 31 December 2011 (Royal Children’s Hospital Melbourne; Pacific Laboratory Medicine Services, Pathology North, NSW Health Pathology, Sydney; Victorian Clinical Genetics Services, Melbourne; and SA Pathology, Adelaide), and carrier frequency among Jewish high school students attending schools participating in TSD screening programs over the same period.

**Main outcome measures**: Jewish TSD carrier frequency; and expected versus observed Jewish TSD-affected births.

**Results**: The 2006 Census indicated that most of the total 88 826 Jewish Australians live in Melbourne (46%) and Sydney (40%). The 7756 Jewish high school students screened for TSD in Sydney and Melbourne during the study period had a carrier frequency of one in 31 (3.26%; 95% CI, 2.89%–3.68%). The estimated expected number of TSD-affected births in Melbourne and Sydney in 1995–2011 was 4.1 for Jewish births and 7.4 for other births (a ratio of Jewish to non-Jewish births of 1.2). The actual number was 12 (four in Sydney and eight in Melbourne), of which two were Jewish (a ratio of Jewish to non-Jewish births of 1:5) of which two were Jewish (a ratio of Jewish to non-Jewish births of 1:5). This finding of fewer than expected Jewish TSD cases coincided with a period during which screening programs were operating. There have been no Jewish TSD-affected children born to parents who were screened previously.

**Conclusion**: Community education, appreciation of autosomal recessive inheritance and genetic carrier screening before pregnancy are the likely factors in our finding of fewer than expected Jewish babies with TSD. Ongoing outcome monitoring must continue.

**Methods**

We retrospectively audited all TSD cases diagnosed in Sydney and Melbourne from 1995 through 2011. All samples were processed by one or more of three Australian laboratories (PaLMS, Pathology North, NSW Health Pathology, Sydney; VCGS, Melbourne; and SA Pathology, Adelaide). Laboratory records from these centres pertaining to each case were reviewed. Records of cascade screening (ie, systematic screening of relatives of affected infants) were identified. We audited all laboratory testing for TSD case diagnosis and carrier screening during the study period. This included diagnosis of TSD cases, cascade screening and screening program referrals. Between 50% and 70% of Jewish high school students attend Jewish high schools that participate in TSD screening programs. Within TSD screening programs, there is 99%
uptake among students offered screening.

Parents of a child with TSD are routinely asked about Jewish heritage, and this information was obtained from laboratory records, medical records and clinical genetics files. Data from testing laboratories and medical records were cross-referenced to eliminate duplication of cases.

We identified all TSD cases diagnosed and obtained the medical and laboratory records for audit. For all TSD cases identified, parental TSD carrier results were on record. We cross-referenced case and cascade screening results with screening program data. Although our audit of laboratory results identified family members of TSD-affected Jewish children who chose to undertake cascade screening and were found to be TSD carriers, no further analysis was performed on the results of extended cascade screening.

**Statistical analysis**

We obtained summary statistics for births registered in Sydney and Melbourne in 1995–2011.15–17 Births for 2011 (Australian Bureau of Statistics [ABS] unpublished data) were estimated based on consecutive data for the previous 15 years.15–17 Jewish births for 1995–2010 were proportionally estimated from the 2006 Australian census report,18,19 and we used the number of 0–4-year-olds identified as Jewish as a proxy measure for births during this 5-year census period.

De-identified data relating to all students screened for TSD in Sydney in 1995–2011 and Melbourne in 1998–2011 were used to calculate TSD carrier frequency among Jewish students. This was used to model expected TSD-affected births among Jewish Australians. Carrier frequency for TSD in the general Australian population has not been measured. We used the World Health Organization estimate for TSD carrier frequencies in mixed populations (1 in 250 or 0.4%)20 to model expected TSD-affected births among non-Jewish Australians.

The predicted numbers of infants born with TSD in Jewish and non-Jewish Australian children were calculated using the Hardy–Weinberg equation.21 All statistical analyses were conducted using SPSS version 15.0 (IBM SPSS Statistics).

**Ethics approval**

Ethics approval was obtained from the Northern Sydney Local Health District Human Research Ethics Committee (HREC) and the Royal Children’s Hospital Melbourne HREC.

**Results**

In the 2006 census, 88 826 of a total 19 855 288 Australians (0.45%) identified themselves as Jewish; 46% of Jewish Australians lived in Melbourne and 40% in Sydney.

Box 1 shows that the 7756 Jewish high school students screened had a TSD carrier rate of one in 31 (3.26%); rates were similar in Sydney (3.39%) and Melbourne (3.15%).

Box 2 shows ABS births data for Melbourne and Sydney in 1995 through 2011.15–17

The 2006 Australian census recorded 4394 Jewish children aged 0–4 years in Melbourne and Sydney.18 The census-based estimate of Jewish births in Melbourne and Sydney in 1995–2011 was 14 940. Box 3 shows numbers of observed and predicted TSD-affected babies born in Sydney and Melbourne in 1995–2011; a total of 12 babies with TSD were born in this period — four in Sydney and eight in Melbourne — of whom two were Jewish.

The observed ratio of Jewish to non-Jewish TSD-affected births was 1.5 compared with the expected ratio of 1:2. No Jewish TSD carrier identified through screening has had a TSD-affected child.

Our audit showed that no parents of TSD-affected Jewish children had participated in screening, and no screening program participants were parents of TSD-affected children.

**Discussion**

Twenty years after the introduction of TSD carrier testing in Australia,15 there have been fewer than expected Jewish TSD-affected births (Box 3). Further, no genetic carrier identified through screening has had a TSD-affected child. As many of these individuals, now aged 16–38 years, have not commenced and/or completed their families, the full impact of the screening program is yet to be realised.
During the study period, most extended families of TSD-affected infants underwent cascade genetic screening, and no parents of an infant with TSD had further TSD-affected children. This is a strong demonstration of the effectiveness of community genetic screening for TSD, supported by appropriate laboratory testing infrastructure.

Current Jewish Australian screening program carrier frequencies are comparable to international Jewish carrier frequencies from 1970 to the present. TSD predominantly affects those of Ashkenazi Jewish ancestry. Of 4105 Australian Jewish high school students screened for TSD in 1995–2007, 78% of participants and 95% of carriers were Ashkenazi. TSD carrier frequencies were 1 in 25 for Ashkenazi Jews and 1 in 97 for those of mixed and non-Ashkenazi Jewish heritage. No distinction is made in census data between Ashkenazi and other Jewish Australians, so subpopulation analysis was not performed. The ABS and Jewish community organisations estimate that Jewish Australians underreport their religion in the census, and usually apply a correction factor of 20% (Eckstein G. Unpublished report; copies available on request from http://www.jca.org.au). We did not apply any correction factor to estimates of Jewish births or expected numbers of Jewish TSD cases.

Our study has limitations relating to the rarity of TSD, the low disease frequency and the small size of the Australian Jewish population. These factors prevented the reduction in observed Jewish TSD cases reaching statistical significance in our study. To demonstrate a significant reduction in cases, it would take 70 years to observe among 12 Jewish TSD cases, using the Poisson model.

TSD testing is now less invasive, and the cost of laboratory testing has fallen over the 16-year period described in this study. Outreach screening strategies to extend the benefits of TSD preconception screening to a wider target population should be considered.

Overall, we found that since TSD screening commenced in Australia, the number of observed TSD cases in Jewish Australians has halved compared with predictions, while carrier frequency remains high (1 in 31). Preconception carrier screening, supported by community education and the appreciation of autosomal recessive inheritance are the likely key factors explaining the fewer than expected Jewish babies born with TSD.

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Competing interests: No relevant disclosures.

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23 Przyluski J, Wilenski H. Homogeneity of results in testing samples from Poisson series: with an application to testing clover seed for dodder. Biometrika 1940; 31: 313-323.
Chapter 6 reports the results of a systematic review of the literature relating to TSD screening in AJ communities. It reports the Australian experience and derives evidence based TSD screening protocols and clinical recommendations.

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REVIEW ARTICLE

Ashkenazi Jewish population screening for Tay–Sachs disease: The International and Australian experience

Raelia M Lew,1 Leslie Burnett,2,3 Anné L Proos,3 Kristine Barlow-Stewart,2,4 Martin B Delatycki,5,6 Agnes Bankier,5 Harry Aizenberg,7 Michael J Field,8 Yemima Berman,2,8 Ronald Fleischer8 and Michael Fietz9

1Department of Obstetrics and Gynaecology, QEII Research Institute for Mothers and Infants, 2Sydney Medical School-Northern, Royal North Shore Hospital E25, University of Sydney, 3Pacific Laboratory Medicine Services (PaLMS), NSW Health Pathology North, 4NSW Centre for Genetics Education, 5Department of Clinical Genetics, Royal North Shore Hospital, 6Wolper Jewish Hospital, Sydney, New South Wales, 7Department of Clinical Genetics, Austin Health, Melbourne, Victoria, 8Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Melbourne, Victoria, 9Department of Biochemical Genetics, SA Pathology, Adelaide, South Australia, Australia

Abstract: Internationally, Tay-Sachs disease (TSD) preconception screening of Ashkenazi Jewish (AJ) individuals and couples has led to effective primary prevention of TSD. In Australia, adolescent preconception genetic screening programs operate mainly in Jewish community high schools. These existing programs offer an effective means of primary prevention of TSD, are cost effective and safe. However, in the broader Australian community TSD screening is not systematically performed and cases still occur in unscreened AJ individuals. In order to improve the effectiveness of Australian screening, there is a need for definitive guidelines for healthcare professionals to facilitate extension of the proven benefits of preconception TSD screening to all AJ individuals at risk. We performed a systematic review of the relevant literature relating to AJ preconception and antenatal screening for TSD. The evidence was assessed using an established National Health and Medical Research Council evidence grading system. Evaluations of efficacy of TSD screening programs design and execution, cost-benefit and cost-utility health economic evaluation, and population outcomes were undertaken. The results have been used to propose a model for universal AJ TSD preconception and antenatal screening for the primary care setting.

Key words: adolescent; education; genetics; metabolic.

Key points

1 Tay–Sachs disease (TSD) carrier screening should be offered to Ashkenazi Jewish (AJ) individuals of reproductive age in order to provide informed reproductive choice. Primary care clinicians’ inquiry of ethnic background is required on history taking to identify AJ individuals who will be at high risk of having children with TSD.

2 Timing of genetic screening should ideally be conducted prior to conception.

3 Primary health providers should be aware of different strategies of TSD screening. Partners of TSD carriers should be offered screening, regardless of heritage. Screening strategies for one- and two-step screening are summarised in Figures 1–3. In order to minimise delay to diagnosis, one-step screening (Fig. 2) is recommended where screening is undertaken during a pregnancy.

Introduction

Tay–Sachs disease

Tay–Sachs disease (TSD) is a neurodegenerative disorder caused by congenital deficiency of β-hexosaminidase enzyme due to mutations in the HEXA gene (OMIM *606869 gene map locus 15q23-q24). TSD exhibits autosomal recessive inheritance, in which heterozygous genetic carriers are phenotypically normal. In a couple where both partners are carriers, 25% of pregnancies will be affected. Most infants with TSD appear healthy at birth.3 After a 3- to 6-month period of normal development, TSD-affected infants regress neurologically secondary to neuronal accumulation of sphingolipid GM2 gangliosides. Most motor and social skills are lost by 18 months of age. Children rarely survive beyond 5 years of age. No effective treatment exists.2 In Ashkenazi Jews (AJ; Jews of Central/Eastern European descent), TSD incidence is one in 2500 (carrier frequency one in 25).3 A total of 93.1–99.1% of Jewish TSD carriers are found to have one of three common HEXA mutations: c.1278insTATC, c.1421+1G>C and c.805G>A.4–6 In non-AJ populations, TSD incidence is one in 250 000 (carrier frequency one in 250).7

International practice

Strong international consensus supports universal TSD AJ preconception/antenatal screening.8–11 In the United States,1 Canada12 and Israel,13 TSD screening of AJ individuals has resulted in >90% reduction in TSD incidence.
The following international bodies recommend that TSD preconception/antenatal screening should be offered to all individuals with AJ heritage:

- The American Congress of Obstetricians and Gynecologists
- The American College of Medical Genetics
- Society of Obstetricians and Gynaecologists of Canada
- United Kingdom National Screening Committee
- State of Israel Ministry of Health

**Australian practice**

Despite strong international support of universal AJ preconception TSD screening, no Australian guideline exists, and AJ TSD screening is not publicly funded.

**Cost–benefit and ethical considerations**

In terms of direct monetary cost, preconception or antenatal screening for TSD is superior to retrospective identification of carrier parents following the birth of an affected infant. The direct tangible monetary costs have been analysed in an Australian cost–utility study, which found in favour of screening. Important ethical benefits of TSD carrier screening include informed reproductive choice and a net increase in health of carrier couples and families by preventing the profound psychological costs of having a TSD-affected infant. These additional benefits are difficult to quantify in terms of monetary value.

**Objective**

Australia is a good model for evaluating the effectiveness of high school TSD screening programmes. Australia has a relatively static Jewish population strongly concentrated to two major centres (Sydney and Melbourne). Australian screening programmes are well funded, have high uptake of services by AJ high school students targeted (>8000 student participants to date) and are highly collaborative with the ongoing analysis of more than 17 years of data.

This paper aims to systematically review the international evidence base supporting universal preconception TSD screening for AJ individuals, to present the Australian experience and to examine the case for supporting universal AJ TSD screening in Australia. Our data and resultant conclusions may be of assistance for screening AJ communities in other countries.

**Australian TSD population genetic screening programmes**

Based on international best practice principles, Australian genetic screening programmes for TSD have targeted senior Jewish high school students in Sydney and Melbourne since 1995. The design of the Sydney programme, on which the Melbourne programme is based, has been described previously. Screening programmes were developed in consultation with the local communities and consist of mandatory on-site education followed by voluntary on-site genetic carrier testing (originally by venepuncture but more recently by mouthwash sampling). Participants may request immediate or deferred disclosure of results. Screening is cost free to participants (philanthropically funded), and participation rates are high (99.6%).

An alternative approach to screening is that of Dor Yeshorim, an international Jewish genetic screening programme accessible to Australians. Dor Yeshorim targets ultra-orthodox Jewish communities world-wide. The programme is accessed prior to marriage to advise on the genetic compatibility of a proposed match. Individuals’ results remain anonymous. A small minority of Jewish Australians identify as ultra-orthodox. Dor Yeshorim is unaffiliated with Australian TSD screening programmes. Dor Yeshorim screening records were unavailable to authors of this paper, so they could not be used for analysis.

In addition to TSD, both Australian Jewish genetic screening programmes and Dor Yeshorim now offer testing for other conditions relevant to the target community.

TSD population genetic screening programmes have proved a successful strategy for primary prevention of TSD. Over the 17 years so far evaluated in health outcome studies, no Australian TSD screening programme participant has had an affected child – representing complete prevention in the 7756 screened between 1995 and 2011. In follow-up analysis five- to 11-year post-high school, screening programme participants were supportive of the programme, retained good knowledge of TSD and were happy with the timing of screening.

An absolute reduction in Australian AJ TSD incidence of approximately 50% occurred between 1995 and 2011, a period corresponding to the introduction and operation of Australian Jewish community population genetic screening programmes in Sydney and Melbourne.

**Access to TSD screening in Australia is incomplete**

The most frequent route of access to TSD screening in Australia is by participation in organised screening programmes in senior high schools and associated outreach programmes. However, even taking into account those who have accessed these programmes, the majority of Australians with AJ heritage of reproductive age have not accessed TSD screening. From Australian Bureau of Statistics and Australian Jewish Community data, it is estimated that approximately 50% of current Australian Jewish high school students attend schools that do not currently participate in TSD screening programmes. (Eckstein G. Demography of the Sydney Jewish community: an overview of information from the 2006 Census. Unpublished report commissioned by the Jewish Community Appeal (JCA); copies available on request from http://www.jca.org.au). AJ individuals who completed high school prior to 1995 in Sydney and 1998 in Melbourne (predating TSD screening programmes) did not have access to any TSD population genetic screening; a subset of this group remains in the reproductive age bracket.

Outside of screening programmes, TSD genetic preconception screening can in theory be accessed through primary care clinician referral. In the Australian context, the primary care clinician is usually a patient’s general practitioner or obstetrician/gynaecologist. In practice, such referral for TSD screening is uncommon (Anné L Proos, unpublished data, 2013). Barriers include lack of clinician education, omission of genetic risk identifying enquiry during medical history taking, lack of patient knowledge, unplanned pregnancy (accounting for 30–50% of all pregnancies in developed countries) and cost of testing. In Sydney, laboratory testing for TSD currently incurs
an out-of-pocket cost to patients of approximately $A100 (L Burnett, AL Proos, pers. comm., 2013).

**Methods**

**Identification of studies**

A Medline-Pubmed, Embase and Google scholar advanced search was conducted using MeSH topics ‘Tay Sachs disease’ AND (‘screening’ OR ‘genetic counselling’ OR ‘cost’ OR ‘GM2’ OR ‘genetic diseases’ or ‘Ashkenazi Jewish’). A Cochrane collaboration review was also conducted. A manual review of abstracts identified articles relevant to genetic screening for TSD in individuals with AJ heritage. These were subsequently reviewed in full. No language restrictions were applied.

Inclusion criteria: all research and review articles reporting experience of AJ TSD screening, including clinical, laboratory, cost analysis and psychological aspects of TSD screening. Exclusion criteria: articles related to non-human subjects, experimental laboratory methods, genetic screening in non-Jewish populations and articles where no online abstract was available.

**Formulation of evidence-based recommendations**

The evidence supporting the proposed guidelines was appraised with reference to the Australian National Health and Medical Research Council (NHMRC) evidence grading system.35

**Results**

The Medline-Pubmed, Embase and Google scholar advanced search identified 288 articles. A manual review of abstracts identified 85 articles relevant to AJ TSD preconception and antenatal screening of which all were reviewed in full. No randomised controlled trials relating to TSD carrier screening were identified. No Cochrane review or meta-analysis of TSD genetic screening has been conducted. Some papers had findings relevant to more than one body of evidence. NHMRC grading system utilised is summarised in Appendix I. Papers included in the review are listed in Appendix II. Grades of recommendations for bodies of evidence are reported in Table 1. The evidence summary is reported in Table 2.

Alternative models of TSD screening are depicted in Figures 1–3.
**Discussion**

Evidence from 17 years experience of TSD screening programmes in Australian Jewish high schools has shown screening to be both effective in TSD primary prevention\(^{20}\) and also cost-effective.\(^{15}\) However, reliance solely on high school-based programmes is an inadequate strategy for population-wide screening as less than half the at-risk target population (AJ individuals of reproductive age) is within this screened cohort (Eckstein G. Demography of the Sydney Jewish community: an overview of information from the 2006 Census. Unpublished report commissioned by the Jewish Community Appeal (JCA); copies available on request from http://www.jca.org.au).

The results of this review found level III-3 evidence for each of the criteria analysed (Table 1). Based on these findings, recommendations for TSD screening were developed (Table 3). Primary care clinician referral for TSD screening is the pragmatic solution to reach this remaining community sector and to facilitate universal access. Challenges to implementation of this strategy include improving clinician and patient education\(^{31,36,37}\) and funding of supporting laboratory testing and health infrastructure.\(^{34}\) Creation of Australian guidelines for AJ preconception genetic screening in the primary care context would assist in realising universal access, and the process of developing such guidelines is underway (Human Genetics Society of Australasia, Joint Human Genetics Society of Australasia/Royal Australian and New Zealand College of Obstetricians and Gynaecologists Prenatal Diagnosis and Screening Committee).

Performing this screening prior to pregnancy should be considered the gold standard as prospective diagnosis allows carrier couples access to a wider range of reproductive options.\(^{38,39}\) As many pregnancies are unplanned,\(^{33}\) clinicians should consider and offer TSD screening opportunistically when at-risk patients present for other reasons (e.g. general or sexual health check).\(^{31}\) Presentation for screening may commonly occur in early pregnancy.\(^{31}\) Where an at-risk couple is identified during pregnancy (both biological parents are found to be TSD carriers), prompt referral for appropriate genetic counselling and maternal fetal medical review should be undertaken. Management may include fetal diagnostic testing.\(^{9}\)

Good pre-test education is imperative to achieve informed consent. In Australian TSD screening programmes, pre-test education is delivered by a qualified genetic counsellor.\(^{25,27}\) Sessions include the delivery of written and multimedia presentations.

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**Fig. 2** One-step couple screening.
Multimedia educational presentations are highly effective in conveying pre-test information in the context of TSD screening programmes. On-line education tools made freely available to primary care clinicians and patients may cost-effectively achieve consistent and quality-assured patient education.

Figures 1–3 summarise the recommended screening pathways applicable to AJ individuals and their partners. These pathways are derived from international best practice screening strategies and offer alternative approaches to different preconception and antenatal scenarios. The Dor Yeshorim model was designed to provide culturally acceptable access to screening for an ultra-orthodox Jewish demographic.

Current practice recommends DNA-based testing for common AJ mutations as the most cost-effective method to identify AJ TSD carriers. Partners of AJ carriers should also be offered TSD preconception screening, with any such non-AJ individuals being tested with enzyme-based assays rather than DNA-based testing, due to the wider range of HEXA variants found in non-AJ heterozygote carriers.

Hexosaminidase A enzyme (Hex A) activity analysis detects 98% of TSD carriers from all ethnic backgrounds. TSD carriers have enzyme activity <52% compared with non-carriers where enzyme activity is >60%. Hex A testing has limitations, which is why this diagnostic technique is not preferred for AJ TSD screening. A blood sample is required, and medications including oral contraceptives and anti-hypertensives may affect detected enzyme levels. There is an inconclusive range in which it is difficult to distinguish carriers from non-carriers, mandating further testing with HEXA DNA testing and/or sequencing. False negative and false positive results can occur with Hex A testing in the presence of B1 and pseudodeficiency alleles, respectively.

Genetic laboratory testing methodology is rapidly advancing. In the immediate future, massively parallel ('next generation') DNA sequencing will likely replace the conventional testing methods for conditions screened in AJ preconception programmes including TSD. This will expand the scope and menu of preconception genetic screening exponentially and pose challenges for the traditional notions of informed consent. Evidence regarding the safety and ethical impact of these developments is currently lacking.

Referral of clients to a local national and regional genetic services may be appropriate; within Australia, a directory of such resources is offered by the Centre for Genetics Education (www.genetics.edu.au), and similar referral directories have been established in many other regions and countries.
Table 1: Grades of recommendations for bodies of evidence

<table>
<thead>
<tr>
<th>Body of evidence</th>
<th>Number of papers</th>
<th>Volume of evidence</th>
<th>Consistency of results</th>
<th>Ability to generalise to target population</th>
<th>Clinical impact</th>
<th>Ability to generalise to the Australian health-care context</th>
<th>Summary grade of recommendation</th>
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<tr>
<td>Ethical and health economic aspects of TSD screening</td>
<td>17</td>
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<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Jewish TSD screening programmes</td>
<td>34</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Timing of TSD screening</td>
<td>23</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>TSD genetic counselling/education</td>
<td>21</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
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<tr>
<td>Management of TSD carriers and carrier couples</td>
<td>15</td>
<td>B</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>III-3</td>
</tr>
<tr>
<td>Laboratory testing methodology</td>
<td>18</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>B</td>
</tr>
</tbody>
</table>

Table 2: Evidence summary

1. Ethical and health economic aspects of TSD screening

   Evidence summary
   TSD preconception screening of Ashkenazi Jewish individuals results in effective primary prevention of TSD, does not cause prolonged psychological harm and is cost-effective.

2. Australian Jewish high school screening programmes for TSD and serious autosomal recessive disorders of reproductive significance

   Evidence summary
   Australian Jewish high school screening programmes for TSD are effective and appropriate. One in 25 Australian Jewish high school students are TSD carriers. Uptake of screening by students is high (99.6%). Disclosure of results may be immediate or deferred. Fewer than 50% of Australian Jewish individuals of reproductive age have accessed existing programmes.

3. Timing of Screening

   Evidence summary
   Preconception screening facilitates informed access to more reproductive strategies for TSD prevention and is associated with less anxiety than testing in pregnancy. For Australians, self-declaration of Ashkenazi Jewish heritage correlates with high risk of TSD. Outside of screening programmes, patient access to screening requires clinician referral. Clinicians’ failure to identify at-risk patients on history taking is a barrier to screening.

4. Pre-test education and genetic counselling

   Evidence summary
   Pre-test education in screening programmes has been validated. Genetic education is provided by a qualified health professional 1 week prior to screening, encompassing TSD clinical features, mode of inheritance, sensitivity and limitations of laboratory testing, implications of positive and negative results, and reproductive options. Written educational material is provided. Multimedia resources have been effective in conveying pre-test information. High knowledge levels correlate with reduced anxiety in TSD carriers. Effective education facilitates informed consent.

5. Models of TSD screening, management of carriers and carrier couples

   Evidence summary
   TSD screening may be conducted using one-step or two-step models (Figures 1–3). Clinician awareness of the pros and cons of different screening strategies maximises cost benefit.

6. Laboratory testing methodology

   Evidence summary
   In Jewish individuals, DNA-based testing has a carrier detection sensitivity of 93.1–99.1% and is the most cost-effective testing method. The small residual risk relates to other HEXA mutations. Most non-Jewish populations exhibit TSD carrier frequencies of one in 250–300. French Canadian, Pennsylvania Dutch and Cajun heritage may convey higher a priori risk. Non-Jewish TSD carriers are most effectively identified by enzyme-based testing for which false negative rate is minimal. HEXA mutations may be characterised by DNA sequencing.

TSD, Tay–Sachs disease.
Conclusion

TSD screening programmes targeting an adolescent AJ population are a highly effective and cost-effective primary prevention strategy. Routine implementation of recommendations summarised in Table 3 by primary care clinicians would optimise informed reproductive choice in AJ individuals and would result in near complete TSD case prevention. The recommendations in Table 3 have been developed through evidence-based review of all published studies irrespective of country of origin and should be portable and applicable to other national jurisdictions. Universal access to TSD genetic screening for all AJ Australians is clinically achievable in the primary care setting using simple practice workflows (Figs 1–3). The conclusions reached based on this Australian data are likely to be transferrable and applicable to other countries and communities.

Multiple Choice Questions

Question 1 Choose the INCORRECT answer. TSD is

a. A neurodegenerative disorder
b. An autosomal recessive condition (heterozygous carriers are unaffected)
c. Less common in AJ populations
d. Lethal in infancy or early childhood
e. Usually diagnosed during the first 6 months of life

Answer 1: c. Less common in AJ populations. TSD was first described in affected individuals of AJ descent. TSD is 100 times more common in AJ populations (carrier frequency one in 25) than in general populations (carrier frequency one in 250). French Canadian populations are also at increased risk of TSD.

Question 2 Choose the CORRECT answer: TSD preconception screening

a. Does not reduce TSD incidence
b. Is not cost-effective in AJ populations
c. Is accessed poorly outside of established Jewish high school-based screening programmes
d. Does not significantly enhance informed reproductive choice
e. Has poor uptake following pre-test counselling

Answer 2: c. Is accessed poorly outside of established Jewish high school-based screening programmes

Question 3 A couple have undergone preconception screening for TSD. Both partners have been found to be TSD carriers. They have been counselled by their doctor that, in each pregnancy, their baby will have a one in four chance of having TSD. Their reproductive options include

a. Using donor sperm or donor egg from a TSD non-carrier
b. First trimester chorion villus sampling for fetal diagnosis
c. Elective IVF with embryo biopsy and pre-implantation genetic diagnosis
d. Adoption
e. All of the above

Answer 3: e. All of the above. Preconception timing of TSD screening optimises the range of reproductive options open to TSD carrier couples.

Acknowledgements

Martin B Delatycki is an NHMRC Practitioner Fellow.

References

Screening for Tay–Sachs disease


35 NHMRC. How to review the evidence: systematic identification and review of the scientific literature, 1999. Reference number CP65 ed. http://www.nhmrc.gov.au/guidelines/publications/cp65 [accessed 16 November 2013]. National Health and Medical Research Council Australia (NHMRC). This handbook describes how to systematically identify scientific literature relevant to a particular question, select and review the most important (highest quality) studies and summarise and present the results for further consideration by the committee that will develop the clinical practice guidelines.


43 Park NJ, Morgan C, Sharma R et al. Improving accuracy of Tay Sachs carrier screening of the non-Jewish population: analysis of 34 carriers


### Appendix I

**NHMRC evidence grading framework**

**NHMRC evidence hierarchy**

<table>
<thead>
<tr>
<th>Key question(s)</th>
<th>Evidence base (number of studies, level of evidence and risk of bias in the included studies)</th>
<th>Consistency (if only one study was available, rank this component as ‘not applicable’)</th>
<th>Clinical impact (indicate in the space below if the study results varied according to some unknown factor (not simply study quality or sample size) and thus the clinical impact of the intervention could not be determined)</th>
<th>Generalisability (How well does the body of evidence match the population and clinical settings being targeted by the guideline?)</th>
<th>Applicability (Is the body of evidence relevant to the Australian health-care context in terms of health services/delivery of care and cultural factors?)</th>
<th>Definition of NHMRC grades of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Key question(s)</td>
<td>Evidence base (number of studies, level of evidence and risk of bias in the included studies)</td>
<td>Consistency (if only one study was available, rank this component as ‘not applicable’)</td>
<td>Clinical impact (indicate in the space below if the study results varied according to some unknown factor (not simply study quality or sample size) and thus the clinical impact of the intervention could not be determined)</td>
<td>Generalisability (How well does the body of evidence match the population and clinical settings being targeted by the guideline?)</td>
<td>Applicability (Is the body of evidence relevant to the Australian health-care context in terms of health services/delivery of care and cultural factors?)</td>
<td>Definition of NHMRC grades of recommendation</td>
</tr>
<tr>
<td>Evidence base (number of studies, level of evidence and risk of bias in the included studies)</td>
<td>A One or more level I studies with a low risk of bias or several level II studies with a low risk of bias</td>
<td>A All studies consistent</td>
<td>A Very large</td>
<td>A Evidence directly generalisable to target population</td>
<td>A Evidence directly applicable to Australian health-care context</td>
<td><strong>Grade of recommendation</strong></td>
</tr>
<tr>
<td>Evidence base (number of studies, level of evidence and risk of bias in the included studies)</td>
<td>B One or two level II studies with a low risk of bias or SR/several level III studies with a low risk of bias</td>
<td>B Most studies consistent and inconsistency can be explained</td>
<td>B Substantial</td>
<td>B Evidence directly generalisable to target population with some caveats</td>
<td>B Evidence applicable to Australian health-care context with few caveats</td>
<td><strong>A</strong></td>
</tr>
<tr>
<td>Evidence base (number of studies, level of evidence and risk of bias in the included studies)</td>
<td>C One or two level III studies with a low risk of bias or level I or II studies with a moderate risk of bias</td>
<td>C Some inconsistency, reflecting genuine uncertainty around question</td>
<td>C Moderate</td>
<td>C Evidence not directly generalisable to target population but could be sensibly applied</td>
<td>C Evidence probably applicable to Australian health-care context with some caveats</td>
<td><strong>B</strong></td>
</tr>
<tr>
<td>Evidence base (number of studies, level of evidence and risk of bias in the included studies)</td>
<td>D Level IV studies or level I–III studies/SRs with a high risk of bias</td>
<td>D Evidence is inconsistent</td>
<td>D Slight/restricted</td>
<td>D Evidence not directly generalisable to target population and hard to judge whether it is sensible to apply</td>
<td>D Evidence not applicable to Australian health-care context</td>
<td><strong>C</strong></td>
</tr>
</tbody>
</table>

**Definition of NHMRC grades of recommendation**

<table>
<thead>
<tr>
<th>Grade of recommendation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Body of evidence can be trusted to guide practice</td>
</tr>
<tr>
<td>B</td>
<td>Body of evidence can be trusted to guide practice in most situations</td>
</tr>
<tr>
<td>C</td>
<td>Body of evidence provides some support for recommendation(s), but care should be taken in its application</td>
</tr>
<tr>
<td>D</td>
<td>Body of evidence is weak, and recommendation must be applied with caution</td>
</tr>
</tbody>
</table>
## Appendix II

### List of papers included in the systematic review

<table>
<thead>
<tr>
<th>Body of evidence</th>
<th>Papers evaluated</th>
</tr>
</thead>
</table>
Appendix II  Continued

<table>
<thead>
<tr>
<th>Body of evidence</th>
<th>Papers evaluated</th>
</tr>
</thead>
</table>
Appendix II  Continued

Body of evidence  Papers evaluated


Timing of TSD screening

Appendix II Continued

Body of evidence Papers evaluated

TSD Genetic counselling/education


Appendix II  Continued

Body of evidence  Papers evaluated


Management of TSD carriers and carrier couples


### Appendix II  
Continued

**Body of evidence**  

<table>
<thead>
<tr>
<th>Papers evaluated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory testing methodology</td>
</tr>
</tbody>
</table>
Chapter 7:

Transition to performing Ashkenazi Jewish population genetic screening using community centred screening strategies and Massively Parallel Sequencing Technologies

Introduction

Tay Sachs disease (TSD), results from a critical deficiency in β-hexosaminidase enzyme. TSD has genetic recessive inheritance and is more common in Ashkenazi Jewish (AJ) populations (carrier frequency 1 in 25). TSD has historically been the prototype genetic condition amenable to preconception screening. ¹

TSD single condition genetic screening

Routine preconception and antenatal screening of AJ individuals for TSD has been offered in centres around the world since the 1970s.¹ Over time and with technological advancement, the method of laboratory diagnosis of carrier risk has changed.

TSD carriers were at first diagnosed by β-hexosaminidase HEXA enzyme assay, most commonly using synthetic fluorimetric substrates. In the 1980s, the HEXA gene was identified and specific genetic mutations common in AJ populations were
characterised.\textsuperscript{2} DNA testing later emerged as the most cost-effective method of TSD screening for AJ populations.\textsuperscript{3,4} Enzyme methods remain in limited use, primarily for non-AJ screening and for functional characterisation of low prevalence and newly described \textit{HEXA} variants.

TSD screening programs are effective in TSD prevention, cost effective and well accepted.\textsuperscript{2,5-7} In AJ populations, disease incidence has reduced by over 90\% since the introduction of preconception and antenatal genetic screening.\textsuperscript{1, 6, 7 8-10} In reference to best practice criteria for justification of screening for disease prevention, the case for TSD genetic screening in AJ populations is clear (\textbf{Table 1}).\textsuperscript{11}
Table 1: Wilson and Jungner classic screening criteria as applied to TSD

<table>
<thead>
<tr>
<th>Wilson and Jungner Criteria</th>
<th>TSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Important health problem</td>
<td>Yes</td>
</tr>
<tr>
<td>2. Intervention possible</td>
<td>Yes</td>
</tr>
<tr>
<td>3. Diagnosis/Intervention infrastructure available</td>
<td>Yes</td>
</tr>
<tr>
<td>4. Carrier state detectable</td>
<td>Yes</td>
</tr>
<tr>
<td>5. Suitable test</td>
<td>Yes</td>
</tr>
<tr>
<td>6. Acceptable test</td>
<td>Yes</td>
</tr>
<tr>
<td>7. Condition understood</td>
<td>Yes</td>
</tr>
<tr>
<td>8. Target group identifiable</td>
<td>Yes</td>
</tr>
<tr>
<td>9. Cost balanced</td>
<td>Yes</td>
</tr>
<tr>
<td>10. Ongoing strategy possible</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Expanded Ashkenazi Jewish preconception screening: beyond TSD

Over the passage of time, concurrent screening for both TSD and also a limited and increasing number of other genetic conditions common to AJ populations became technically feasible. Commercially produced multi-disease screening panels, including TSD, became widely used in AJ preconception and antenatal genetic screening to detect known disease-causing founder mutations.\textsuperscript{12} For reasons of laboratory reagent costs, it can now paradoxically be more expensive to test for a single condition e.g. TSD than for a panel of multiple conditions.\textsuperscript{12} When given the choice of limited screening vs. screening using an expanded panel, after counselling 95% of Jewish individuals opt for an expanded panel.\textsuperscript{13} Conditions included in expanded panels can vary in disease severity and penetrance, making pre-test genetic counselling more complex, a barrier to community based screening strategies initiated by primary health care clinicians. In the genomic era, genetic screening performed using Massively Parallel Sequencing (MPS) technologies presents the option to expand AJ screening panels further still. MPS also presents the possibility to offer preconception genetic screening for carrier trait diagnosis of rare genetic diseases in unselected general populations. Algorithms to assist clinicians in choosing the most appropriate method of genetic testing and screening have been proposed.\textsuperscript{14} For Australian AJ individuals, primary health care clinicians are now supported by the HGSA AJ genetic screening position paper, presented in Chapter 8 of this thesis. Conditions recommended for AJ preconception genetic screening in the HGSA ratified AJ panel are recommended for screening based on
their clinical impact and relatively high carrier frequencies. Patient demand for genetic screening technologies is however unprecedented, including direct to consumer (DTC) screening models which screen for more than 100 conditions.\textsuperscript{15}
Transitioning AJ genetic screening from local screening programs to a broader clinical practice setting

Chapter 8 of this thesis represents an important resource to educate and empower primary health care clinicians with confidence in offering preconception genetic screening to AJ patients. Remaining barriers requiring future redress include the lack of Medicare funding item numbers for preconception genetic screening as a health promotion initiative and a relative lack of clinical genetic literacy amongst primary health care clinicians. This is in part explained by the rapid expansion and evolution of genetic diagnostic technologies and test menu that has occurred within the working lifetime of a single generation of practitioners.

Massively Parallel Sequencing (MPS)

Genetic diagnosis by MPS is now possible due to several scientific developments of the 21st century. The Human Genome Project provided a complete reference genome for comparison with test sequences. Large databases of genomic information from healthy individuals and patients with disease have been interrogated to contextually assess observed genetic variants. Technologies and platforms have been developed, at reducing cost to facilitate simultaneous sequencing of multiple regions of fragmented RNA or DNA in a single assay.

To perform MPS, a typical workflow would involve DNA from a patient is being fragmented. Common adapters are ligated to the fragment ends. DNA fragments are amplified by Polymerase Chain Reaction (PCR). Sequencing-by-synthesis then
occurs, where as each base is added, a signal is generated and read by software – recording the genetic sequence of each PCR fragment. The sequence of each fragment is mapped against the human genome. This allows identification of genetic variants present in the test subject’s DNA. Databases of genetic variation now exist allowing characterization of the “human variome”, observed genetic variability within human populations.\textsuperscript{17-20} These include ethnically matched reference genome sequences. Novel and rare variants may be more likely to be pathogenic, while common variants can more often be characterized as normal population genetic variability.\textsuperscript{21} The data generated from MPS is then analyzed and interpreted in context of the patient’s family history and the disease prevalence and pattern of inheritance.

“Third generation” technologies are developing methods of MPS without a preceding step of DNA amplification, aiming to improve MPS speed and accuracy.\textsuperscript{22}

MPS was developed as a research tool for whole-genome sequencing (WGS).\textsuperscript{23} The sequenced genome of an individual can be regarded as a personal reference library that could be re-interrogated over an individual’s lifetime as a diagnostic tool. This concept has generated many complex clinical, ethical and bioinformatics challenges, which are yet to be fully addressed.\textsuperscript{24} The American College of Medical Genetics and Genomics recommends that the application of exome or whole genome sequencing should be limited to clinical cases which elude targeted interrogation.\textsuperscript{25} For clinical applications in genetic screening and diagnosis, targeted sequencing
may be faster, more cost-effective and less likely to result in the detection of secondary or incidental findings (IFs) of uncertain significance.\textsuperscript{26}

In targeted MPS, prior to amplification, a library of DNA or RNA fragments containing regions of particular interest may be selected. Probes are designed to anneal to specific chromosomal regions of interest. Targeted regions are identified and isolated for PCR amplification and sequencing.

Massively Parallel Sequencing for Genetic Screening: Supporting evidence guiding current practice

Preconception genetic screening is a natural application of MPS technology. MPS methods permit screening for an expanded panel of genetic disorders simultaneously and with high fidelity, quick turnaround time, and reducing costs.\textsuperscript{27} Studies are needed to provide evidence of net benefit and to reassure clinicians that the practice of MPS based targeted genetic screening does not cause harm.

The selection of disorders on expanded carrier screening panels should be rigorously based on health promotion principles.\textsuperscript{28} The basis for which disorders were selected for inclusion should be disclosed to screening participants.
Prevalence and severity of diseases phenotypes should be considered when assessing whether a condition should be included in a screening panel. Inclusions in screening panels of disorders with mild phenotypes, variable expression, low penetrance, or with an adult onset of disease manifestations is controversial. Where such conditions are included, patients should be fully informed of the nature of these conditions and be given the option to “opt out” of receiving specific test results.

**Direct to Consumer MPS Screening**

Currently, MPS screening technology is available commercially “direct to consumer” and has been utilised widely, despite the fact that there exists no expert consensus regarding whether MPS expanded screening is on balance medically indicated in general populations and, if so, which genetic conditions should be included in screening panels.29 In the USA, the Food and Drug Administrative authority (FDA) have defined some DTC genetic testing platforms in the category of “medical device” (e.g. 23andMe) and have thus restricted company marketing for medical genetic screening (marketing of DTC genetic sequencing for genealogical applications is unrestricted).30 Internet based marketing and sales give DTC genetic screening models the potential to have global reach and transcend regulation in any single jurisdiction. /unregulated DTC genomic testing could potentially result in misuse and even fraud or non-consensual testing. In response, there have been calls for
countries, including Australia to legislate a criminal offence relating to non-consensual genetic testing, although currently no such legal framework exists. In May 2014, the Australian National Pathology Accreditation Advisory Council published a position paper to guide providers of DTC testing in Australia. In June 2014, the National Health and Medical Research Council published a draft statement for DTC providers and a statement of information to guide consumers of DTC genetic testing.

**Is MPS based pre-conception genetic screening warranted in general populations?**

In 2008 Andermann et al. revisited the longstanding screening criteria proposed by Wilson and Jungner and proposed additional screening criteria for the genomic era (Box 1); While these criteria have not been universally ratified, they form a valid framework within which general population genetic screening may be considered.
**Box 1: Andermann et al., Synthesis of emerging screening criteria**

1. The screening programme should respond to a recognized need.
2. The objectives of screening should be defined at the outset.
3. There should be a defined target population.
4. There should be scientific evidence of screening programme effectiveness.
5. The programme should integrate education, testing, clinical services and programme management.
6. There should be quality assurance, with mechanisms to minimize potential risks of screening.
7. The programme should ensure informed choice, confidentiality and respect for autonomy.
8. The programme should promote equity and access to screening for the entire target population.
9. Programme evaluation should be planned from the outset.
10. The overall benefits of screening should outweigh the harm.

Criterion 4 states that scientific evidence of screening program effectiveness is required. Evidence of genetic screening program effectiveness using a limited screening panel in ethnically identifiable sub-populations such as AJ genetic screening programs is strong. In these populations, in which carrier frequencies of particular serious genetic diseases (e.g. TSD) are high, the effectiveness of limited screening for specific mutations is high. In an unselected general population, carrier
frequencies for specific inherited single targeted mutations, as are targeted in AJ screened populations, would be expected in many cases to be reduced. However, the reality remains that all humans carry several recessive lethal or disease causing genetic mutations. While the majority of recessive mutations are inherited, a proportion of these may arise spontaneously in an individual.\textsuperscript{35} These mutations are responsible for a significant proportion of human disease.\textsuperscript{36} A given individual – regardless of their ethnic origin has a strong chance of carrying one or more serious genetic mutations that has been either inherited or acquired. In a general population as compared to an AJ population, we are less likely to prospectively predict the specific nature of these mutations. Thus highly limited DNA screening has limited clinical application in the prevention of disease in general populations. Casting a wider net using MPS expanded genetic screening has promising utility in the reduction of genetic disease, with the potential, if routinely used in the future, to have net health benefit on a population level. While on a population level, carrier frequencies for specific gene mutations are low, at-risk couples may have a 1 in 4 chance of having severely affected children.

\textbf{Table 2} examines the compliance of MPS expanded panel genetic screening to Andermann et al adapted criteria.
<table>
<thead>
<tr>
<th>Andermann criteria</th>
<th>MPS screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Recognised need</td>
<td>Yes, and consumer demand</td>
</tr>
<tr>
<td>2. Defined objectives at outset</td>
<td>Yes, but strong potential for unexpected results exists</td>
</tr>
<tr>
<td>3. Defined target population</td>
<td>Yes (Direct to consumer genetic screening targets a general population)</td>
</tr>
<tr>
<td>4. Evidence of program effectiveness</td>
<td>Limited. Existing evidence relates to existing genetic screening programs for a more limited number of conditions</td>
</tr>
<tr>
<td>5. Integrated program: Education, testing, follow-up, management.</td>
<td>Variable: Structured screening program vs. DTC models</td>
</tr>
</tbody>
</table>
Challenging Andermann et al. proposed criteria: MPS expanded panel genetic screening

Preventative medicine and health promotion

Genetic screening for serious recessive genetic conditions allows couples to have advanced warning of their risk of having a child affected with a lethal or severely debilitating genetic illness. These couples have the potential to have healthy, unaffected children. Reproductive technologies exist to assist an at-risk couple to achieve this. Without advanced knowledge of their genetic risk and treatment options, individuals and couples are denied access to the full range of existing reproductive options. These include antenatal diagnosis in the first trimester of pregnancy, pre-implantation genetic diagnosis prior to conception (through IVF with embryo biopsy), the use of donor oocyte or sperm to achieve a healthy pregnancy, or adoption.

The need for this intervention is to improve the quality of life of individuals and couples by improving their chances of having a healthy child. Even when no intervention is pursued, couples benefit from their reproductive choices being informed.  

Arguments in favour of MPS expanded panel genetic screening include long-term population level cost-benefit. By accessing reproductive technologies to assist at-risk
couples to have healthy families, on a health system level, fewer resources will be required to treat and palliate children with lethal and severely debilitating genetic disease. Fewer support services will be required to assist families of affected children (e.g. respite, counsellor services, carer pensions) and fewer families will be forced to leave the workforce.  

With MPS technologies, a pan-ethnic approach to accessing preconception genetic screening strategies will be feasible in the future. This will potentially extend the benefits of preconception genetic screening strategies demonstrated in small ethnically defined populations to all Australians.

As genetic sequencing technology evolves and costs reduce, in the future it is foreseeable that an argument will be made for pan-ethnic preconception genetic screening strategies as part of the movement for preventive medicine and health promotion. Evidence from expanded panel screening within Ashkenazi Genetic screening programs is an important resource to guide the development of future screening protocols and extend benefits to people of all ethnic origins. When mainstream pan-ethnic screening strategies are adopted, knowledge of personal genetic heritage will become less important in assessing genetic risk. This will be timely in the context of our multicultural “melting pot” Australian society, where ethnic origins are often mixed and carrier status for recessive conditions will be less reliably predicted by health care providers.
MPS in Personalised Medicine: Changes to our medical paradigm

The aim of evidence-based medicine is to determine therapy for a patient’s treatment that has been validated to be the most effective, safe and cost efficient on the basis of expert consensus and trial data. In the past, we have relied on clinicians and researchers experience of the effectiveness of interventions in groups of patients with a common diagnosis, based on disease phenotype or syndrome.

The gold standard interrogation of an intervention’s validity has been the double blinded randomised controlled trial.\textsuperscript{39} In this model, patients are normally analyzed within the treatment group to which they were randomly allocated, irrespective of whether they experienced the intended intervention (intention to treat analysis).\textsuperscript{40}

Despite inclusion and exclusion criteria for studies, multifactorial individual patient differences exist, meaning that the intervention and control groups are never identical. We also now know that due to genetic and epigenetic differences, no two individuals are identical. In acknowledgement of this, the concept of blinded randomisation aims to minimise potential biases that might obscure the findings of a study for or against the benefit of an intervention.

This concept is based on the ideal of choosing interventions that are the most likely to be of benefit in the majority of cases to have an optimised net effect on a population level.
Personalised medicine is a concept that challenges the normalised “best fit” aims of population based studies. The aim is to analyse the genetic (and it the future potentially the epigenetic) make up of an individual, or disease process (e.g. genetic sequencing of cancer clone populations) and target therapy for the individual based on these findings. In this scenario, interventions selected to target disease ontologies based on genetic classification may replace interventions selected based on syndromic phenotype. Future individual-specific targeting of intervention strategies in this way will be hoped to have positive effects on patient outcomes and quality of life. With continued progress in our collective understanding on the effect of our genetic make-up on disease outcomes, pan-ethnic population screening may play a role in health promotion, disease prevention and more effective treatment strategies in multifactorial genetic disease, beyond it’s current application in the detection and management of Mendelian genetic disease associated risk. This concept challenges the notion of defining a “target population” for screening strategies (Table 2, Criterion 3), as we consider each individual as a target for genetic screening and health promotion strategies.

**MPS: Secondary and Incidental findings (IFs)**

As exome and genome sequencing become integrated into mainstream medical care, genetic changes that predispose a healthy individual to develop diseases will be better understood. While instituting MPS technologies to answer specific diagnostic questions, the potential exists for recognition and reporting of findings
unrelated to the indication for ordering the sequencing (incidental or secondary findings) that may be of medical value for patient care.\textsuperscript{42}

The American College of Medical Genetics and Genomics (ACMG) in 2013 published a policy statement for the clinical application of whole exome or whole genome sequencing that emphasized the importance of prospectively explaining the possibility of MPS derived unexpected findings. ACMG initially recommended that clinical laboratories actively search and evaluate for certain pathogenic variants in 56 genes and advised these should be reported, regardless of patient preference for results disclosure.\textsuperscript{42} 24 of these IFs related to genes predisposing to cancers or cardiovascular disease. ACMG recommended that those who did not agree to learn of these IFs could choose to forego the entire test. This statement was subsequently modified in March 2014 to align with European guidelines and to address concerns about preserving patient autonomy.\textsuperscript{43}

The ultimate goal of ACMG recommendations is to positively impact patient care. To prioritize incidental reporting on gene variants of potential benefit to patients, while also allowing personal choice in decisions of IFs disclosure. Bioinformatics systems can apply data processing filters, limiting data analysis to the portion of the genome that the patient has chosen to learn about. Filters can be tailored to reflect individual patient choices. There may be great variability regarding the breadth of genomic disclosure individuals request.\textsuperscript{44}
Informed consent describes the goal that patients are aware of the potential risks and benefits of a test, treatment or study and that they voluntarily consent to participate. MPS based genetic screening prompts a review of traditional notions of informed consent. MPS generates a large amount of data, with a wide range of potential results. MPS has the capacity to detect nearly any disease-causing gene variant, imparting a broad range of relative risks for disease vary from high to zero. However, it will take time and collaborative scientific effort to definitively categorize all disease variants and to clarify the clinical importance of Variants Of Unknown Significance (VOUS). The novel aspect of MPS based testing is the generation of genomic data of enormous scale and scope, which is of potential medical relevance to a patient. Rapid technological progress and expansion of scientific and medical knowledge make it impossible to give a detailed explanation of or even to predict all the ways that stored genomic data might be used in the future at the time that biospecimens are collected.

In traditional genetic screening programs, screening objectives, risks and outcomes have been satisfactorily discussed in the context of screening for a limited range of conditions. Informed consent for genetic screening using MPS may only be achieved in a more generic sense than has previously been the accepted standard in genetic counselling and testing. Depending on the number of conditions screened for, the majority rather than a minority of participants can be expected to be diagnosed as a carrier of one or more conditions.
Many groups around the world are working on models for optimising the process of informed consent in the genomic era.\textsuperscript{45} No consensus exists on how this can best be achieved. Studies documenting the experiences of medical professionals and patients using exome sequencing in diagnostics will provide insight into the next steps needed to optimise the informed consent procedure in this context.\textsuperscript{49}

\textbf{Sydney Community Genetics Ashkenazi Jewish Preconception Screening: Evolution of a TSD screening program}

In Sydney, an AJ preconception genetic screening program targeting senior Jewish high school students has been operating since 1995. The original program design was based on international best-practice principles and screened for TSD only. Between 1996 and 2004, screening was conducted for TSD +/- cystic fibrosis. From 2005 onwards, screening was conducted for five genetic conditions (TSD, cystic fibrosis, Fanconi anaemia, Canavan Disease, familial dysautonomia), with carrier 12% detection rate for one or more conditions.

In 2013, a pilot study was proposed using an AJ MPS expanded screening panel, now underway. MPS screening was conducted in parallel to the existing screening program. The MPS panel studied consists of 25 genetic conditions (\textbf{Table 3}). The Sydney Community Genetics MPS screening panel design was based on International AJ screening program practice\textsuperscript{50,51,52-54} and PaLMS (later acquired by
Pathology North) Department of Laboratory and Community Genetics infrastructure and MPS testing capabilities.

The 2015 routine clinical panel (non MPS) is aligned to the HGSA recommended panel (Chapter 8).
Table 3: Current and pilot MPS panels for AJ preconception genetic screening

<table>
<thead>
<tr>
<th>Disease (* = Included in current screening panel)</th>
<th>Gene</th>
<th>No of mutations</th>
<th>Jewish Carrier Frequency</th>
<th>Disease phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tay Sachs disease (TSD)*</td>
<td>HEXA</td>
<td>14</td>
<td>1 in 27</td>
<td>Lethal in infancy/early childhood</td>
</tr>
<tr>
<td>Canavan disease (CD)*</td>
<td>ASPA</td>
<td>4</td>
<td>1 in 55</td>
<td>Lethal in infancy/early childhood</td>
</tr>
<tr>
<td>Familial dysautonomia (FD)*</td>
<td>IKBKAP</td>
<td>2</td>
<td>1 in 31</td>
<td>Severe disability</td>
</tr>
<tr>
<td>Bloom disease*</td>
<td>BLM</td>
<td>1</td>
<td>1 in 134</td>
<td>Severe disability, cancer susceptibility</td>
</tr>
<tr>
<td>Fanconi anaemia (FA)*</td>
<td>FANCC</td>
<td>4</td>
<td>1 in 100</td>
<td>Various birth defects, cancer susceptibility</td>
</tr>
<tr>
<td>Niemann-Pick disease (A&amp;B)*</td>
<td>SMPD1</td>
<td>4</td>
<td>1 in 115</td>
<td>Lethal in childhood</td>
</tr>
<tr>
<td>Mucolipidosis type 4*</td>
<td>MCOLN</td>
<td>2</td>
<td>1 in 89</td>
<td>Severe psychomotor retardation</td>
</tr>
<tr>
<td>Glycogen storage disease 1a</td>
<td>G6PC</td>
<td>2</td>
<td>1 in 64</td>
<td>Severe metabolic dysfunction; early diagnosis improves outcomes ++</td>
</tr>
<tr>
<td>Cystic fibrosis (CF)*</td>
<td>CFTR</td>
<td>11</td>
<td>1 in 29</td>
<td>Spectrum of severe to milder disease. Reduced life expectancy</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>GBA</td>
<td>6</td>
<td>1 in 15</td>
<td>Multi-organ system disease. Treatment improves outcomes ++</td>
</tr>
<tr>
<td>Maple syrup urine disease Type 1B</td>
<td>BCKDH</td>
<td>3</td>
<td>1 in 97</td>
<td>Lethal in infancy if metabolic crisis untreated. Treatment improves outcome ++</td>
</tr>
<tr>
<td>Hyperinsulinism</td>
<td>ABCC8</td>
<td>2</td>
<td>1 in 68</td>
<td>Lethal in infancy if untreated. Treatment improves outcome +</td>
</tr>
<tr>
<td>Dihydrolipoamide dehydrogenase deficiency (E3)</td>
<td>DLD</td>
<td>1</td>
<td>1 in 107</td>
<td>Lethal in infancy if metabolic crisis untreated. Treatment sometimes improves outcome</td>
</tr>
<tr>
<td>Usher syndrome type 3</td>
<td>CLRN1</td>
<td>1</td>
<td>1 in 120</td>
<td>Progressive visual and hearing loss with vestibular dysfunction</td>
</tr>
<tr>
<td>Usher syndrome type 1F</td>
<td>PCDH1</td>
<td>5</td>
<td>1 in 147</td>
<td>Progressive visual and hearing loss, vestibular dysfunction</td>
</tr>
<tr>
<td>Nemaline myopathy (NM)</td>
<td>NEB</td>
<td>1</td>
<td>1 in 168</td>
<td>Neuromuscular dystrophy. Severe disability</td>
</tr>
<tr>
<td>Joubert syndrome T2</td>
<td>TMEM2</td>
<td>1</td>
<td>1 in 92</td>
<td>Motor disability +/- mental retardation</td>
</tr>
<tr>
<td>Spinal muscle atrophy</td>
<td>SMN1</td>
<td>1</td>
<td>1 in 41</td>
<td>Progressive muscle weakness, severe disability</td>
</tr>
<tr>
<td>Walker Walburg syndrome</td>
<td>FKTN</td>
<td>1</td>
<td>1 in 149</td>
<td>Severe psycho-motor retardation</td>
</tr>
<tr>
<td>Haemophilia C</td>
<td>F11</td>
<td>3</td>
<td>1 in 23</td>
<td>Mild haemophilia.</td>
</tr>
<tr>
<td>Familial Mediterranean fever</td>
<td>MEFV</td>
<td>15</td>
<td>1 in 5</td>
<td>Painful attacks, AA amaloidosis / chronic renal failure</td>
</tr>
<tr>
<td>Deafness</td>
<td>GJB2</td>
<td>2</td>
<td>1 in 25</td>
<td>Deafness</td>
</tr>
<tr>
<td>Glycogen storage disease Type 3</td>
<td>GLD</td>
<td>1</td>
<td>1 in 35</td>
<td>Organomegally, variable psycho-motor retardation, hypotonia, cardiovascular abnormalities</td>
</tr>
<tr>
<td>Alpha-1 antitrypsin deficiency</td>
<td>SERPINA1</td>
<td>4</td>
<td>1 in 30</td>
<td>Lung and liver failure. Reduced life expectancy</td>
</tr>
</tbody>
</table>
Australian AJ Preconception genetic screening programs provide an environment to assess the clinical and psychosocial impact of using MPS-based genetic screening in a pilot study. Multiple studies are underway assessing the clinical, psychosocial, safety implications of MPS based screening in an Australian adolescent AJ target population. Preliminary results are expected to be available in late 2016, however ongoing assessment of this cohort will continue into the future to assess longer-term impacts and reproductive outcomes.

In the planning phase of this study, I undertook research to model the predicted diagnostic and medical workforce implication of switching from targeted genetic testing for the 5 recessive conditions included in routine screening until 2015, to an expanded MPS based panel for 25 conditions in an AJ cohort (Appendix 1). This research assisted in planning of the AJ MPS pilot study currently underway.

MPS expanded screening differs from previous AJ screening program models in three ways;

1. A greater number of genetic conditions are screened for simultaneously.
2. Targeted conditions are serious, but many are of relatively low prevalence compared to TSD and cystic fibrosis.
3. Targeted genomic data is recorded; this resource could be subject to reinterpretation and discovery of unexpected results that fall outside of the original screening objectives.
Whether and how unexpected results should be reported has been the subject of vigorous debate.\textsuperscript{55-57} Informatics tools are currently being developed to assist in management of result reporting.\textsuperscript{58, 59} In Appendix 1 modelling of primary, secondary and incidental findings expected in a 25 condition AJ MPS screening panel are explored. A summary of the major findings reported in this work is outlined below.

**Modelling of primary, secondary and incidental findings expected in a 25 condition AJ MPS screening panel**

Massively Parallel DNA Sequencing for Ashkenazi Jewish community pre-conception genetic screening programs: Predicted outcomes, ethical and workforce implications (Poster 1)

I estimated the clinical impact of AJ preconception genetic screening using an MPS panel, designed to include 25 autosomal recessive (AR) genetic conditions where the homozygous phenotype has serious health implications. Conditions included those having an AJ carrier frequency ranging from 1 in 5 (\textit{MEFV} Familial Mediterranean fever, variable penetrance) to 1 in 168 (\textit{NEB} Nemaline myopathy). The panel was designed based on international practice, principles for screening and the diagnostic capabilities at PaLMS Pathology North, Royal North Shore Hospital.

In the modelling, the average number of AJ individuals screened per annum was estimated from AJ screening program data. Internationally published AJ allele prevalence for conditions (Appendix 1, Poster 1) were used to calculate the
predicted number of carriers of one or more conditions that would be detected using the expanded MPS panel versus the current screening method targeting five conditions (TSD (carrier frequency 1 in 27), cystic fibrosis (carrier frequency 1 in 29), Canavan disease (carrier frequency 1 in 55), Fanconi anaemia (carrier frequency 1 in 100), familial dysautonomia (carrier frequency 1 in 31)).

It was found that while the five conditions AJ panel has an a priori carrier risk for at least one condition of 12%, the expanded MPS panel has an a priori carrier risk for at least one condition of 51%. The probability calculation used was $1 - [(1-P_1)*(1-P_2)*\ldots*(1-P_x)]$, where $P_1$ to $P_{25}$ refer to the carrier frequency of conditions listed in Poster 1, presented below in Figure 1.
INTRODUCTION

Mature minors can provide informed consent for conventional DNA testing for a limited number of conditions screened for and method of laboratory experience implementing this model (2005-12), the observed number of carrier of one or more conditions.

A panel of conditions was designed for use in AJ preconception genetic screening for multiple relevant autosomal recessive conditions (Table 2).

Some 50% (1 in 2) AJ individuals screened with the proposed MPS panel would be diagnosed a carrier for a priori risk carrier risk (for at least 1 condition) of 12%.

Conventional DNA testing for 5 conditions of conditions that would be detected using the expanded panel, vs. the current screening program data.

The probability calculation used was 1- \[(1-P1)*(1-P2)*…(1-Px)\], where p1 to p25 refer to the number of participant, where P represents the probability of not being a carrier for a particular condition.

Predicted outcomes, ethical and workforce implications of the implementation of diagnostic genome sequencing technologies  (MPS) as the routine modality,  extending screening to  a broader range of conditions.

In the proposed MPS screening model, ((Table 1, conditions 1 to 25), the a priori risk of conditions screened for and method of laboratory

DISCUSSION

Predicted outcomes, ethical and workforce implications of the implementation of diagnostic genome sequencing technologies  (MPS) as the routine modality,  extending screening to  a broader range of conditions.

In the proposed MPS screening model, ((Table 1, conditions 1 to 25), the a priori risk of conditions screened for and method of laboratory

Study of the clinical impact of  AJ preconception genetic screening using an MPS practice.

Nucleic Acids Res

1)

Development of health policy regarding ethical, legal and workforce implications of the implementation of diagnostic genome sequencing technologies  (MPS) as the routine modality,  extending screening to  a broader range of conditions.

In the Sydney program's experience implementing this model (2005-12), the observed number of carrier

Outcome Summary

<table>
<thead>
<tr>
<th>Year</th>
<th>Participants</th>
<th>Carriers</th>
<th>Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>170</td>
<td>6</td>
<td>TSD</td>
</tr>
<tr>
<td>1996</td>
<td>123</td>
<td>13</td>
<td>TSD, CF</td>
</tr>
<tr>
<td>1997</td>
<td>181</td>
<td>4</td>
<td>TSD</td>
</tr>
<tr>
<td>1998</td>
<td>178</td>
<td>13</td>
<td>TSD, CF</td>
</tr>
<tr>
<td>1999</td>
<td>175</td>
<td>11</td>
<td>TSD, CF</td>
</tr>
<tr>
<td>2000</td>
<td>166</td>
<td>13</td>
<td>TSD, CF</td>
</tr>
<tr>
<td>2001</td>
<td>172</td>
<td>6</td>
<td>TSD</td>
</tr>
<tr>
<td>2002</td>
<td>192</td>
<td>4</td>
<td>TSD</td>
</tr>
<tr>
<td>2003</td>
<td>215</td>
<td>10</td>
<td>TSD</td>
</tr>
<tr>
<td>2004</td>
<td>242</td>
<td>6</td>
<td>TSD</td>
</tr>
<tr>
<td>2005</td>
<td>245</td>
<td>28</td>
<td>CF,TSD,CD,FD,FA</td>
</tr>
<tr>
<td>2006</td>
<td>298</td>
<td>34</td>
<td>CF,TSD,CD,FD,FA</td>
</tr>
<tr>
<td>2007</td>
<td>254</td>
<td>32</td>
<td>CF,TSD,CD,FD,FA</td>
</tr>
<tr>
<td>2008</td>
<td>281</td>
<td>22</td>
<td>CF,TSD,CD,FD,FA</td>
</tr>
<tr>
<td>2009</td>
<td>252</td>
<td>43</td>
<td>CF,TSD,CD,FD,FA</td>
</tr>
<tr>
<td>2010</td>
<td>220</td>
<td>23</td>
<td>CF,TSD,CD,FD,FA</td>
</tr>
<tr>
<td>2011</td>
<td>278</td>
<td>37</td>
<td>CF,TSD,CD,FD,FA</td>
</tr>
<tr>
<td>2012</td>
<td>252</td>
<td>28</td>
<td>CF,TSD,CD,FD,FA</td>
</tr>
</tbody>
</table>

Carriers detected(%)

<table>
<thead>
<tr>
<th>Carriers detected(%)</th>
<th>TSD</th>
<th>TSD,CF</th>
<th>CF,TSD,CD,FD,FA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>36(3%)</td>
<td>50(8%)</td>
<td>247(12%)</td>
</tr>
</tbody>
</table>

Figure 1: Outcome Summary Data, Sydney Jewish community genetic screening programs (presented in Poster 1)
To estimate the clinical impact of AJ preconception genetic screening using an MPS practice.

**Nucleic Acids Res**

**Australian Jews.**

1) to the carrier frequency of each condition referenced 1 to 25 in Table 1. The probability calculation used was \(1 - [(1-P_1)*(1-P_2)*…(1-P_{25})]\), where \(p_1\) to \(p_{25}\) refer for genetic counselling. This escalation highlights future workforce experience implementing this model (2005-12), the observed number of carrier participants (12% - see Table 2) was in agreement with this predicted number, and current practice is to offer all identified carriers a referral for genetic counselling.

**AIM**

Current practice is to offer all identified carriers a referral for genetic counselling. If this practice were to continue with the introduction of MPS based expanded screening, a more-than-four-fold increase in referrals for genetic counselling would be generated from screening of the same target population.

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### Table 1: Disease (\(^*\) = Included in current screening panel)

<table>
<thead>
<tr>
<th>Probability</th>
<th>Disease ((^*) = Included in current screening panel)</th>
<th>Gene</th>
<th>No of mutations</th>
<th>Jewish Carrier Frequency ((P))</th>
<th>(1-P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Tay Sachs disease (TSD)*</td>
<td>HEXA</td>
<td>14</td>
<td>1 in 27</td>
<td>0.96</td>
</tr>
<tr>
<td>P2</td>
<td>Canavan disease (CD)*</td>
<td>ASPA</td>
<td>4</td>
<td>1 in 55</td>
<td>0.98</td>
</tr>
<tr>
<td>P3</td>
<td>Familial Dysautonomia (FD)*</td>
<td>IKBKAP</td>
<td>2</td>
<td>1 in 31</td>
<td>0.97</td>
</tr>
<tr>
<td>P4</td>
<td>Bloom disease</td>
<td>BLM</td>
<td>1</td>
<td>1 in 134</td>
<td>0.99</td>
</tr>
<tr>
<td>P5</td>
<td>Fanconi Anaemia (FA)*</td>
<td>FANCC</td>
<td>4</td>
<td>1 in 100</td>
<td>0.99</td>
</tr>
<tr>
<td>P6</td>
<td>Niemann-Pick (A&amp;B)*</td>
<td>SMPD1</td>
<td>4</td>
<td>1 in 115</td>
<td>0.99</td>
</tr>
<tr>
<td>P7</td>
<td>Mucolipidosis type 4</td>
<td>MCOLN</td>
<td>1</td>
<td>1 in 89</td>
<td>0.99</td>
</tr>
<tr>
<td>P8</td>
<td>Glycogen storage disease 1a</td>
<td>G6PC</td>
<td>2</td>
<td>1 in 64</td>
<td>0.98</td>
</tr>
<tr>
<td>P9</td>
<td>Cystic fibrosis (CF)*</td>
<td>CFTR</td>
<td>11</td>
<td>1 in 29</td>
<td>0.97</td>
</tr>
<tr>
<td>P10</td>
<td>Gaucher Disease</td>
<td>GBA</td>
<td>6</td>
<td>1 in 15</td>
<td>0.93</td>
</tr>
<tr>
<td>P11</td>
<td>Maple Syrup disease Type 1B</td>
<td>BCKDHB</td>
<td>3</td>
<td>1 in 97</td>
<td>0.99</td>
</tr>
<tr>
<td>P12</td>
<td>Hyperinsulinism</td>
<td>ABCC8</td>
<td>2</td>
<td>1 in 68</td>
<td>0.99</td>
</tr>
<tr>
<td>P13</td>
<td>Dihydrolipoamide Dehydrogenase Deficiency (E3)</td>
<td>DLD</td>
<td>1</td>
<td>1 in 107</td>
<td>0.99</td>
</tr>
<tr>
<td>P14</td>
<td>USH3</td>
<td>CLRN1</td>
<td>1</td>
<td>1 in 120</td>
<td>0.99</td>
</tr>
<tr>
<td>P15</td>
<td>USH1F</td>
<td>PCDH15</td>
<td>1</td>
<td>1 in 147</td>
<td>0.99</td>
</tr>
<tr>
<td>P16</td>
<td>Nemaline Myopathy(NM)</td>
<td>NEB</td>
<td>1</td>
<td>1 in 168</td>
<td>0.99</td>
</tr>
<tr>
<td>P17</td>
<td>Joubert Syndrome T2</td>
<td>TMEM2</td>
<td>1</td>
<td>1 in 92</td>
<td>0.99</td>
</tr>
<tr>
<td>P18</td>
<td>Spinal Muscle atrophy</td>
<td>SMN1</td>
<td>1</td>
<td>1 in 41</td>
<td>0.98</td>
</tr>
<tr>
<td>P19</td>
<td>Walker Walburg</td>
<td>FKTN</td>
<td>1</td>
<td>1 in 149</td>
<td>0.99</td>
</tr>
<tr>
<td>P20</td>
<td>Haemophilia C</td>
<td>F11</td>
<td>3</td>
<td>1 in 23</td>
<td>0.96</td>
</tr>
<tr>
<td>P22</td>
<td>Familial Mediterranean fever</td>
<td>MEFV</td>
<td>15</td>
<td>1 in 5</td>
<td>0.80</td>
</tr>
<tr>
<td>P23</td>
<td>Deafness</td>
<td>GJB2</td>
<td>2</td>
<td>1 in 25</td>
<td>0.99</td>
</tr>
<tr>
<td>P24</td>
<td>Glycogen storage disease T3</td>
<td>GDE/AGL</td>
<td>1</td>
<td>1 in 35</td>
<td>0.97</td>
</tr>
<tr>
<td>P25</td>
<td>Alpha 1 antitrypsin Deficiency</td>
<td>SERPINA</td>
<td>1</td>
<td>1 in 30</td>
<td>0.98</td>
</tr>
</tbody>
</table>
While it has been recognised that traditional notions of informed consent are challenged by the magnitude of genetic information generated by MPS based screening, no consensus exists as to how this issue should be navigated. While there is robust evidence supporting the safety, efficacy and cost efficiency of targeted genetic screening in mature minors, prior to pregnancy planning, translational research is needed to assess the safety and ongoing psychosocial impacts of MPS based expanded genetic screening in adolescent populations.

Reporting findings of Massively Parallel Sequencing: A model for genetic screening of autosomal recessive conditions in at-risk communities (Poster 2)

MPS based genetic screening was arbitrarily limited in our previous work to encompass 25 autosomal recessive conditions (Poster 1). However as MPS technology attains greater speed, greater accuracy and lower cost, parallel screening for an even broader range of conditions may be expected to become routine.

We developed and validated a mathematical model to predict the AR genetic carrier rate in a dataset or population as the testing panel expands. This model was used to study the effects of increasing the number of AR conditions included in population screening programs, using data from Australian AJ genetic screening program as a prototype.
To simulate the effect of increasing the number of conditions tested for, each carrier frequency for the 26 genetic conditions most prevalent in the AJ community was added to the model one-by-one from most to least frequent, and the cumulative AR carrier rate was calculated. The simulation panel represented the 25 AR conditions listed in Figure 2, with the addition of one semi-dominant condition, familial hypercholesterolaemia (LDLR). There was good agreement between the predictions of our model and the actual rate of AR genetic carriers detected in the Australian AJ community genetic screening program. Expansion of the AJ testing menu to include 26 conditions was predicted to result in 58% of the AJ screened population to be diagnosed as a carrier for one or more conditions (Appendix 1, Poster 2). We showed the primary driver of the AR genetic carrier rate is the number of tested conditions. A mathematical model was developed based on the binomial distribution: 

\[ p(X) = 1 - [(1-P_1) \cdot (1-P_2) \cdot ... (1-P_n)] \]

where \( P_1 \) to \( P_n \) are the carrier frequencies of various genetic conditions and \( p(X) \) is the probability of being a genetic carrier for any one of these conditions (assuming all conditions are inherited independently).

The model was validated *in silico* using the testing panels and data from Australian AJ community genetics screening programs. Known or best estimate carrier frequencies for the 26 most prevalent AR conditions in the AJ community were obtained from published literature. The predicted annual detection rate of AR genetic carriers was compared to the actual number of carriers.

To simulate the effect of increasing the number of tested conditions, each carrier frequency was iteratively added to the model one by one, from the most to least frequent, to calculate the cumulative AR carrier rate. The carrier frequency was
varied over a 4-fold range to simulate the effect of uncertainty in carrier prevalence or errors in sequencing on the predicted AR carrier rate.

This is in contrast to autosomal dominant conditions where the proportion of individuals with a pathogenic variant plateaus as the number of conditions tested for increases.\textsuperscript{6}


(Presented in Poster 2)
Figure 4: Predicted proportion of AR genetic carriers with increasing number of AR conditions tested for (Presented in Poster 2)

Individuals of AJ heritage represent a minority of Australians, and health system implications due to the expected increased number of AJ genetic carriers identified by MPS based screening will be modest overall. However our mathematical models, validated with data generated from longitudinal experience of Australian AJ genetic screening programs, highlight important workforce and health system infrastructural implications expected when MPS based genetic testing and screening become integrated into mainstream genomic preventative medicine.

Reporting Incidental Findings of Massively Parallel sequencing: Predicted implications of the American College of Medical Genetics and Genomics (ACMG) recommendations (Poster 3)
The ACMG has recently published recommendations for the clinical reporting of 24 specific genetic conditions if diagnosed incidentally during MPS based testing.\textsuperscript{7,8} We applied our mathematical modelling to a simulated MPS diagnostic panel to calculate the proportion of individuals screened who would require supplementary consultation and genetic counselling due to the detection of 1 or more of the ACMG’s 24 incidental findings (IFs) recommended to be reported (Figure 5).
### Table 2: Carrier frequency estimates of recommended conditions

<table>
<thead>
<tr>
<th>Probability</th>
<th>Disease Phenotype</th>
<th>Carrier Frequency (Low estimate)</th>
<th>Carrier Frequency (Most likely estimate)</th>
<th>Carrier Frequency (High estimate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Hereditary Breast and Ovarian Cancer</td>
<td>0.1060%^3,4</td>
<td>0.4589%</td>
<td>2.8820%^4,5</td>
</tr>
<tr>
<td>P2</td>
<td>Li-Fraumeni Syndrome</td>
<td>0.0050%^6</td>
<td>0.0100%</td>
<td>0.0200%^6</td>
</tr>
<tr>
<td>P3</td>
<td>Peutz-Jeghers Syndrome</td>
<td>0.0004%^7</td>
<td>0.0012%</td>
<td>0.0040%^7</td>
</tr>
<tr>
<td>P4</td>
<td>Lynch Syndrome</td>
<td>0.0500%^8</td>
<td>0.1066%</td>
<td>0.2273%^8</td>
</tr>
<tr>
<td>P5</td>
<td>Familial adenomatous polyposis</td>
<td>0.0023%^9</td>
<td>0.0027%</td>
<td>0.0032%^9</td>
</tr>
<tr>
<td>P6</td>
<td>MYH-Associated Polyposis; Adenomas, multiple colorectal, FAP type 2, Colorectal adenomatous polyposis, autosomal recessive, with pilimatricomas</td>
<td>1.0000%^10</td>
<td>1.4142%</td>
<td>2.0000%^10</td>
</tr>
<tr>
<td>P7</td>
<td>Von Hippel Lindau syndrome</td>
<td>0.0014%</td>
<td>0.0028%^11</td>
<td>0.0056%</td>
</tr>
<tr>
<td>P8</td>
<td>Multiple Endocrine Neoplasia Type 1 (MEN1)</td>
<td>0.0017%</td>
<td>0.0033%^12</td>
<td>0.0067%</td>
</tr>
<tr>
<td>P9</td>
<td>Multiple Endocrine Neoplasia Type 2 (MEN2)</td>
<td>0.0014%</td>
<td>0.0029%^13</td>
<td>0.0057%</td>
</tr>
<tr>
<td>P10</td>
<td>Familial Medullary Thyroid Cancer (FMTC)</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>P11</td>
<td>PTEN Hamartoma Tumor Syndrome</td>
<td>0.0003%</td>
<td>0.0005%^14</td>
<td>0.0010%</td>
</tr>
<tr>
<td>P12</td>
<td>Retinoblastoma</td>
<td>0.0067%^15</td>
<td>0.0058%</td>
<td>0.0050%^15</td>
</tr>
<tr>
<td>P13</td>
<td>Hereditary Paraganglioma-Pheochromocytoma Syndrome</td>
<td>0.0001%^16</td>
<td>0.0003%</td>
<td>0.0009%^16</td>
</tr>
<tr>
<td>P14</td>
<td>Tuberous Sclerosis Complex</td>
<td>0.0086%</td>
<td>0.0172%^17</td>
<td>0.0345%</td>
</tr>
<tr>
<td>P15</td>
<td>WT1-related Wilms tumor</td>
<td>0.0005%^18</td>
<td>0.0006%</td>
<td>0.0006%^18</td>
</tr>
<tr>
<td>P16</td>
<td>Neurofibromatosis type 2</td>
<td>0.0025%^19</td>
<td>0.0028%</td>
<td>0.0030%^19</td>
</tr>
<tr>
<td>P17</td>
<td>EDS - vascular type</td>
<td>0.0005%^20</td>
<td>0.0010%</td>
<td>0.0020%^20</td>
</tr>
<tr>
<td>P18</td>
<td>Marfan Syndrome, Loeys-Dietz Syndromes, and Familial Thoracic Aortic Aneurysms and Dissections</td>
<td>0.0102%^21,22,23</td>
<td>0.0144%</td>
<td>0.0205%^21,22,23</td>
</tr>
<tr>
<td>P19</td>
<td>Hypertrophic cardiomyopathy, Dilated cardiomyopathy</td>
<td>0.1100%^24</td>
<td>0.1241%</td>
<td>0.1400%^24</td>
</tr>
<tr>
<td>P20</td>
<td>Catecholaminergic polymorphic ventricular tachycardia</td>
<td>0.0050%^25</td>
<td>0.0058%</td>
<td>0.0066%^25</td>
</tr>
<tr>
<td>P21</td>
<td>Arrhythmogenic right ventricular cardiomyopathy</td>
<td>0.0800%^26</td>
<td>0.2530%</td>
<td>0.8000%^26</td>
</tr>
<tr>
<td>P22</td>
<td>Romano-Ward Long QT Syndromes Types 1, 2, and 3, Brugada Syndrome</td>
<td>0.0143%^27</td>
<td>0.0218%</td>
<td>0.0333%^27</td>
</tr>
<tr>
<td>P23</td>
<td>Familial Hypercholesterolemia</td>
<td>0.1500%</td>
<td>0.3000%^28</td>
<td>0.6000%</td>
</tr>
<tr>
<td>P24</td>
<td>Malignant hyperthermia susceptibility</td>
<td>0.0010%</td>
<td>0.0071%^29</td>
<td>0.0500%</td>
</tr>
</tbody>
</table>

Each probability, P1 – P24, represent the combined probabilities of the conditions listed. E.g. P1 represents the combined probabilities of BRCA1 and BRCA2. Frequency estimates with a white background represent values found in the literature, while those with grey backgrounds indicate calculated estimates (see Methods).

* Probabilities for FMTC included with those of the related syndrome MEN2

We found approximately 2.7% (1.5 to 6.5%) of individuals screened would require supplementary counselling because of the detection of IFs. As the list of IFs recommended for clinical reporting is likely to expand over time, the issue of MPS identified IFs of clinical relevance is likely to result in major net increases in health...
care resources. Further studies, medical infrastructure and workforce planning around this issue should be considered.

Implications of Massively Parallel Sequencing in screening for Autosomal Recessive conditions: the risk of being a “genetic wallflower” (Poster 4)

We undertook mathematical modelling to better understand the impact of expanded AJ MPS genetic screening on the number of at-risk carrier couples expected to be identified via screening. We also explored the likelihood of genetic wallflowers emerging (Appendix 1, Poster 4). Mathematical modelling based on binomial distribution was used to predict the number of AR carriers based on prevalence of pathogenic variants and number of tested conditions. Monte Carlo simulation was used to calculate the probability of any two individuals in this screened population being carriers of exactly the same AR genetic conditions.
Figure 6:

A. Predicted rate of AR genetic carriers with increasing number of AR conditions tested

![Graph showing cumulative carrier rate with increasing number of genes tested](image-url)
B. Average number of variants in an individual with increasing number of AR conditions tested

![Graph showing average number of variants in an individual with increasing number of AR conditions tested.](image)

While the number of genetic carriers for AR conditions is expected to expand quickly with expanded MPS based screening panels, the expansion of the number of at-risk carrier couples is predicted to expand more slowly (Figure 7). We are currently undertaking work to further quantify this. These findings have significant implications for health economic evaluation and planning.
Figure 7: Average proportion of couples at-risk of an AR affected child with increasing number of AR conditions tested

Education strategies for AJ preconception screening programs are highly effective. Participants understand in depth the natural history and recessive inheritance of screened conditions and are introduced to potential reproductive interventions. Knowledge has been demonstrated to be well retained in 5 to 11 year follow-up studies.\(^8\) The same depth of understanding for a panel of 25+ inherited conditions may not be a reasonable expectation. Studies of participants’ knowledge and perceptions prior to and after pre-test education, as well as longer-term studies of knowledge retention and quality of life impacts are needed.

Should MPS screening pilot studies support the further application of MPS based genetic screening programs to an expanded target group, longitudinal access planning (including costing and potential health budget funding) and ongoing impact evaluation will be mandated in the future.
The planning and primary analysis of MPS based pre-conception genetic screening strategies represents a first step in adapting this technology to diagnostic applications to benefit individuals and population health. The publications described in Appendix 1 outline work undertaken in the planning phase of the MPS AJ preconception screening pilot study. Expansion of this work is underway, and will progress beyond the scope of this thesis.

References


Chapter 8 Human Genetics Society of Australasia position paper: Australasian Ashkenazi Jewish genetic screening

These guidelines were prepared by invitation for the Joint Human Genetics Society of Australasia/Royal Australian and New Zealand College of Obstetricians and Gynaecologists Prenatal Diagnosis and Screening Committee. They are designed to support the transition of AJ preconception genetic screening strategies to universally accessible primary health care settings.

As chairperson and first author of these guidelines I convened a committee with multidisciplinary expertise to devise a framework to devise and to debate these guidelines. I then wrote the guidelines and brought them back to the committee for review. I instituted suggested revisions from both the committee and peer review.

Recommendations are designed to be relevant for all individuals who are involved in pre-pregnancy and antenatal care, including, but not limited to General Practitioners, Obstetricians and Gynaecologists, Clinical Geneticists and Genetic Counselors.

In 2015, this guideline was ratified by the HGSA, and published with unrestricted access (https://www.hgsa.org.au/documents/item/6092).
Position Paper

Ashkenazi Jewish Population Screening

Document Number 2015PS01
Publication Date February 2015
Replaces NEW

This document has been produced by a committee convened by the Joint Human Genetics Society of Australia/Royal Australian and New Zealand College of Obstetricians and Gynaecologists Prenatal Diagnosis and Screening Committee.

The committee members were:
Raelia Lew – Chair (NSW/VIC RANZCOG CREI Fellow)
Harry Aizenberg (NSW, pathologist, Community Liaison and Lay Member)
Leslie Burnett (NSW, pathologist, chemical and genetic pathology)
Megan Cotter (VIC, genetic counsellor)
Martin Delatycki (VIC, clinical geneticist)
Anné L. Proos (NSW, biochemical and molecular genetics scientist)

These guidelines are relevant for all individuals who are involved in pre-pregnancy and antenatal care, including, but not limited to, General Practitioners, Obstetricians and Gynaecologists, Clinical Geneticists and Genetic Counsellors.

The process:
The HGSA Ashkenazi Jewish population screening committee met by teleconference on two occasions between January and May 2014. Each element of population-based carrier screening in the Ashkenazi Jewish community was debated and consensus reached. The draft policy was circulated to each committee member for individual comment. The policy was submitted to the Joint Human Genetics Society of Australasia/Royal Australian and New Zealand College of Obstetricians and Gynaecologists Prenatal Diagnosis and Screening Committee and was approved for publication by the Executive of the Human Genetics Society of Australasia in March 2015.
Summary of Recommendations

The HGSA TSD Population Screening Committee recommends that:

1. All AJ individuals of reproductive age should be made aware of the availability of AJ preconception genetic screening for severe monogenetic conditions and offered access to screening for TSD, CF, FD, FA NPD, BLM, CD and MPLIV.

2. For all screened conditions except TSD, screening is by mutation detection. For TSD, screening can be by mutation detection or enzyme analysis for AJ individuals but should be by enzyme analysis for non-AJ individuals screened.

3. The gold standard should be considered to be a combination of high school genetic screening programs, outreach community programs, opportunistic screening by medical practitioners and preconception screening. No single approach is likely to be sufficient in providing a comprehensive screening strategy for the Australian AJ population.

4. Preconception screening is preferable to antenatal screening. Where screening is conducted during pregnancy, screening of both parents concurrently and without delay is preferred.

5. Where an AJ individual is diagnosed as a carrier for one or more severe monogenetic conditions, their partner should be offered screening for the condition(s) in question, regardless of AJ heritage. Residual risk counselling is required when screening indicates that a non-AJ partner is a non-carrier for the condition(s) in question.

6. Best practice testing methodology will differ for AJ and non-AJ individuals, as will test sensitivity and residual risk. AJ/non-AJ patient background and any known family history of AJ genetic disease should be documented on pathology requests and communicated to the testing laboratory. Testing laboratories should comply with international best-practice standards and quality assurance procedures.

7. Primary care clinicians, including but not limited to General Practitioners, and Obstetricians and Gynaecologists, are encouraged to provide access to genetic screening in AJ patients. Enquiry of ethnic background should be made on medical history taking. Clinicians should consider and offer AJ genetic screening opportunistically when at-risk patients present for other reasons (i.e. general or sexual health check).

8. Primary health care providers should be aware of different screening strategies to maximise cost-benefit.

9. Pre-test education should be delivered by a qualified health professional. Details of conditions screened for including clinical features, mode of inheritance and implications for relatives should be discussed. Information should be imparted regarding reproductive options for at-risk couples. Voluntary informed consent should be obtained from the patient and should be documented.

10. Carriers of one or more conditions detected by AJ genetic screening should be fully informed of the significance of their results. Current and future partners of carriers should be offered screening. Cascade screening should be offered to relatives of these genetic carriers.

11. Carrier couples should be referred for specialist genetic counselling. In the context of pregnancy, carrier couples should be referred for Obstetrician +/- subspecialist Maternal Fetal Medicine review without delay.

12. These recommendations should be promulgated to relevant learned Medical Colleges and health care professional Associations and Societies, as well as to genetic support groups and consumer health organisations (section 10).
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1.4 Non-Standard Abbreviations

AJ – Ashkenazi Jewish
TSD – Tay Sachs disease
CF – Cystic fibrosis
CD – Canavan disease
FD – Familial dysautonomia
NPDA - Niemann Pick disease type A
BLM – Bloom syndrome
MLPIV - Mucolipidosis Type IV
FA - Fanconi anaemia
GD - Gaucher disease
ACOG - The American Congress of Obstetricians and Gynecologists
ACMG - The American College of Medical Genetics and Genomics
SOGC - Society of Obstetricians and Gynaecologists of Canada
UK NSC - United Kingdom National Screening Committee
2. Introduction

2.1 Background
Population screening refers to testing for heterozygous carrier status in individuals without a family history of disease. The health implications are not for the individuals tested but for their children. For serious monogenetic diseases, knowledge of carrier status prior to pregnancy allows carrier couples to be informed and exercise reproductive choice. Classic principles of population genetic screening (Wilson & Jungner), reviewed for the genomic age (World Health Organization) are relevant to preconception and antenatal genetic screening.(1)

The 2011 Australian Bureau of Statistics (ABS) census estimates the current overall population of Jewish Australians to be 97,335 representing a 9.58% increase from the previous 5-year census period.(2) The term ‘Ashkenazi’ describes Jewish populations with ancestry from Central and Eastern Europe. The majority of Jewish Australians have AJ heritage. Disease-causing founder mutations for a range of recessive genetic conditions have been identified in AJ populations. These conditions may be more common in AJ populations (e.g. Tay Sachs disease, AJ carrier frequency 1 in 25), almost exclusive to AJ individuals (e.g. familial dysautonomia, AJ carrier frequency 1 in 40) or present at similar prevalence in AJ individuals to other communities (e.g. cystic fibrosis, AJ carrier frequency 1 in 23). In AJ individuals, however, a small number of identified AJ founder mutations are responsible for the vast majority of disease. This small number of founder mutations simplifies many of the technical complexities involved in laboratory testing and screening. Genetic carrier screening for severe monogenetic conditions is well accepted by AJ communities worldwide (reviewed below). Although individual conditions screened for may be rare in themselves, about 1 in 5 AJ individuals screened with an AJ mutation panel will be carriers for one or more of these conditions.(3)

2.2 International Practice
Tay Sachs disease (TSD) screening of AJ individuals has resulted in >90% reduction in TSD incidence in the United States(4), Canada(4, 5), Israel.(6) Most recently, a reduction in AJ TSD case frequency has been documented in Australia.(7) The success of TSD preconception and antenatal screening programs has led to the expansion of screening programs over time to offer simultaneous screening for TSD and a range of other severe monogenetic conditions with known causative AJ founder mutations.

The American Congress of Obstetricians and Gynecologists (ACOG)(8), the American College of Medical Genetics and Genomics (ACMG)(9), the Society of Obstetricians and Gynaecologists of Canada (SOGC)(10), the United Kingdom National Screening Committee (UK NSC) (11) and the State of Israel Ministry of Health(12) all recommend that TSD preconception/antenatal screening should be offered to all individuals with AJ heritage.

In addition to screening for TSD, ACOG further recommends routine AJ screening for cystic fibrosis (CF), Canavan disease (CD) and familial dysautonomia (FD)(8), while ACMG endorses routine screening for 9 conditions; TSD, CF, CD, FD, Niemann Pick disease type A (NPDA), Bloom syndrome (BLM), Mucolipidosis Type IV (MLIV), Fanconi anaemia (FA) and Gaucher disease (GD).(3) (9) Centres in the United States (e.g. Mt Sinai Centre for Jewish Genetic Diseases, Tufts Medical Centre Victor Outreach and Screening Program for Ashkenazi Jewish Genetic Diseases) routinely offer screening for 16 to 19 conditions, including TSD, BLM, CD, CF, FD, FA, GD, MPIV, NP, dihydrolipoamide dehydrogenase deficiency, familial hyperinsulinism, glycogen storage
disease type 1A, Joubert syndrome, maple syrup urine disease, nemaline myopathy, spinal muscular atrophy, Usher syndrome type 1F, Usher syndrome type III and Walker Warburg syndrome.(13-16)

Table 1: International Practice for AJ carrier screening

<table>
<thead>
<tr>
<th>Country</th>
<th>UK</th>
<th>USA</th>
<th>ACMG</th>
<th>Private**</th>
<th>ACMG*</th>
<th>SOCG</th>
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<tr>
<td>Supporting guideline</td>
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<td>ACO G</td>
<td>ACM G</td>
<td>Private**</td>
<td>ACMG*</td>
<td>SOCG</td>
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<td>X</td>
<td>X</td>
</tr>
<tr>
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<td>Dihydrolipoamide dehydrogenase deficiency</td>
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<td>Nemaline myopathy</td>
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<td>Usher syndrome type 1F</td>
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<tr>
<td>Usher syndrome type III</td>
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</tr>
<tr>
<td>Walker Warburg syndrome</td>
<td>X</td>
<td></td>
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</tr>
</tbody>
</table>

* Non-government funding
** Endorsed for all conditions other than GD
*** Mt Sinai Centre for Jewish Genetic Diseases, Tufts Medical Centre Victor Outreach and Screening Program for Ashkenazi Jewish Genetic Diseases

In 2013, the ACMG published a position statement(17) on prenatal/preconception expanded carrier screening, endorsing the concept of screening for a far wider range of conditions, made possible by next-generation genetic sequencing technology (NGS, now increasingly known as Massively Parallel Sequencing [MPS]). MPS genetic screening offers high test fidelity, lower costs and reduced laboratory turn-around time, compared to previous diagnostic techniques.(17) The ACMG position statement stipulates that the basis for selection of disorders for inclusion in expanded
screening panels should be disclosed completely and transparently. Disorders with mild phenotypes, variable expression, low penetrance, and/or characterised by an adult onset may be included in screening panels where the implications of screening for such conditions are fully understood. Patients must be able to selectively opt out of receiving results of these tests and be fully informed of the concept of residual risk where test results are negative. (17)

2.3 Health economic considerations
An Australian study found pre-conception or antenatal screening for CF and TSD to be cost-effective when compared to retrospective identification of carrier parents following the birth of an affected infant. (18) It is difficult to assign a monetary value to other significant but intangible benefits, such as informed reproductive choice (19) and a net increase in health of families by preventing the profound psychological costs of having an infant affected by a fatal or severely debilitating genetic condition.

2.4 Outcomes of screening
The Australian evidence for AJ preconception screening derives predominantly from evaluation of TSD screening programs. These programs target senior students at Jewish high schools. Within existing Australian TSD screening programs, AJ individuals self-identify correctly. (20) Testing has been demonstrated to be acceptable to students with extremely high levels of participation (>98%) and low levels of anxiety associated with diagnosis of TSD carrier status. (21-24)

Over 17 years evaluation by health outcome studies to date, no Australian screening program participant has had a TSD affected child, representing 100% disease prevention in this cohort. (7) As expected, AJ heterozygous genetic carrier frequencies for TSD in the screened cohort remained high and were not impacted by screening. The implication is that the Australian AJ community remains at high risk without ongoing intervention. Access to TSD screening programs is limited to less than half their ideal target population. The ideal target population for AJ genetic screening would include all AJ individuals of reproductive age. (25) Two AJ infants were diagnosed with TSD during the study period. None of their four AJ parents had participated in Jewish community high school based genetic screening programs, or been screened privately for TSD carrier status. (7)

3. The recommended Australasian AJ screening panel (version 2014)

The rationale for AJ carrier testing is to identify carriers and to provide individuals and couples with optimised reproductive choice. Care must be taken to do no harm. Based on international experience and recommendations (1, 3, 6, 8, 9, 17, 26), this committee has deliberated that, at this time, evidence from TSD screening programs support preconception/prenatal genetic screening of AJ individuals and their partners for conditions that meet the following criteria:

1. A high AJ carrier frequency due to founder mutations (>1%)
2. Severe monogenetic condition of juvenile onset at the latest
3. >90% diagnostic sensitivity
4. Limited or no available treatment

Following review of potential genetic conditions meeting these criteria, as well as review of International practice (see 2.2, above), AJ carrier screening for the following conditions is recommended (Table 2):

Table 2: HGSA recommended panel for AJ carrier screening
<table>
<thead>
<tr>
<th>Condition</th>
<th>AJ Carrier frequency</th>
<th>Clinical features</th>
<th>Life expectancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tay-Sachs disease (TSD)</td>
<td>1 in 25</td>
<td>Progressive neurodegeneration</td>
<td>Infancy to early childhood</td>
</tr>
<tr>
<td>Cystic fibrosis (CF)</td>
<td>1 in 25</td>
<td>Pulmonary disease, pancreatic insufficiency, reduced fertility</td>
<td>Variable childhood to mid-adulthood</td>
</tr>
<tr>
<td>Bloom syndrome (BLM)</td>
<td>1 in 102*</td>
<td>Dysmorphic features, reduced intellect, cancer susceptibility, low fertility</td>
<td>Childhood to young adulthood</td>
</tr>
<tr>
<td>Familial dysautonomia (FD)</td>
<td>1 in 30</td>
<td>Progressive neurodegeneration, autonomic dysfunction</td>
<td>Childhood to young adulthood</td>
</tr>
<tr>
<td>Niemann-Pick disease type A (NPD)</td>
<td>1 in 80</td>
<td>Progressive neurodegeneration</td>
<td>Infancy to early childhood</td>
</tr>
<tr>
<td>Canavan disease (CD)</td>
<td>1 in 40</td>
<td>Progressive neurodegeneration</td>
<td>Infancy to early childhood</td>
</tr>
<tr>
<td>Fanconi anaemia (FA)</td>
<td>1 in 80</td>
<td>Dysmorphic features, cancer susceptibility, pancytopenia</td>
<td>Childhood to young adulthood</td>
</tr>
<tr>
<td>Mucolipidosis type IV (MLPIV)</td>
<td>1 in 100</td>
<td>Dysmorphic features, progressive neurodegeneration</td>
<td>Childhood to young adulthood</td>
</tr>
</tbody>
</table>

*Bloom syndrome has been included (AJ carriers frequency 1.02%). Severe phenotype with reduced life expectancy.

4. Expanded summary of conditions in the recommended AJ screening panel

4.1. Tay-Sachs disease
TSD is due to congenital deficiency of ß-hexosaminidase enzyme, caused by mutations of HEXA OMIM *606869 (gene map locus 15q23-q24). TSD has autosomal recessive inheritance. 93.1 to 99.1% of Jewish TSD carriers are found to have one of three common HEXA mutations: c.1278insTATC, c.1421+1G>C and p.Gly269Ser.(27-34). Heterozygous carriers are unaffected. Where both parents are carriers, 25% of pregnancies will be affected. Most infants with TSD appear healthy at birth and develop normally for three to six months.(4) Neuronal accumulation of sphingolipid GM2 gangliosides then is associated with progressive neurological regression. Most motor and social skills are lost by 18 months of age. Children rarely survive beyond 5 years of age. No effective treatment exists.(35-37)

1 in 25 AJ Australians is a heterozygous carrier for TSD.(20) In general populations TSD carrier frequency is 1 in 250.(38) French Canadian, Pennsylvania Dutch, Irish, and Cajun heritage may convey higher a priori risk. TSD population genetic screening programs have proved a successful strategy for primary prevention of TSD.(4, 7, 39, 40)

4.1.1. TSD carrier detection
DNA testing for AJ founder HEXA mutations (c.1278insTATC, c.1421+1G>C and p.Gly269Ser) is the most cost-effective and efficient approach to carrier screening for TSD in individuals of confirmed AJ ancestry, with sensitivity of 93.1 to 99.1%.(28, 34) A small residual risk relates to
other HEXA mutations beyond these three that are being tested. (41) HEXA mutations may then be characterised by DNA sequencing, to identify the mutation (or pseudomutation) present. (42)

HEXA enzyme testing is the gold standard for TSD screening and diagnosis, yielding 98% sensitivity in pan-ethnic populations and in the presence of rare or de novo mutations. (9) However, enzyme testing should not be first line for TSD screening in AJ individuals. Enzyme testing is technically complex, requires laboratory expertise and may yield indeterminate, (41) false negative (43, 44) and false positive results. (45) Unlike DNA testing, which can utilise either venepuncture or cheek brush/mouthwash sampling, enzyme testing requires venepuncture. This may limit test uptake (22) and also complicate timing of testing, sample collection and transport of samples. Medications such as the combined oral contraceptive pill may affect HEXA enzyme concentration. (46)

Non-AJ Jewish individuals of Sephardi or Moroccan descent may have alternative mutations for TSD at relatively high frequencies. These individuals and AJ partners of known TSD carriers may request enzyme testing to optimise test sensitivity and avoid the very small possibility of a false negative result. Enzyme testing is recommended for non-Jewish individuals being screened for TSD carrier status (due to the lower false negative rate). (47)

It should also be noted that TSD testing in a reference laboratory for the purpose of making or confirming a diagnosis of TSD may utilise slightly different diagnostic algorithms and reference limits for test results than those used for genetic carrier testing and screening. The recommendations in this current document are focussed on carrier testing and screening.

4.2. Cystic fibrosis
CF is an autosomal recessive disorder causing multi-organ dysfunction and reduced life expectancy. CF is due to mutations in CFTR (gene map locus 7q31.2, MIM# 602421). The CFTR gene product is a trans-membrane conductance regulator protein. CF has a wide clinical spectrum depending on the mutation profile and degree of protein product depletion/dysfunction in affected individuals. CF is characterised by progressive disruption of exocrine function, affecting the pancreas (pancreatic insufficiency), bronchial glands (chronic bronchopulmonary infection with emphysema), biliary tree (biliary cirrhosis), intestinal glands (meconium ileus) and sweat glands (high sweat electrolyte content). Males are infertile due to congenital bilateral absence of the vas deferens. Female subfertility may also occur. (48) Despite advances in treatment, median survival is 37 years. (49) AJ CF carrier frequency is 1 in 23. (50)

4.2.1. CF carrier detection
The mutation profile of AJ CF carriers differs from other Caucasian populations. (51) 23 mutations were included in the pan-ethnic screening panel endorsed by ACMG in 2004, which achieves 94% AJ carrier detection sensitivity (Table 3). (26) Inclusion of additional AJ mutations not included in the ACMG panel (Table 3, marked with *) further increases AJ carrier detection sensitivity. (51) Non-AJ individuals may accurately be counselled of their residual carrier risk when mutation testing is negative, based on their ethnicity. For non-AJ, non-Hispanic Caucasians ACMG panel mutation carrier detection sensitivity is 88% with 12% residual risk. (26)

Table 3: Common CFTR mutations
<table>
<thead>
<tr>
<th>ACMG CF Screening panel, AJ mutations absent from ACMG panel*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AJ and shared</strong></td>
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<tr>
<td>W1282X p.Trp1282ter</td>
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<tr>
<td>3849+10kbC&gt;T c.3718-2477C&gt;T</td>
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<tr>
<td>D1152H* p.Asp1152His</td>
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<td></td>
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<tr>
<td>405 + 1G--&gt;A* c.273+1G&gt;A</td>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Y1092* p.Tyr1092ter</td>
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</tbody>
</table>

### 4.3. Bloom syndrome

BLM results from a founder mutation in *RECQL3* (gene map locus 15q26.1, MIM# 604610) which encodes for RecQ protein-like-3, a DNA helicase. *RECQL3* is tightly linked to the proto-oncogene FES. BLM is a chromosome instability disorder with autosomal recessive inheritance. One third of all reported cases of BLM have occurred in AJ subjects (carrier frequency 1 in 110). A single AJ founder mutation (c.2207_2212delATCTGAinsTAGATTC) causing a complex frameshift in *BLM* has been identified in all AJ cases.(52)

Babies with BLM exhibit proportionate intrauterine and postnatal growth restriction. BLM is associated with reduced life expectancy; chromosomal instability predisposes to malignancy, often leukaemia, in childhood or early adulthood. Other features of BLM include reduced intelligence, reduced fertility, photosensitivity, telangiectasia and uneven skin pigmentation.

### 4.4 Familial dysautonomia/Riley Day syndrome (FD)

FD is a progressive sensorimotor neuropathy with sympathetic autonomic dysfunction. FD is caused by mutation of *IKBKAP* gene (locus 9q31.3, MIM# 603722). FD has autosomal recessive inheritance and occurs almost exclusively in individuals of AJ descent (AJ carrier frequency 1 in 32). 99.5% of AJ individuals with FD demonstrate homozygous inheritance of a single founder mutation, c.2204+6T>C. c.2204+6T>C creates a donor splice site, which leads to deletion of exon 20 from mRNA.(53, 54) A second *IKBKAP* gene mutation identified is p.Arg696Pro.(55)

Clinically FD is evident from birth with early feeding difficulties and hypotonia. Gastro-oesophageal reflux disease can be associated with aspiration and resultant chronic lung disease. Autonomic dysfunction may include absence of tears, severe episodes of nausea and vomiting and rapid swings in blood pressure from severe hypertension to postural hypotension and resultant cardiovascular arrhythmias. Other characteristic features include decreased pain and temperature...
perception and absent lingual fungiform papillae with impaired taste. Intelligence is normal. Life expectancy is reduced. (56)

4.5. Niemann Pick disease type A (NPDA)
NPDA is a lysosomal storage disease with recessive inheritance, caused by mutations in *SMPD1* (gene locus 11p15.4, MIM# 607608). In NPDA, a deficiency of sphingomyelinase enzyme results in lysosomal accumulation of sphingomyelin. NPDA is characterised by severe neurodegeneration from infancy leading to death by early childhood (usually by age 3 years). (57) NPDA affected infants appear normal at birth but develop early and persistent jaundice. Other features usually develop by 6 months. Early signs include enlarging abdomen with hepatosplenomegaly, failure to thrive and failure to reach developmental milestones. Later signs include psychomotor retardation with hypotonia and spasticity. (57) Three AJ founder point mutations have been identified (c.911T>C(p.Leu304Pro), c996delC and c.1493G>T(p.Arg498Leu)) accounting for 97% of AJ carriers. (58, 59, 60)

4.6. Canavan disease
CD is a recessive neurodegenerative disease associated with central nervous system (CNS) leukodystrophy and demyelination. Spongiform cerebral degeneration is characteristic. High levels of N-acetylaspartic acid are found in CNS tissue and in urine. (61) CD is caused by mutations in *ASPA* (gene map locus 17p13.2 MIM# 608034), which encodes for aspartoacylase (ASPA) enzyme. In AJ populations, 97% of CD is caused by two mutations: c.854A>C(p.Glu285Ala) (missense mutation) and c.693C>A(p.Tyr231ter) (nonsense mutation). (62, 63) AJ carrier frequency is 1 in 40. Affected infants appear normal at birth but subsequently become hypotonic with persistent poor head control and failure to meet developmental milestones. Other clinical features of progressive neurodegeneration include megaloecephaly, spasticity characterised by lower limb extension and upper limb flexion, sleep disturbance, feeding difficulty, inability to sit, walk or talk and optic atrophy resulting in severe visual impairment. CD is universally fatal; average life expectancy for affected infants is 18 months.

4.7. Fanconi anaemia complementation group C (FANCC)
FANCC is a genome instability disorder with autosomal recessive inheritance. FANCC is associated with congenital skeletal and other abnormalities with varying penetrance (absent thumbs, radial hypoplasia, scoliosis, café au lait spots, horseshoe kidney, cardiac, gastrointestinal and neurological abnormalities) aplastic anaemia and cancer susceptibility (acute myeloid leukaemia and solid tumours). Infants may appear normal with normal blood cell parameters; however 90% develop pancytopaenia in the first decade of life. (64)

The gene product of *FANCC* (locus 9q22.32 MIM# 613899) is the FACC protein. The FACC protein is thought to be involved in multiple pathways for DNA repair (65) and to play a regulatory role in the growth, differentiation and survival of haematopoietic progenitor cells. (66) At a cellular level, FANCC is associated with a hypersensitivity to DNA cross-linking agents. Conventional chemotherapeutic agents are extremely toxic to FANCC patients. AJ carrier frequency for the single AJ founder mutation c.456+4A>T is 1 in 89. (67) FANCC due to homozygous c.456+4A>T mutations is always associated with a severe phenotype. (67) The main causes of death are haemorrhage or infection. Median survival is 23 years, (64) however some patients have survived longer when successfully treated with haematopoietic stem cell transplantation. (68).
4.8. Mucolipidosis type IV (MLPIV)
MLPIV is a lysosomal storage disease with autosomal recessive inheritance characterised by psychomotor retardation and ophthalmic abnormalities. MLPIV is caused by mutations in MCOLN1 (gene map locus, 19p13.2 #MIM 605248). The exact role of the mucolipin-1 protein product in the lysosome pathway is uncharacterised.(69) The protein localises to the cell plasma membrane and may have a role in endocytosis(70) and/or clearance of intra-mitochondrial free radicals.(71) Over 80% of affected individuals are of AJ descent. Two founder mutations cause 95% of AJ MLPIV cases; c.406-2A>G, a splice site mutation and g.511-6943del, a 6.4-kb deletion.(72) Overall AJ carrier frequency is approximately 1 in 103.(72)

5.0 Limits to Inclusion in recommended AJ panel

The Committee considered whether there was sufficient evidence or ethical need to include various other conditions within these guidelines. Consideration of particular disorders, with the Committee’s assessment, is described below.

5.1 DFNB1 Sensorineural deafness
The gene GJB2 (locus 13q12.11, MIM# 121011) encodes the gap junction protein connexin 26. Mutations in GJB2 cause non-syndromic deafness in many populations. The most common GJB2 in European populations is c.35delG (carrier frequency 1 in 51).(73) The founder mutation c.167delT, is found exclusively in AJ populations at a carrier frequency of 2-4%.(74)

Recommendation: The Committee did not recommend that this condition be included in the 2014 version of the HGSA recommended panel for AJ genetic screening.

Basis for exclusion:
Affected individuals have normal intelligence and life expectancy.

5.2. Gaucher disease

GD is a lysosomal storage disease with autosomal recessive inheritance caused by mutations in GBA gene (locus 1q22. MIM# 606463) that codes for beta-glucocerebrosidase enzyme. GD is characterised by intracellular accumulation of glucosylceramide, particularly in cells of macrophage lineage.(75) “Gaucher cells” may accumulate in multiple tissues with various effects.

GD has 3 main subtypes. In GD type 1 “Gaucher cell” infiltrations can cause hepatosplenomegaly, pancytopenia, and physical manifestations of bone marrow infiltration (e.g. avascular necrosis of the femoral head). No neurological involvement is present. There is a wide spectrum of clinical severity, ranging from symptomatic infants to unaffected adults. GD types II and III are associated with CNS involvement. AJ populations are predominately at risk of type I GD, with a carrier frequency of 1 in 10. The four most common AJ GBA mutations account for 96% of GD in AJ populations: c.1226A>G, c.84insG, c.115+1G>A and c.1448T>C.(76) DNA testing for GD in Non-AJ individuals has 73% diagnostic sensitivity. A combination of DNA and enzyme testing provides improved diagnostic sensitivity in non-AJ partners of AJ GD carriers.(77)

Recommendation: The Committee did not recommend that this condition be included in the 2014 version of the HGSA recommended panel for AJ genetic screening.
**Basis for exclusion:**
The majority of homozygotes and compound heterozygotes with AJ GBA mutations will predictably have mild or even asymptomatic GD. Based on AJ gene frequencies, an estimated 60% of c.1226G homozygotes with GD are undiagnosed, presumably due to an extremely mild phenotype. (78) Phenotypic variability, however, cannot be fully explained or predicted by genotype analysis. (78) Arends et al. (2013) reported that Gaucher patients have an increased relative risk (RR) of malignancy in general (1.7 95%CI 1.3 to 2.3) and multiple myeloma (RR 25 to 51) and haematological malignancies (RR 3.5 to 12.7) specifically when compared to the general population. (79) Significant biases cannot be accounted for in this meta-analysis, including selection bias of index cases with increased severity of Gaucher phenotype associated with clinical diagnosis and related reporting bias. Zuckerman et al. (2007) reported the vast majority of couples diagnosed with a GD affected fetus in pregnancy choose to continue the pregnancy. (80)

5.3 Expanded AJ MPS screening
It is likely Massively Parallel Sequencing (MPS) technologies will become the routine testing modality in existing AJ genetic screening programs targeting high school students. MPS will allow diagnostic testing and screening to be carried out with higher resolution, more quickly and more cost effectively. (81) MPS screening using an expanded panel of conditions will most likely become available in the future. It is recognized that economic considerations may make it cheaper or more efficient to design MPS assays utilizing expanded screening panels, than to design restricted panels. Discussion of expansion of AJ genetic screening beyond the recommended minimal panel is outside the scope of this guideline.

**Recommendation:** The Committee did not recommend that such additional conditions be included in the 2014 version of the HGSA recommended panel for AJ genetic screening. However, it does not oppose the inclusion of additional conditions, provided there is full disclosure and informed consent.

6. Routes of access to Ashkenazi Jewish preconception/antenatal genetic screening in Australia
Routes of access to AJ preconception/antenatal genetic screening in Australia include:
1. Participation in Australian Jewish community genetic screening programs in participating Jewish community high schools. (20, 21, 23, 82)
2. Participation in outreach programs associated with Australian Jewish community genetic screening programs.
3. Participation in the international Dor Yeshorim screening program.
5. Genetics services facilitated screening at an Australian public hospital.

6.1. Australian Ashkenazi Jewish population genetic screening programs
The two largest Australian Jewish population centres are located in Melbourne and Sydney. Australian Jewish community genetic screening programs based in Melbourne and Sydney (23, 82) have been designed based on international best-practice principles (28, 29, 39, 83) and target senior AJ high school students who attend Jewish community high schools. Programs have up to 18 years of experience in screening for TSD. More recently, these programs have offered screening for a panel of conditions. The Sydney screening program currently tests for TSD, CF, FD, FA and CD, and is in the process of introducing BLM, NPD and MLIV; as their assay system is based on MPS genetic testing, GSD1A will also be offered to those who consent to its inclusion.
The Melbourne screening program currently tests for TSD, CF, FA, CD, BLM and NPD. Screening is not Medicare funded. Through a combination of philanthropic sponsorship and public health funding, screening is provided cost free to high school students within established screening programs. Participation is high (99.6%) (21) and health outcome studies within screening programs demonstrate disease prevention. (7)

In Melbourne and Sydney, fewer than 50% of current Australian Jewish high school students attend Jewish community high schools. (Eckstein G. Demography of the Sydney Jewish community: an overview of information from the 2006 Census. Unpublished report commissioned by the Jewish Community Appeal (JCA); copies available on request from http://www.jca.org.au). (7) Overall, more than 50% of Australian AJ individuals in the reproductive age group have not had the opportunity to participate in existing Jewish high school based screening programs. In Australian Jewish communities living outside of Melbourne and Sydney, where no high school based screening programs are conducted, the proportion of unscreened AJ individuals of reproductive age is likely to be even higher. Table 4 summarizes the Australian Jewish population by State of residence as reported in the 2011 ABS Census of Community and Housing. (2) Table 5 summarizes the New Zealand Jewish population by region. (84)

Table 4. 2011 ABS Census: Australian Jewish population by State of residence

<table>
<thead>
<tr>
<th>Australian State/Territory</th>
<th>Number of Jewish residents</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSW</td>
<td>39730</td>
</tr>
<tr>
<td>VIC</td>
<td>45149</td>
</tr>
<tr>
<td>QLD</td>
<td>4442</td>
</tr>
<tr>
<td>SA</td>
<td>1090</td>
</tr>
<tr>
<td>WA</td>
<td>5854</td>
</tr>
<tr>
<td>TAS</td>
<td>248</td>
</tr>
<tr>
<td>ACT</td>
<td>673</td>
</tr>
<tr>
<td>NT</td>
<td>146</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>97335</td>
</tr>
</tbody>
</table>

Table 5: 2013 New Zealand Census (Statistics New Zealand): Jewish Religious affiliation by Region

<table>
<thead>
<tr>
<th>Region of New Zealand</th>
<th>Number of Jewish residents</th>
</tr>
</thead>
<tbody>
<tr>
<td>All regions</td>
<td>6867</td>
</tr>
<tr>
<td>Auckland City</td>
<td>3102</td>
</tr>
<tr>
<td>Wellington City</td>
<td>756</td>
</tr>
<tr>
<td>Christchurch City</td>
<td>435</td>
</tr>
<tr>
<td>Lower Hutt City</td>
<td>171</td>
</tr>
<tr>
<td>Dunedin City</td>
<td>171</td>
</tr>
<tr>
<td>Hamilton City</td>
<td>156</td>
</tr>
<tr>
<td>Tauranga City</td>
<td>160</td>
</tr>
<tr>
<td>All other regions total (Individual regions Jewish population &lt;100 people per region)</td>
<td>1944</td>
</tr>
</tbody>
</table>

6.2. Outreach programs

Outreach screening services can be accessed by AJ individuals in Sydney and Melbourne.
Contact details:
Sydney: Wolper Jewish Hospital (www.wolper.com.au)
Melbourne: Austin Health (megan.cotter@austin.org).

There are currently no community screening programs or associated outreach services operating outside of Sydney and Melbourne. Access to testing for those outside of Sydney and Melbourne can be arranged either via the local Genetics Services (see section 6.5), or by medical referral to a Pathology Service, who can arrange for collection of the appropriate blood (or in some cases, cheek-brush) sample. AJ communities in other major populations centres that wish to consider establishing local outreach screening services are welcome to approach the established Sydney and Melbourne programs for advice and assistance.

6.3. Dor Yeshorim
Dor Yeshorim(83) is an international Jewish genetic screening program based overseas but accessible to Australians. Sample collection and laboratory testing may be conducted overseas. The program targets an ultra-orthodox demographic and is accessed by clients prior to marriage. The genetic compatibility of a proposed couple is disclosed without revealing the results of the individuals. In this way, program participants' results remain anonymous.

Contact details:
Dor Yeshorim Institute

New York Office
429 Wythe Ave
Brooklyn, NY 11249
Telephone: (+718) 384-6060/ (+718) 384-2332

Jerusalem Office
21 Strauss Street, Jerusalem,
P.O. Box 91057 Israel,
Telephone: (+972) 26499888
https://www.jewishgenetics.org/dor-yeshorim

6.4. General Practitioner/Obstetrician Gynaecologist initiated screening
General Practitioner/Obstetrician Gynaecologist initiated genetic screening is the most readily accessible route to screening for AJ Australians outside of high school based genetic screening programs. Barriers to screening in the primary care context include lack of clinician and patient education,(85, 86) incomplete genetic history taking, low patient knowledge(46) , cost of testing(87) and unplanned pregnancy; 30 to 50% of all pregnancies in developed countries are unplanned. (88)

Genetic risk (AJ heritage) should be identified on medical history taking. Appropriate patient education and counselling should be provided. Discussion should cover the disorders being screened, including their clinical features, mode of inheritance, and implications for relatives. Reproductive options for at risk couples should be discussed. Voluntary informed consent should be obtained from the patient(s) and documented.
Non-AJ partners of AJ carriers for one or more monogenetic autosomal recessive condition should also be offered screening. Ideally, where results of screening are not time critical, the AJ individual should be tested first (Figure 1: Two step screening). The non-AJ partner should subsequently be tested for any condition(s) for which the AJ partner was found to be a carrier. During pregnancy when results are needed with urgency, simultaneous screening of the AJ client and their non-AJ partner is appropriate. For conditions other than TSD and CF non-AJ individuals must be counselled regarding imprecise residual risk when found to be non-carriers based on analysis of common AJ mutations. In the case of TSD the enzyme test identifies nearly all carriers and thus the non-AJ partner of a TSD carrier should be offered enzyme testing rather than genetic testing. The mutation detection rate and carrier frequencies among different ethnic groups is known for CF and thus precise test sensitivity and residual risk counselling can be offered.

For couples with a known family history of a particular AJ monogenetic condition, testing is not “screening” per se, and a referral should be arranged to a Genetics service for genetic counselling.

6.4.1 Laboratory referral
Testing should be performed through laboratory service accredited for testing of the relevant genetic disorders. The Royal College of Pathologists of Australasia (RCPA) and the HGSA maintain a database of tests (rcpamanual.edu.au) and laboratories performing genetic tests (http://genetictesting.rcpa.edu.au/). Note that the listing of a laboratory in this database does not necessarily indicate it has undergone accreditation, and before referring patients or specimens for testing, specific enquiries should firstly be made of the laboratory, or alternatively its scope of accreditation can be viewed at the accrediting authorities’ website. In Australia, accreditation is undertaken by the National Association of Testing Authorities, Australia (NATA, www.nata.com.au), in a joint program with the Royal College of Pathologists of Australasia (RCPA, www.rcpa.edu.au). In New Zealand, accreditation is undertaken by International Accreditation New Zealand (IANZ, www.ianz.govt.nz). Pathology requests should specify the patient’s AJ heritage in the clinical notes. Best-practice testing methodology will at times differ for AJ and non-AJ individuals (e.g. TSD: DNA testing for c.1278insTATC, c.1421+1G>C and p.Gly269Ser in AJ individuals, and HEXA enzyme testing for non-AJ individuals). In couple screening, where logistically possible, both partners’ samples should be tested in the same laboratory. Where a family member has previously been affected by, or diagnosed to be a carrier of a genetic condition being screened, full details of the clients’ genetic and family history should be provided to the testing laboratory, to ensure that the most appropriate tests are performed.

Currently AJ genetic screening in Australia is not Medicare-funded and may incur a cost to patients for diagnostic services.

AJ genetic testing (not screening) is also not Medicare funded at present, but some State genetic services may offer to defray the costs of testing; in other cases, testing may incur a cost to patients.
6.5. Referral for specialist genetic services
AJ genetic screening can be facilitated via specialist genetics services referral. This method of screening is optimal where a family history for a serious genetic condition is present. Referral for genetic counselling and testing through a public hospital genetics service is cost-free to patients, although there may be waiting-lists at some busier clinics and services.

**Australia**

**New South Wales**
Genetic Services, New South Wales Health
[www.genetics.edu.au/Genetics-Services](http://www.genetics.edu.au/Genetics-Services)
Royal North Shore Hospital Community Health Centre
+61 (0) 2 9462 9599
Email: contact@genetics.edu.au

**Victoria**
Victoria Clinical Genetics Services
[www.vcgs.org.au](http://www.vcgs.org.au)
Royal Children’s Hospital
+61 (0) 3 8341 6201
Email: vcgs@vcgc.org.au

**Australian Capital Territory**
ACT Genetics Service
Canberra Hospital
+61 (2) 6174 7630
Email: genetics@act.gov.au

**Queensland**
Genetic Health Queensland
Royal Brisbane and Women’s Hospital
+61 (0) 7 3646 1686
Email: GHQ@health.qld.gov.au

**South Australia**
South Australian Clinical Genetics Service
Paediatric and Reproductive Genetics Unit
Women’s and Children’s Hospital Adelaide
+61 (0) 8 8161 7375
Email: sapathology.prgu@health.sa.gov.au

**Western Australia**
Genetic Services of Western Australia
King Edward Memorial Hospital Perth
+61 (0) 8 9340 1525
Email: gswa@health.wa.gov.au
6.6. Direct-to-consumer genetic testing
Direct-to-consumer (DTC) genetic and genomic testing is available in Australia and overseas. Simultaneous genetic screening is offered for a broad range of conditions, targeting a pan-ethnic population, without mandatory medical involvement or genetic counselling. AJ high risk conditions may be included in DTC screening models. Ethical and regulatory issues relating to DTC screening remain unresolved.(89) Australians surveyed regarding their opinions and knowledge of DTC genetic testing revealed that genetic screening is perceived to be important and over one quarter of respondents would be interested to pursue this testing modality.(90) Australian Guidelines for health providers on DTC genetic testing have been developed by the National Health and Medical Research Council (NHMRC) ([https://www.nhmrc.gov.au/guidelines/publications/g7](https://www.nhmrc.gov.au/guidelines/publications/g7)) and the National Pathology Accreditation Advisory Council (NPAAC)
7. Screening models
Three screening models are described in Figures 1 to 3:

**Figure 1: Two-step screening**

- Test Partner A (usually female)
  - Is Partner A genetic carrier?
    - YES: Offer Partner B genetic carrier testing. Discuss cascade screening of A's relatives
    - NO: Negligible residual risk of tested genetic diseases for offspring. Ensure couple understands that low genetic risk applies ONLY in current partnership. Future partnerships will need to be retested. Note that the risk in a different future partnership remains low for the individual who has tested negative. No referral for genetic counselling required. No referral for specialist reproductive medicine required.

- Is Partner B Jewish?
  - YES: Aj and J relevant testing
  - NO: Non-Aj and non-J relevant testing

- Is Partner B genetic carrier?
  - YES: Each pregnancy of this partnership has 25% risk of tested genetic disease. As appropriate, refer for:
    - Genetic counselling advice or Clinical Geneticist opinion
    - Specialist reproductive medicine opinion
    - Access to educational resources
    - Cascade screening of relatives
  - NO:
Figure 2: One-step couple screening

One-step screening model
(suitable for antenatal screening)

Test both Partner A and B simultaneously

Are both A and B genetic carriers?

YES

Each pregnancy of this partnership has 25% risk of genetic disease

Explain results and, as appropriate, refer for:
- Genetic counselling advice or Clinical Geneticist opinion
- Specialist reproductive medicine opinion
  Discuss:
  - Access to educational resources
  - Cascade screening of relatives

Negligible residual risk of tested genetic disease for offspring
(Ensure couple understands that low genetic disease risk applies ONLY in current partnership)

No referral for genetic counselling required
No referral for specialist reproductive medicine required

No

Is exactly ONE of A or B genetic carrier?

YES

(Only if consented):
- Disclose individual results
- Cascade screening of relatives

No

Nothing further indicated
Figure 3: One step couple screening ( ultra-orthodox unmarried couple)

The advantages and disadvantages of different screening strategies should be considered in the context of a couple’s circumstances to maximise cost benefit (Table 6).
Table 6: Advantages and disadvantages of AJ monogenetic autosomal recessive disease screening strategies

Please refer to Figures 1, 2 and 3 for definitions of two-step and one-step screening models

<table>
<thead>
<tr>
<th>Model</th>
<th>Two step</th>
<th>One step (established couple)</th>
<th>One step (potential couple)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals tested</td>
<td>Partner A initially. Partner B where Partner A is a carrier</td>
<td>Partners A and B</td>
<td>Partners A and B</td>
</tr>
<tr>
<td>Member of couple informed of their own results</td>
<td>One or both</td>
<td>Both</td>
<td>Neither</td>
</tr>
<tr>
<td>Laboratory testing required</td>
<td>Less</td>
<td>More</td>
<td>More</td>
</tr>
<tr>
<td>Counselling required (medical/genetics/reproductive)</td>
<td>Less</td>
<td>More</td>
<td>Least</td>
</tr>
<tr>
<td>Total cost</td>
<td>Least</td>
<td>Most</td>
<td>Less</td>
</tr>
<tr>
<td>Elapsed time before result available</td>
<td>Slower</td>
<td>Faster</td>
<td>Faster</td>
</tr>
<tr>
<td>Suitability for pre-conception testing</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Suitability for antenatal testing</td>
<td>Less desirable</td>
<td>Good</td>
<td>N/A</td>
</tr>
<tr>
<td>Autonomy of result reporting</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cascade testing of relatives</td>
<td>Suboptimal</td>
<td>Optimal</td>
<td>No</td>
</tr>
</tbody>
</table>

8. Timing of screening

Preconception screening in an informed consenting adult or mature minor(91, 92) should be considered the gold standard.(47, 93) Preconception screening maximises reproductive choice for at-risk couples (Table 7) and is associated with lower patient anxiety than screening conducted during pregnancy.(19) Clinicians should consider and offer AJ genetic screening opportunistically when at-risk patients present for other reasons (e.g. general or sexual health check).(86)

Presentation for screening commonly occurs in early pregnancy.(86) A significant proportion of pregnancies are unplanned.(88) Even where pregnancy is planned, genetic risk may not be considered pre-conception. Where an at-risk couple is identified during pregnancy, prompt referral for appropriate genetic counselling and, if indicated, maternal fetal medical review should be undertaken. Management may include fetal diagnostic testing.(8)
<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>No diagnostic testing</td>
<td>Natural conception</td>
<td>Avoids pre-natal diagnostic testing and associated risk of pregnancy loss.</td>
<td>25% risk of an affected child with each pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avoids termination of pregnancy.</td>
<td></td>
</tr>
<tr>
<td>(PGD)</td>
<td>condition. Healthy embryo is returned to mother's uterus.</td>
<td>Avoids termination of pregnancy.</td>
<td>Significant costs; currently no government rebate for PGD.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detection of free fetal DNA in Maternal circulation</td>
<td>Testing performed on maternal serum sample.</td>
<td>Avoids costs and complications associated with IVF.</td>
<td>Currently in experimental use only.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Possible in unplanned pregnancy.</td>
<td>Emotional and medical complications associated with termination of disease affected pregnancy.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avoids pre-natal diagnostic testing and associated risk of pregnancy loss.</td>
<td></td>
</tr>
<tr>
<td>Pre-natal diagnosis: CVS (11 to 14 weeks gestation)</td>
<td>Natural conception. Testing of biopsied pregnancy tissue to diagnose disease risk during pregnancy.</td>
<td>Avoids costs and complications associated with IVF.</td>
<td>1 in 200 risk of pregnancy loss associated with diagnostic procedures.</td>
</tr>
<tr>
<td>Amniocentesis (&gt;15 weeks gestation)</td>
<td></td>
<td>Possible in unplanned pregnancy.</td>
<td>Emotional and medical complications associated with termination of disease affected pregnancy.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stress associated with uncertainty faced throughout the first trimester of pregnancy.</td>
</tr>
<tr>
<td>Donor gamete</td>
<td>Donor ovum or sperm (Donor is non-carrier for the condition).</td>
<td>May avoid risks associated with IVF. Avoids risks</td>
<td>One partner is non-biological parent to offspring.</td>
</tr>
</tbody>
</table>

Table 7: Reproductive options for couples at risk of a pregnancy affected by a severe monogenetic autosomal recessive disease
<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
<th>Benefits</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adoption</td>
<td>Child is adopted.</td>
<td>Avoids disease risk.</td>
<td>Many barriers to adoption exist.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avoids pregnancy-associated risks.</td>
<td>Child is not biologically related to the adoptive parents.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avoids termination of pregnancy.</td>
<td></td>
</tr>
<tr>
<td>Decision not to proceed with union</td>
<td>Some individuals may be screened prior to a potential arranged introduction/marriage.</td>
<td>Avoids disease risk.</td>
<td>Undesirable for the majority of couples.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avoids stigma associated with disclosure of carrier status.</td>
<td></td>
</tr>
<tr>
<td>Decision not to have children</td>
<td>No children</td>
<td>Avoids disease risk.</td>
<td>May not represent reproductive wishes of parent couple</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avoids pregnancy-associated risks.</td>
<td></td>
</tr>
</tbody>
</table>

9. **Internet resources**

Internet resources that may be useful for genetic counselling and patient education can be found at the following websites:

- [http://www.jewishgeneticdiseases.org](http://www.jewishgeneticdiseases.org)

Many State Health Departments offer on-line educational resources for genetic conditions. Examples are:

- NSW: [http://www.genetics.edu.au](http://www.genetics.edu.au)

10. **Audience Organizations**

Medical Colleges
- Royal Australasian College of General Practitioners
- Royal Australia and New Zealand College of Obstetricians and Gynaecologists
- Royal Australasian College of Physicians
- Royal College of Pathologists of Australasia

Professional Societies
- Human Genetic Society of Australasia
- Australasian Association of Clinical Biochemists

Associations
- Australian Medical Association
- Genetic Alliance Australia (formerly Association of Genetic Support of Australasia)
- Rare Voices Australia
11. References:


84. Religious affiliation (total responses) by age group and sex for the census usually resident population count, 2001, 2006 and 2013 Censuses (RC, TA, AU) [Internet]. 2013 [cited 12/12/2014].
89. Samuel GN, Jordens CF, Kerridge I. Direct-to-consumer personal genome testing: ethical and regulatory issues that arise from wanting to 'know' your DNA. Internal medicine journal. 2010;40(3):220-4.
91. Gillick v West Norfolk and Wisbech Health Authority 1 AC 112 (1986).
Chapter 9: Discussion and conclusions

The broad aim of this thesis was to critically assess processes of Ashkenazi Jewish population screening for Tay Sachs disease (TSD) and to establish an evidence based approach to guide the future evolution of AJ preconception genetic screening in Australia. Working in collaboration with the two established Australian TSD screening programs. I have researched and documented the Australian experience.

Existing TSD screening programs in Australia target Ashkenazi high school students who attend independent Jewish high schools in Sydney and Melbourne. Outreach opportunities co-ordinated by existing TSD screening programs offer access to TSD screening outside of this limited target population.

During the course of this PhD I investigated the effectiveness of screening in this specific target population (adolescent AJ high school-based screening program participants). In terms of risk identification, I compared student’s self-reporting of Ashkenazi Jewish heritage with documented grandparents country of birth and TSD mutation carrier frequencies.

I identified a sustained high TSD carrier frequency amongst Australian Jewish high school students and young adults of reproductive age, comparable to international study findings conducted since the 1970s.
I assessed Australian Bureau of Statistics collated census data to achieve a best possible estimate of the proportion of all Australian Ashkenazi Jewish students who access TSD screening via existing programs and of Australian Ashkenazi Jewish students and adults of reproductive age who have not accessed these programs. Through this research, it was found that a majority of Australian AJ individuals of reproductive age had not had the opportunity to access AJ community screening programs facilitated mainly in a limited number of private school settings in Sydney and Melbourne.

I designed a strategy to enable auditing of all cases of TSD diagnosed in Victoria and NSW from 1995 to 2012 inclusive, this being the period Australian Ashkenazi Jewish TSD population screening programs were in operation. I determined that fewer than expected new Jewish TSD cases occurred during the study period. I found that of the two Ashkenazi Jewish cases of TSD that were diagnosed during the study period, none of their four Ashkenazi Jewish parents had undergone preconception or antenatal TSD screening. I determined that no TSD screening program participant had gone on to parent a child affected by TSD.

Having proved the effectiveness of Australian Ashkenazi Jewish TSD preconception screening programs and having identified that a major pitfall of these programs was restriction of access to only a fraction of the Australian Ashkenazi Jewish population of reproductive age at risk of TSD, I endeavoured to research a practical solution to extend the benefits of TSD screening to the entire Australian Ashkenazi Jewish population at high risk of TSD.
Australian Ashkenazi Jewish populations are concentrated within metropolitan areas, the largest communities residing in Melbourne, Sydney and Perth. Australian cities have existing health care infrastructure offering excellent access to the services of primary health care clinicians such as general practitioners and obstetricians/gynaecologists. No recent systematic review of the literature relating to TSD preconception screening research and practice had been published in the international literature. I endeavoured to systematically and comprehensively assess the Australian and international evidence for TSD preconception and antenatal screening. Resulting from my original research and systematic review of the Australian and international literature, I have presented the first evidence-based practice model for TSD preconception/antenatal screening in the Australian primary health care setting.\(^5\)

By invitation, I have authored the first open access Australasian clinical practice guideline for AJ preconception screening, ratified and published in 2015 by HGSA (Chapter 8).

I have ongoing involvement with the planning and preliminary analysis of the first Australasian AJ preconception genetic screening pilot study using MPS based diagnostic technology (Appendix 1).

I have presented new arguments about:
• Persisting high risk of TSD in Australian Ashkenazi Jewish populations (Chapter 4)
• High accuracy of risk identification in Australian Ashkenazi Jews (Chapter 4)
• Effectiveness and cost efficacy of existing Australian Ashkenazi Jewish TSD population genetic screening programs (Chapter 5)
• The unequal access to preconception/antenatal TSD screening across the Australian Ashkenazi Jewish populations at high risk of TSD (Chapter 5)
• The pivotal role of primary health care clinicians in extending preconception/antenatal TSD screening to all Australian Ashkenazi Jewish individuals of reproductive age. (Chapter 6)
• The need for an evidence based guideline to assist primary care clinicians in the achievement of ubiquitous access to TSD screening of Ashkenazi Jewish individuals at high risk (Chapter 6, Chapter 7, Chapter 8)
• The imperative to reassess screening testing methodology and supporting clinical infrastructure with the emergence of massively parallel sequencing diagnostic technologies (Chapters 7, Appendix 1).

In the following sections I will discuss each of the specific aims of this thesis, the respective study outcomes and the contributions they have made to the literature.

**Answering the aims of this thesis:**

**Aim 1:** To assess the ability of Australian Jewish individuals screened for TSD to self-report Ashkenazi Jewish heritage. To assess how AJ heritage and grandparental
country of origin correlates with TSD risk, both overall and in the case of specific common AJ HEXA gene mutations.

Over the 12-year period 1995 to 2007, 4105 Jewish high school students in Melbourne and Sydney participated in the Australasian Community Genetics Program and were provided with TSD preconception genetic screening (Chapter 3). At the time of sample collection, students were required to complete a questionnaire. Demographic information collected included nominated ethnicity (Ashkenazi Jewish, Sephardi Jewish, mixed heritage, non-Jewish heritage) and birthplace of the student’s parents and grandparents. I correlated and statistically analysed the relationship between TSD carrier frequency, including subgroup analysis for 3 common Ashkenazi specific HEXA mutations: c.1278insTATC, p.Gly269Ser and c.1421+1G>C.

**Study Outcomes**

It was determined that screening of self-declared Ashkenazi Jewish subjects identified 95% of TSD carriers within a Jewish cohort of students who identified as either Ashkenazi, Sephardi or mixed heritage.

Self-declaration of Ashkenazi Jewish heritage was more predictive of TSD carrier status (carrier frequency 1:25) than was analysis of grandparents’ country/countries of birth. Having mixed Ashkenazi and non-Ashkenazi heritage reduced TSD carrier frequency to 1:97.
In the case of the specific *HEXA* mutation c.1421+1G>C, Ashkenazi Jewish subjects with South African heritage were found to have a four-fold increased risk of carriage compared with other Ashkenazi Jewish subjects (Odds ratio 4.19; 95% confidence interval 1.83–9.62, \( p=0.001 \)). This was the only case where geographic ancestral origin was associated with greater diagnostic sensitivity than Ashkenazi Jewish heritage alone. Overall TSD carrier frequencies (3 mutation) in Ashkenazi Jewish subjects with South African heritage did not differ significantly from other Ashkenazi Jewish subjects.

Carriers of c.1278insTATC mutations were more likely to have Western European heritage (OR, 1.65 (95% CI, 1.04–2.60), \( p=0.032 \)) or South Eastern European heritage (OR, 1.77 (95% CI, 1.14–2.73), \( p=0.010 \)). Heritage from specific European countries investigated did not significantly alter the overall odds of TSD carrier status.

**Impact of study outcomes on the literature**

Historically, Australian Ashkenazi Jewish populations formed and expanded with relation to specific world events. The largest event was immigration of Jewish refugees to Australasia from Europe post World War II in the mid to late 1940’s. A second event was immigration from former Eastern European members of the Soviet Union in the 1960s. A third event occurred in the 1980’s and 1990’s with immigration from Apartheid South Africa and the former Soviet Union.
The majority of the current generation of Australian Ashkenazi Jewish individuals of reproductive age were born in Australia, and in many cases, their parents were also born in Australia.

It is an important finding that Ashkenazi Jewish heritage has been accurately reported in the current generation at risk of TSD. It is an equally important finding that reporting of Ashkenazi Jewish heritage in this cohort correlates with identification of 95% of TSD carriers identified by screening, and that carrier frequencies of HEXA mutations among this cohort remain high (1:25).

These findings are central to the potential to translate the successes of Ashkenazi Jewish TSD population genetic screening programs from the limited screening program setting to the primary health care setting. A general practitioner or obstetrician/gynaecologist may identify a patient at increased risk of having TSD affected offspring by simply inquiring if that patient has Ashkenazi Jewish heritage. Where this risk factor is identified, TSD preconception/antenatal genetic screening is cost-effective and can potentially be facilitated in a primary healthcare setting. If existing barriers were identified, assessed and tackled, all Australian Ashkenazi Jewish individuals of reproductive age could potentially gain access to TSD preconception/antenatal genetic screening through their own doctor. The publication of Chapter 8 of this thesis is a supporting aid for primary care clinicians to overcome some of the existing access barriers to community based AJ preconception genetic screening.
Aim 2: To perform the first complete and consecutive audit of all cases of TSD diagnosed in Melbourne and Sydney during the period of Jewish TSD screening programs operation. To identify Jewish and non-Jewish heritage of historical TSD cases. To assess for a reduction in observed versus expected Jewish TSD cases in the era of AJ preconception genetic screening program operation. Predicted TSD cases were determined on the basis of Australian Bureau of Statistics census population data and known TSD carrier frequencies in Jewish and general populations.

I designed a strategy for a retrospective national audit of all laboratory testing results for TSD case diagnosis and carrier screening from 1995 through to 2011. This included diagnosis of TSD cases, cascade screening and screening program referrals. In order to achieve this, I collaborated with all of the three Australian laboratories in which all samples were processed (by one or more sites) during the study period (PaLMS, Pathology North, NSW Health Pathology, Sydney; VCGS, Melbourne; and SA Pathology, Adelaide). Ethics approval was separately obtained from the Melbourne and Sydney sites, and was acknowledged and approved by the third South Australian testing site. The database I created had never been accessed, collated or synthesized by any previous investigator. Past attempts by these three national laboratories to perform such a study had been unsuccessful due to the scale and complexity of the proposed project and the difficulties in identifying and accessing the required data (Leslie Burnett, personal communication).
12 TSD cases diagnosed in Sydney (four cases) and Melbourne (eight cases) from 1995 through 2011 were identified. Laboratory records pertaining to each case were reviewed. All existing archived clinical patient history files were reviewed by me on site in NSW and Victoria. Two cases were Jewish (a ratio of Jewish to non-Jewish births of 1:5). The estimated expected number of TSD-affected births in Melbourne and Sydney in 1995–2011 was 4.1 for Jewish births and 7.4 for other births (a ratio of Jewish to non-Jewish births of 1:2). WHO published TSD carrier frequency of 1 in 250 was used to model expected TSD cases in non-Jewish Australians. This estimate was calculated using Australian Bureau of Statistics population data and known Australian Jewish TSD carrier frequencies. As the Australian Bureau of Statistics 2006 census population data quoted did not distinguish between Ashkenazi and non-Ashkenazi Jewish individuals, the carrier frequency of 1:31 (3.26%; 95% CI, 2.89%–3.68%) was used for TSD carrier risk in Australian Jewish individuals.

This finding of fewer than expected Jewish TSD cases corresponded to the period during which screening programs were operating. There were no Jewish TSD-affected children born to parents who were screened previously. TSD preconception/antenatal carrier screening, supported by community education and the appreciation of autosomal recessive inheritance are the likely key factors explaining the fewer than expected Jewish babies born with TSD. As screening program participants are currently aged 16 to 40 years, many have not yet completed their families. The full benefit of Australian TSD screening programs is yet to be realized.
While fewer than expected TSD Jewish babies have been born since the introduction of Australian AJ preconception genetic screening programs, the prevention of virtually all AJ cases of TSD should be achievable. As AJ preconception screening is highly protective and cost-effective, the following question was framed: In the Australian context: how can preconception genetic screening for TSD be extended to all individuals of reproductive age with AJ heritage?
Aim 3: To systematically review the Australian and international literature and develop an Australasian best-practice model for primary care clinician-lead AJ TSD screening.

No systematic review of the Australian and International literature relating to TSD preconception genetic screening had been undertaken prior to my PhD candidature. In order to facilitate a potential future application for a Medicare item number to be created to fund TSD preconception screening, experts in the area of genetic screening have the onus of proof that the practice is beneficial, cost effective, evidence based best practice and relevant within the Australian health care setting. I conducted a systematic review of the literature using the NHMRC framework to assess the impacts of TSD screening internationally and in the Australasian context. This review allowed me to generate recommendations that were published in the Journal of Paediatric and Child Health, a PubMed indexed journal with a wide circulation amongst Australian physicians. The dual aim of this review was:

1) To raise awareness of AJ preconception genetic screening strategies amongst Australian Medical practitioners, and

2) To provide supportive evidence based material to promote community based TSD screening. This was achieved.

Via my undertaking of this extensive work, the collaborations involved created the opportunity for me to chair a subcommittee of the HGSA and to author the first Australasian clinical practice guideline for AJ preconception genetic screening. The
first edition of this guideline was published in 2015 and is included in this thesis (Chapter 8).
Aim 4: To compare current with projected future rates of significant genetic findings using a conventional DNA mutation testing panel versus a massively parallel sequencing model to conduct Ashkenazi Jewish pre-conception genetic screening. To evaluate the clinical and workforce implications of adopting modernised DNA sequencing technologies for TSD carrier screening in the context of an expanded Ashkenazi Jewish panel.

Chapter 7 summarises major challenges pre-conception screening strategies will pose to the Australian Health system moving forward. In this chapter I explored the clinical, ethical and workforce implications of adopting MPS based expanded panel preconception genetic screening strategies. Such strategies will in the future undoubtedly form part of a pan-ethnic preconception preventive medicine and health promotion strategy to help couples achieve healthy families. I explain how existing AJ preconception genetic screening programs may be an educative model for policy development surrounding the changing practice of “multi-condition” simultaneous genetic screening. I outline my preliminary research in this area, presented in Appendix 1.
Future Directions

Through the work presented in this thesis, I have provided definitive answers to the aims and questions developed during my PhD candidature. Developments and progress in genetic diagnostic technology have now opened new areas for research and intervention. In the future, I plan to extend my work as follows:

1) I am involved in ongoing research to analyse and report findings of the AJ preconception genetic screening MPS pilot study underway through the Sydney Community Genetic Screening program (Chapters 7 and Appendix 1).

2) I am undertaking a collaborative audit to report all cases of TSD carrier couples who have utilised PGD/ART to achieve the live birth of a healthy child (free of TSD) and also all cases where prenatal diagnosis has been used to detect TSD by CVS/Amniocentesis. This work is a follow-up study of the work reported in Chapter 5 of this thesis.

3) In February 2015, I was awarded a fellowship of the Australia and New Zealand College of Obstetricians and Gynaecologists (FRANZCOG). I am completing the Royal Australia and New Zealand College of Obstetricians and Gynaecologists further subspecialty medical qualification, Certificate of Reproductive Endocrinology and Infertility (CREI). My aim is to perpetuate an ongoing commitment to research in the area of pre-conception health promotion as a clinician-scientist. With this aim, as part of my final CREI training year in 2016, I will be working with VARTA (Victorian
Assisted Reproductive Technology Authority) in the area of preconception health promotion research and strategic planning.

References


Appendices

List of Appendices

Appendix 1 Posters (ACMG, ASHG, HGSA, BSHG)

Appendix 2 Poster BCGIP

Appendix 3 Australian Doctor

Appendix 4 Australian Jewish News

Appendix 5 Northern Clinical School Newsletter

Appendix 6 Wolper Newsletter

Appendix 7 Individual co-author and co-supervisor signed statements of

Contribution relating to published works included within this thesis
Appendix 1:  

Appendix 1 is a collection of 4 posters, presented at international peer review forums and reporting preliminary work relating to exploration of MPS based expanded panel preconception genetic screening of Australian AJ consenting mature minors planned to be conducted under research conditions.

The Posters contained in this Appendix do not form an examinable part of my Thesis, but are included for relevant background and supplementary information.

Poster 1  

Lew R, Burnett L, Proos, A, Massively Parallel DNA Sequencing for Ashkenazi Jewish community pre-conception genetic screening programs: Predicted outcomes, ethical and workforce implications, British Society of Genetic Medicine
(BSGM) Liverpool, UK, September 2013

Poster 2  

**Poster 3**


**Poster 4**

Massively Parallel DNA Sequencing for Ashkenazi Jewish community pre-conception genetic screening programs: Predicted outcomes, ethical and workforce implications

Raelia Lew,⁎, 1 Leslie Burnett, 1,2 Anné Proos, 1,2

1 The University of Sydney, Sydney, NSW, 2006, Australia 2Pacific Laboratory Medicine Services (PaLMS), Pathology North, NSW Health Pathology, Royal North Shore Hospital, St Leonards NSW 2065, Australia.

INTRODUCTION

Ashkenazi Jewish (AJ) individuals are at high risk of several genetic conditions. 1 Preconception genetic screening programs were introduced in Jewish high schools in Sydney (1995) 1 and Melbourne (1998) 2 to detect asymptomatic Tay Sachs disease carriers. The scope of these screening programs has expanded to now assess for multiple relevant autosomal recessive conditions (Table 2). 2, 3

Effectiveness in primary prevention 1, 4, 6 and cost effectiveness 6 of these programs have been validated. Conditions screened for and method of laboratory testing has changed over time (1995-2004 TSD: Enzyme testing + Restriction Length Fragment Polymorphism (RFLP), 2005-2012: Amplification Refractory Mutation System (ARMS)). 7

In Sydney, future testing may be conducted using massively parallel DNA sequencing technologies (MPS) as the routine modality, extending screening to a broader range of conditions.

AIM

To estimate the clinical impact of AJ preconception genetic screening using an MPS expanded panel.

METHODS

A panel of conditions was designed for use in AJ preconception genetic screening for common autosomal recessive conditions.

Criteria for inclusion was according to accepted principles for screening. 5

For modelling, the average number of students screened per annum was based on actual screening program data.

The known AJ prevalence of conditions included in the current and expanded panel (Table 1) was used to calculate the predicted number of carriers of one or more conditions that would be detected using the expanded panel, vs. the current screening method.

The probability calculation used was 1-[(1-P1)*(1-P2)*…(1-Px)], where p1 to p25 refer to the carrier frequency of each condition referenced 1 to 25 in Table 1.

The proportion of individuals screened that would require referral for genetic counselling services for both screening models was calculated.

RESULTS

Conventional DNA testing for 5 conditions (Table 1, marked with *) has an AJ calculated a priori risk carrier risk (for at least 1 condition) of 12%. In the Sydney program’s experience implementing this model (2005-12), the observed number of carrier participants (12% - see Table 2) was in agreement with this predicted number, and these were referred for genetic counselling.

In the proposed MPS screening model, ((Table 1, conditions 1 to 25), the a priori risk of being diagnosed a carrier for at least 1 condition is 50.8%.

DISCUSSION

Some 50% (1 in 2) AJ individuals screened with the proposed MPS panel would be diagnosed a carrier for ≥1 recessive condition, which predicts a more-than-four-fold (12% to 50%) increase in referrals for genetic counselling. This escalation highlights future workforce demands predictable with whole population uptake of genomic preventive medicine.

MPS also poses challenges to traditional concepts of informed consent. Currently, Jewish Community Genetic screening programs target Senior high school students. 2, 4, 5, 8 Mature minors 5 can provide informed consent for conventional DNA testing for a limited number of conditions. 9 Studies are now underway to ensure that informed consent is achievable in Community Genetics screening programs utilising MPS for multiple conditions simultaneously.

CONCLUSION

Development of health policy regarding ethical, legal and workforce implications of the implementation of diagnostic genome sequencing technology must be addressed with some urgency.

REFERENCES


TABLE 1: Current and proposed expanded AJ panel for preconception genetic screening

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Probability</th>
<th>Participants (30)</th>
<th>Carriers (6)</th>
<th>Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSD</td>
<td>1/32</td>
<td>110</td>
<td>6</td>
<td>TSD, CF</td>
</tr>
<tr>
<td>CF</td>
<td>1/23</td>
<td>172</td>
<td>13</td>
<td>CF, TSD</td>
</tr>
<tr>
<td>TSD</td>
<td>1/32</td>
<td>172</td>
<td>6</td>
<td>TSD</td>
</tr>
<tr>
<td>CF</td>
<td>1/23</td>
<td>202</td>
<td>13</td>
<td>CF, TSD</td>
</tr>
<tr>
<td>TSD</td>
<td>1/32</td>
<td>215</td>
<td>6</td>
<td>TSD</td>
</tr>
<tr>
<td>CF</td>
<td>1/23</td>
<td>215</td>
<td>13</td>
<td>CF, TSD</td>
</tr>
<tr>
<td>TSD</td>
<td>1/32</td>
<td>242</td>
<td>6</td>
<td>TSD</td>
</tr>
<tr>
<td>CF</td>
<td>1/23</td>
<td>242</td>
<td>13</td>
<td>CF, TSD</td>
</tr>
</tbody>
</table>

TABLE 2: Outcome summary Sydney Jewish community genetic screening programs

<table>
<thead>
<tr>
<th>Year</th>
<th>Participants</th>
<th>Carriers</th>
<th>Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>110</td>
<td>6</td>
<td>TSD, CF</td>
</tr>
<tr>
<td>1996</td>
<td>123</td>
<td>13</td>
<td>TSD, CF</td>
</tr>
<tr>
<td>1997</td>
<td>141</td>
<td>6</td>
<td>TSD, CF</td>
</tr>
<tr>
<td>1998</td>
<td>176</td>
<td>13</td>
<td>TSD, CF</td>
</tr>
<tr>
<td>1999</td>
<td>179</td>
<td>13</td>
<td>TSD, CF</td>
</tr>
<tr>
<td>2000</td>
<td>184</td>
<td>13</td>
<td>TSD, CF</td>
</tr>
<tr>
<td>2001</td>
<td>172</td>
<td>6</td>
<td>TSD</td>
</tr>
<tr>
<td>2002</td>
<td>202</td>
<td>13</td>
<td>CF, TSD</td>
</tr>
<tr>
<td>2003</td>
<td>215</td>
<td>6</td>
<td>TSD</td>
</tr>
<tr>
<td>2004</td>
<td>242</td>
<td>6</td>
<td>TSD</td>
</tr>
<tr>
<td>2005</td>
<td>242</td>
<td>13</td>
<td>CF, TSD</td>
</tr>
<tr>
<td>2006</td>
<td>238</td>
<td>4</td>
<td>CF, TSD</td>
</tr>
<tr>
<td>2007</td>
<td>234</td>
<td>4</td>
<td>CF, TSD</td>
</tr>
<tr>
<td>2008</td>
<td>253</td>
<td>4</td>
<td>CF, TSD</td>
</tr>
<tr>
<td>2009</td>
<td>252</td>
<td>4</td>
<td>CF, TSD</td>
</tr>
<tr>
<td>2010</td>
<td>224</td>
<td>4</td>
<td>CF, TSD</td>
</tr>
<tr>
<td>2011</td>
<td>278</td>
<td>4</td>
<td>CF, TSD</td>
</tr>
<tr>
<td>2012</td>
<td>278</td>
<td>4</td>
<td>CF, TSD</td>
</tr>
</tbody>
</table>

Carriers detected (%): 8% (TSD, CF), 12% (CF, TSD, CD, FA)
Massively parallel sequencing (MPS) has dramatically lowered the cost of genomic sequencing for multiple genetic conditions. Best ethical and clinical practice for clinical diagnostic testing using traditional targeted sequencing technologies requires informed consent and availability of genetic counselling.

High infant mortality from severe autosomal recessive (AR) childhood conditions has led to the establishment of genetics screening programs in communities with increased risk of AR conditions due to ancestral origins. For example, Australian Ashkenazi Jewish (AJ) preconception and carrier screening programs test for conditions relevant to the AJ community and have been effective in decreasing the incidence of AR-affected births (1).

The greater speed, lower costs and parallel nature of genetic testing using MPS technologies allows the testing panel of screening programs to be expanded to encompass a broader range of conditions. The effects of broader testing on carrier detection rates are yet to be fully evaluated.

To develop and validate a model to predict the AR genetic carrier rate in a dataset or population as the testing panel expands. This model was used to estimate carrier frequencies for the 26 most prevalent AR conditions in at-risk communities.

We validated the model using data from an Australian AJ community genetics screening program. Our model closely predicts the observed rates of AR genetic carriers detected in the Sydney AJ community screening program over an 18-year period.

Using our model, we studied the likely impact on carrier detection rates of the introduction of MPS into screening. We show that the primary driver of the AR genetic carrier rate is the number of tested conditions. This is in contrast to autosomal dominant conditions where the proportion of individuals with a pathogenic variant plateaus as the number of tested conditions is increased (4). Increasing the number of AR conditions screened from the current panel of 5 up to 26 would result in a 4-fold increase in the proportion of AR genetic carriers detected.


For further information please contact: leslie.burnett@sydney.edu.au
INTRODUCTION

Massively parallel sequencing (MPS), also called next generation sequencing, has dramatically lowered the cost of nucleic acid sequencing for multiple genetic conditions.

Best ethical and clinical practice for clinical diagnostic testing using traditional targeted sequencing technologies requires obtaining informed consent and ensuring availability of genetic counselling.

Testing for specific genetic conditions using MPS may incidentally discover off-target genetic conditions. The American College of Medical Genetics and Genomics (ACMG) has recently published recommendations for the clinical reporting of incidental findings for a list of 24 specific conditions. 1, 2

AIM

To predict the clinical impact of expanded genomic testing with MPS using the ACMG recommendations for Clinical Reporting of Incidental Findings.

METHODS

A diagnostic panel was simulated based on the ACMG recommended minimum list of genes to be reported, regardless of the original indication for the clinical sequencing (incidental findings).

The number of patients with significant variants in one or more conditions that would be detected using the screening panel was calculated from known (or best estimate) prevalence. Where a range of variant prevalence data was available, we selected the lowest and highest values, and calculated the most likely estimate as the geometric mean. Alternatively, when only a single datum was available, we selected half and twice this prevalence as the low and high estimates.

Assuming all disorders were inherited independently, the probability calculation used was 1- (1-P1)(1-P2)...(1-P24), with P1 to P24 as the carrier frequencies of the 24 proposed ACMG conditions. Calculations were repeated separately for the lower and higher limits.

The proportion of individuals screened who would require supplementary consultation and genetic counselling was calculated.

DISCUSSION

Our modelling of the ACMG Recommendations for Clinical Reporting of Incidental Findings has shown that a non-trivial percentage (1.5% - 6.5%) of screened individuals will have a significant reportable finding. These individuals would require confirmatory testing, education, counselling and potentially treatment but might derive significant benefit from the incidental findings.

All the conditions on the current ACMG list are relatively rare. Information about the population carrier frequencies of such rare conditions is necessarily limited, and calculations based on such limited information must be regarded with caution. Many of the estimates of carrier frequency are based on the prevalence of a particular disorder. The true carrier frequency may be much higher if the disorder has incomplete penetrance; this would lead to a falsely low estimation of the predicted number of incidental findings. Similarly, some pathogenic phenotypes will be due to genetic variations not yet described or currently considered of uncertain significance; these will lead to a falsely high estimation of the number of true incidental findings. However, over time, with increasing experience and larger population sample numbers, the true rate of incidental findings will become apparent.

Ongoing research continues to reveal the genetic basis for more and more conditions. The ACMG list of Recommendations is likely to grow, not shrink, over time. The implementation of the ACMG Recommendations for Clinical Reporting of Incidental Findings will require major increases in resources for the health system. To adopt the ACMG Recommendations, these increased resource requirements need to be identified, costed and addressed. The costs to implement the recommendations need to be compared to the potential benefits, cost offsets and utility of reporting and acting on these findings.

REFERENCES

TABLE 1: Carrier Frequency Estimates of Recommended Conditions

<table>
<thead>
<tr>
<th>Probability</th>
<th>Disease Phenotype</th>
<th>Carrier Frequency Low estimate</th>
<th>Carrier Frequency Most likely estimate</th>
<th>Carrier Frequency High estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>hereditary breast and ovarian cancer</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>2</td>
<td>Li-Fraumeni syndrome</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>3</td>
<td>retinoblastoma syndrome</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>4</td>
<td>Lynch syndrome</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>5</td>
<td>syndrome adenomatous polyposis</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>6</td>
<td>SMN-associated polyadenyl, adenosine, multiple colorectal, type 2; SMN2 adenosine, multiple colorectal, type 1</td>
<td>1.0000% (0.0000%)</td>
<td>1.4202% (0.0000%)</td>
<td>2.0000% (0.0000%)</td>
</tr>
<tr>
<td>7</td>
<td>von Hippel Lindau syndrome</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>8</td>
<td>multiple endocrine neoplasia type 1 (MEN1)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>9</td>
<td>multiple endocrine neoplasia type 2 (MEN2)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>10</td>
<td>familial medullary thyroid cancer (FMTC)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>11</td>
<td>familial retinoblastoma syndrome</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
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<tr>
<td>12</td>
<td>hereditary paroxysmal phaeochromocytoma syndrome</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
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<tr>
<td>13</td>
<td>neurofibromatosis type 1</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>14</td>
<td>neurofibromatosis type 2</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
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<tr>
<td>15</td>
<td>oculodermal melanocytosis, kindreds; S. Ellis-van Creveld Syndrome</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>16</td>
<td>symmetric disorder, axenic anemia and dysregulation</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>17</td>
<td>hyperparathyroidism, isolated</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>18</td>
<td>lynch syndrome polyposis</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>19</td>
<td>hereditary reticular vascular cancer</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
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<tr>
<td>20</td>
<td>neurofibromatosis type 1,2,3,4</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>21</td>
<td>familial hypercholesterol</td>
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<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
<tr>
<td>22</td>
<td>myotonic dystrophy</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
<td>0.0000% (0.0000%)</td>
</tr>
</tbody>
</table>

TABLE 2: Expected Incidental Findings Using ACMG Panel

<table>
<thead>
<tr>
<th>Carrier Frequency Estimate</th>
<th>Low</th>
<th>Most likely</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of individuals who screen positive for at least one of the 24 conditions</td>
<td>1.52%</td>
<td>2.70%</td>
<td>6.48%</td>
</tr>
</tbody>
</table>

RESULTS

The proposed ACMG recommended screening panel would require supplementary consultation and genetic counselling for approximately 2.7% (range 1.5%-6.5%) of screened individuals.

For further information please contact: leslie.burnett@sydney.edu.au
Genetic screening for autosomal recessive (AR) genetic carriers is available in many communities “at risk” due to high prevalence of pathogenic variants. Examples include Tay-Sachs disease testing in the Ashkenazi Jewish (AJ) community. The range of tests included in screening programs is broadening, and will accelerate with the introduction of Massively Parallel Sequencing.

We recently modelled the frequency of autosomal dominant (AD) genetic conditions arising as Incidental Findings (IF) in Whole Genome Sequencing. We found that the proportion of tested individuals with significant IFs plateaus to a limit even as the number of genes tested increases beyond those in the ACMG IF Recommendations (1).

In contrast, modelling of AR conditions showed that the carrier rate continues to increase with the number of genes tested (2). As more gene panels are included in screening programs, a risk emerges of being a “genetic wallflower”, someone rejected by every suitor because of the common AR variants they carry.

AIM

To model the rate of increase in AR genetic carriers with increasing numbers of AR genes in test menus. We also explore the likelihood of genetic wallflowers emerging.

METHODS

We developed a computer simulation model to create a virtual population of individuals with randomised genomes. The simulation model could be adjusted to vary population size (we used 10^2-10^4), the number of genes tested (each genome consisted of 1-27 genes of known AJ prevalence, supplemented by 10^2 - 10^6 simulated genes, so that at maximal values, the simulated genome approximated the size of the human exome), the prevalence of pathogenic AR variants (using actual gene prevalence for known AJ genes, and 10^-2 - 10^-4 for simulated genes) and number of iterations (range 10-100). Random couples were then chosen from each population to ‘mate’. The average AR genetic carrier rate, number of variants present per individual and proportion of at-risk couples (i.e. both partners being carriers for the same pathogenic AR genetic condition) for a population were calculated.

The simulation model was validated using actual testing panels and data from Australian AJ community genetics screening programs. Known or best estimate carrier frequencies for the 26 most prevalent AR conditions in the AJ community were obtained from published literature (3,4). The simulated annual rate of AR genetic carriers was compared to the actual rate of carriers and our previous mathematical modelling results (2).

The effect of increasing the number of genes tested on the overall carrier rate and on the proportion of at-risk couples was simulated on a population of 10,000 individuals and averaged from 100 iterations.

RESULTS

Our simulation model correctly predicts the observed rates of AR genetic carriers detected in the Australian AJ community over an 18-year period, and closely tracks our mathematical model. Figs 1-4 illustrate the behaviour of AR conditions in our simulated populations.

CONCLUSIONS

As the number of AR conditions included in testing panels is increased, the number of AR genetic carriers identified in a population will increase. This behaviour is in marked contrast to that for AD conditions, where the proportion of a population with a reportable IF will plateau. These findings have significant implications for health economic evaluation and planning. However, regardless of the number of AR conditions tested, the number of “at-risk” carrier couples increases more slowly, lessening the risk of emergence of genetic wallflowers. We are currently quantifying this risk.

REFERENCES


For further information please contact: leslie.burnett@sydney.edu.au
Appendix 2:

Appendix 2 is a poster reporting preliminary work that was later expanded on and published in Chapter 4 of this thesis.

The Poster contained in this Appendix does not form an examinable part of my Thesis, but is included for relevant background and supplementary information.

Poster 5

Tay Sachs Disease: Evaluating the impact of genetic screening on disease incidence in Australia

Raelia Lew1,2,3, Anne Proos1,2, Leslie Burnett1,2, Martin Delataycki1,4, Agnes Bankier2,5, Michael Fietz6

1 The University of Sydney, 2Pacific Laboratory Medicine Services (PaLMS), Pathology North, 3Royal Prince Alfred Hospital, 4Victorian Clinical Genetics Service (VCGS), Murdoch Children’s Research Institute, 5Royal Children’s Hospital (RCH), 6Austin Health Clinical Genetics Service, * Department of Biochemical Genetics, SA Pathology (at WCH)

INTRODUCTION

Tay Sachs disease (TSD) is a lethal, recessive, neurodegenerative lysosomal sphingolipid storage disorder1, where mutations of HEXA MIM *606869 (15q23-q24) result in hexosaminidase A enzyme deficiency2. TSD is more common in Jewish individuals. We investigated the effect of 17 years of preconception genetic screening programs3,4 for TSD in Jewish communities in Sydney and Melbourne on disease incidence

METHODS

• Ethics approval was obtained.
• A comprehensive audit of medical case and laboratory records (RCH, VCGS, PaLMS, SA Pathology) identified all infantile and intermediate TSD cases diagnosed in Sydney and Melbourne (1995-2011).
• TSD carrier frequency was calculated for Jewish Australians using screening program data (Sydney/Melbourne, 1995-2011).
• We used TSD carrier frequency 1 in 250 (0.4%) for other Australians (WHO)6.
• Population demographic data was extracted for estimated Jewish (2.6, 34%) and general and Jewish populations (Hardy-Weinberg equation).

RESULTS

• Of 88,826 Australians who reported their religion as “Jewish” in the 2006 census, 46% live in Melbourne and 40% in Sydney.
• 7756 Jewish High school students screened for TSD (1995-2011) in Melbourne and Sydney demonstrated TSD carrier frequency 1 in 31 (3.3%) (95% CI 2.9-3.7%).
• TSD expected incidence (1995-2011) was calculated for estimated Jewish (2.6, 34%) and other births (5.0, 66%) in Melbourne and Sydney.
• Our comprehensive audit (1995-2011) found 12 TSD cases diagnosed in Sydney (4) and Melbourne (8), of which 2 (17%) were Jewish.
• No Jewish TSD case was born to parents who has undertaken preconception genetic screening previously.

TAY-SACHS DISEASE (TSD)

Preconception Screening Programs

TABLE 1: TSD PRECONCEPTION JEWISH PROGRAMS MELBOURNE AND SYDNEY 1995-2011

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<tr>
<td>Students screened</td>
<td>275</td>
<td>92</td>
<td>183</td>
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<tr>
<td>Carriers detected</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Carriers frequency</td>
<td>1 in 137.5</td>
<td>1 in 92</td>
<td>1 in 121.5</td>
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DISCUSSION

The Majority of Jewish Australians reside in Melbourne (46%) and Sydney (40%)9,10. 50% of young Jewish adults in Sydney and 70% in Melbourne attend schools that access preconception genetic screening programs for TSD and other recessive conditions more common in Jewish people (Gaucher disease, cystic fibrosis, mucolipidosis type IV, Fanconi anemia, familial dysautonomia, Canavan disease, Bloom syndrome).

78% of Jewish students screened for TSD in Melbourne and Sydney (1995-2008) identified as Ashkenazi11.

Screening programs are co-ordinated in Melbourne (1997-2012) by Victorian Clinical Genetics Services, Murdoch Children’s Research Institute, Royal Children’s Hospital, Melbourne and in Sydney (1995-2012) by The Australian Community Genetics Program through the Wolear Jewish Hospital using the Laboratory and Community Genetics Department, Pacific Laboratory Medicine Services [PaLMS, Pathology North], Royal North Shore Hospital.

• Genetic material is now collected by mouth wash sampling.
• Following pre-screening education and counselling, participation rates approach 100%12. Jewish TSD cases in Sydney and Melbourne were audited over the period 1995-2011, corresponding to widespread young Jewish adult participation in TSD preconception screening programs.

The clinically evident 50% reduction in Jewish TSD cases has not yet achieved statistical significance due to the small size of the Australian population. During the 17 year study period, N=12 TSD cases were diagnosed. Using the binomial test to show a 50% reduction in Jewish cases (80% power, p<0.05) would require N=43 TSD cases.

Adoption of TSD preconception genetic screening program models may be expected to show similar disease incidence reductions when applied to other mendelian disorders with similar carrier frequencies in target populations such as cystic fibrosis in Caucasian populations.

Our study has limitations: retrospective design, low TSD prevalence, relative small sample size, limited Jewish population census data.

CONCLUSIONS

• The effectiveness of community education, appreciation of autosomal recessive inheritance and carrier screening pre-pregnancy are likely factors to explain fewer than the expected number of Jewish babies born affected with TSD.
• Ongoing monitoring must be continued, so as to ensure outcomes evaluation.
• Carrier frequency for TSD in Australian Jewish high school students screened between 1995-2011 was 1 in 31. Without ongoing intervention through community screening, this population remains at significant risk for TSD.

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10. Australian Bureau of Statistics 2006, 2006 Census of Population and Housing, Australia (Australia) Religious affiliation (a) broad by sex, Cat. No. 2068.0, ABS Canberra

Correspondence to
rlew2064@uni.sydney.edu.au
Appendix 3

The article contained in Appendix 3, written by medical journalist David Brill appeared in the magazine Australian Doctor published on the 10th December 2012, in response to the publication of chapter 5 of this thesis.

Screening sees demise of Tay-Sachs

Tay-Sachs disease has been all but eliminated among Australian Jews, thanks to a high school genetic screening program, research shows.

Just two affected infants have been born to Jewish families since screening began in 1995, neither of whose parents had partaken in screening, according to a study published Monday in the Medical Journal of Australia.

Tay-Sachs disease is an autosomal recessive lysosomal storage disorder characterised by slow neurological decline from early infancy. It is typically fatal by age four.

The gene mutation is disproportionately common among Jews, particularly those of Ashkenazi descent, one in 25 of whom carry it.

It is found in one in 97 Jews of mixed or non-Ashkenazi heritage, and one in 250 people in the general population.

Study author Dr Raelia Lew said the screening program carried out in the Jewish community had been a clear success.

Population modelling showed that without screening, there would have been four infants born to Jewish families over the study period.

"It not only facilitates individuals’ awareness of their carrier statues, but it also increases the awareness of Tay-Sachs disease for the community and increases understanding of the mode of inheritance," said Dr Lew, a senior obstetrics and gynaecology registrar at Sydney’s Royal Prince
Alfred Hospital, who is researching Tay-Sachs disease for a PhD.

The community-funded screening program, which targets students at Jewish high schools free of charge, was launched in Sydney in 1995 and Melbourne in 1998.

It also screens for cystic fibrosis, Fanconi anaemia, Canavan disease and familial dysautonomia.

Uptake is extremely high, at more than 99%. About 7750 students were screened from 1995 to 2011.

The research could not establish what decisions had been made by families in which both partners were identified as carriers.

Dr Lew said they had several options: adoption, IVF with preimplantation genetic screening, use of eggs or sperm from a non-carrier donor, or proceeding with a natural pregnancy and having chorionic villus sampling and amniocentesis, if necessary.

The choice was often influenced by people’s level of religious observance, she said.

Professor Leslie Burnett, honorary medical director of the screening program, said the research showed it had been “a remarkable success”.

"It’s a dreadful disease. The child is born healthy and you see it wither and die before your eyes," said Professor Burnett, a consultant pathologist at NSW Health and clinical professor in pathology at the University of Sydney.

"[This research] shows that by introducing a screening program, particularly one that’s ethically sound, based on education, knowledge and informed consent, you can avoid families having to go through the trauma."

Ten infants with Tay-Sachs disease were born to non-Jewish parents over the period of the study, compared to a predicted number of seven.

The study looked only at births in Sydney and Melbourne, where the majority of Australia’s Jewish population lives.

Professor Burnett said the program could be expanded to all Australians in future.

He hoped to submit an application to list the test on the MBS next year.

Previous research had shown that community-wide screening for Tay-Sachs disease was more cost effective than targeting only families with an affected child.

Screening in Sydney is funded by the Wolper Jewish Hospital, which runs the school-based program and once-monthly screening sessions.

CEO Harry Aizenberg said the project had cost about $500,000 since its launch in 1995 — roughly $30,000 per year.


Australian Doctor on Twitter

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Call to ‘forget GPs’ and self
Dismissed
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Several deaths.
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Our secure...
Appendix 4 contains a newspaper article written by journalist Gareth Narunsky was published in the 19th August 2011 edition of the Australian Jewish News in response to work published as chapter 4 of this thesis:

Scientists' Tay-Sachs find

GARETH NARUNSKY

A RECENTLY released study on Tay-Sachs disease has revealed the risk among Jews of being a carrier is not linked to geographic ancestry, contradicting previously assumed knowledge.

Genetic researchers had thought the risk of carrying or contracting the illness was highest among Jews whose families hailed from Poland, Hungary, Lithuania or surrounds.

But the new analysis, conducted over 13 years by researchers at Royal North Shore Hospital and the University of Sydney's department of obstetrics and gynaecology, has found that all Ashkenazi Jews faced a similar risk of carrying the Tay-Sachs gene.

Professor Leslie Burnett, of Royal North Shore Hospital, said the findings were a great surprise, both to his team and to the genetic world in general.

"We had assumed that the geographic location of one's ancestors would affect one's risk," he said. "But what we have found is that simply being an Ashkenazi Jew is sufficient information to know one's risk."

Professor Burnett said many Jews were reluctant to be tested, assuming they were not at risk because their ancestors were not from a particular part of Europe.

"Our findings now make it very clear," he said. "We are all part of one community, and over the centuries, our genes have become blended across national borders."

"This means that the only question one needs to ask oneself is whether one is Jewish, in which case it is important to participate in the community genetics screening program and to be tested."

Around one in 27 healthy Ashkenazi adults is a carrier of Tay-Sachs disease. If two carriers have a child, there is a 25 per cent chance that the child will be born with the disease, which causes severe disability and death by the age of three or four.

Inquiries: (02) 9328 6077.
Appendix 5

Appendix 5 contains a magazine article by Claire Bridgeman, entitled Congratulations to Dr Raelia Lew, that appeared in the Sydney University Northern Clinical School Newsletter in February 2013 in response to the work published in Chapter 5 of this thesis:


The article can also be accessed via the following link:
FEATURE ARTICLE

Congratulations to Dr Raelia Lew, a PhD student of Professor Leslie Burnett and an O&G Registrar at Sydney's Royal Prince Alfred Hospital, who has just published a paper in the Medical Journal of Australia 2012; 197:652-54, reviewing almost two decades of genetic screening for Tay-Sachs disease.

E-NEWSFLASH - FEBRUARY 2013

Happy New Year!

We are very excited to be starting a new year in 2013, with our new stage 1 students starting in a few weeks time, and our stage 3 students already back and immersing themselves on the wards!

We look forward to sharing another great year with you all, don’t forget if you have any news you would like to see published in the newsletter we would love to hear from you. Please send any stories to claire.bridgman@sydney.edu.au

A Sydney Medical School – Northern celebration

Save the date for the upcoming Sydney Medical School – Northern celebration, Medical Education – All Together Better Health.

On the evening of Tuesday the 19th March we would love you to join us at this annual event where we celebrate medical education in all its facets, and award prizes over a glass of wine and canapés.

Invitations will go out shortly but in the meantime make sure you have the date (19th March 2013) and time (5:30pm) in your diaries. We look forward to seeing you there!

Congratulations to Dr Raelia Lew

Congratulations to Dr Raelia Lew, a PhD student of Professor Leslie Burnett and an O&G Registrar at Sydney’s Royal Prince Alfred Hospital, who has just published a paper in the Medical Journal of Australia 2012; 197:652-54, reviewing almost two decades of genetic screening for Tay-Sachs disease (a classic neurodegenerative autosomal recessive disease, which affects newborn children).

Screening of parents for Tay-Sachs disease was introduced into Australia by Professor Burnett and Ms Anné Proos in 1992, and was first offered to the Sydney population via community genetic screening in 1995 then, following Professor Burnett’s and Ms Proos’ joining the Kolling Institute in 1997, offered to the rest of Australia in 1998. Their laboratory is currently housed in PaLMS Pathology on the RNS Hospital campus, but continues to publish its work under the auspices of the University of Sydney.
Professor Burnett explained the significance of Dr Lew’s findings: “While our past research has demonstrated genetic screening is medically effective and cost-effective, we had not been able to demonstrate “outcomes effectiveness”, i.e. that genetic screening actually works when applied to an entire population. Raelia’s paper has now done just that. She has shown that, since the introduction of genetic screening to the entire Australian population, there has not been a single case of Tay-Sachs disease in the screened population, while those who did not take up screening continued to have children affected with the disease at the baseline rate. This is a very important demonstration, as it now means we have an evidence base for effectiveness of this new form of diagnosis and treatment.’

Further information can be found in Australian Doctor 10 December, 2012.

If you would like to contribute to this newsletter, or have any feedback, please contact Claire Bridgman.

Sydney Medical School - Northern

Phone: 02 9926 4678
Email: claire.bridgman@sydney.edu.au
Web: http://sydney.edu.au/medicine/northern
Appendix 6

Appendix 6 contains an article that appeared in the Wolper Hospital Newsletter in response to the publication of work published in Chapter 5 of this thesis:


The Wolper Hospital together with the Sydney Jewish Community Genetics screening program, co-ordinates outreach screening strategies in Sydney for TSD and other genetic conditions relevant to Jewish communities.
Wolper winning against Tay Sachs disease

The Medical Journal of Australia has just published a research paper on community testing for Tay-Sachs disease in Australia. Its findings have documented the success of the genetic screening program currently offered in Sydney to all Jewish Day schools and at a Sunday Clinic at Wolper Jewish Hospital.

Tay-Sachs disease is one of a number of genetically inherited diseases which can affect children of any ancestry, but with a much higher genetic carrier rate in the Jewish community, where 1 in every 31 people can carry the Tay-Sachs genetic mutation. A genetic carrier of Tay-Sachs does not have, and will not develop Tay-Sachs disease. However, when both parents carry the Tay-Sachs affected gene, there is a 1-in-4 chance that their children may have Tay-Sachs disease. Diagnosis is usually made after the child reaches 6 months of age and the family must deal with the knowledge that their child will deteriorate in health significantly and will die by the time they are five years old – there is no cure.

In the Australia-wide research coordinated by Dr Raelia Lew on behalf of the Sydney and Melbourne Tay-Sachs screening programs it was found there had been no Tay-Sachs disease affected children born to any parents who had participated in the genetic screening program during the study period 1995 to 2011. However, there were two Tay-Sachs affected children in Victoria born to families who had unfortunately not participated in the screening program. During this same period, there were 10 Tay-Sachs affected children born in Australia to non-Jewish families. Even though the carrier rate for the Tay-Sachs genetic mutation in the Jewish community remained high at 1 in 31 people, the actual number of Tay-Sachs affected children born to Jewish families was well below the number expected if the screening program had not been in operation. And for those members of the Jewish community who participated in the community screening program, there was not a single case of a Tay-Sachs affected child in the 16 years analysed by the research study.

Harry Aizenberg, CEO of Wolper Jewish Hospital, which has funded the program since 1995, stated “we are delighted with the results. What a wonderful outcome for the Community. It vindicates our decision to support the program. Through offering the screening program to all students in our Jewish Day Schools, as well as making the program available via our monthly Sunday Community Genetics program at Wolper, we have ensured that no Jewish family need suffer the trauma of a child being born with Tay-Sachs disease”.

For an information booklet on the Sydney Genetic Screening Program contact Wolper Jewish Hospital on 9328 6077.
Appendix 7

Appendix 7 contains the individual signed statements of contribution from co-supervisors of this thesis and co-authors of published works included in this thesis. These statements make reference specifically and accurately to the nature and extent of co-authors’ contributions to published chapters.
Contribution Statement and Co-Authors

The roles played by the co-authors in chapters 3, 4, 5, 6 and 8 were as follows:

1. Professor Leslie Burnett was my primary research supervisor. He provided intellectual input into the review of chapters 3, 4, 5, 6 and 8.
2. Professor Lucy Raymond was an associate supervisor. She provided support during my period of study in Cambridge, UK.
3. Dr Robert Markham was an associate supervisor. He provided intellectual input into review of this thesis.
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15. Dr Michael Field was a co-investigator. He provided intellectual input into the review of chapter 6.

The final editorial authority remained my own.

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