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Offering fragile X syndrome carrier screening: a prospective mixed-methods observational study comparing carrier screening of pregnant and non-pregnant women in the general population

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Check
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	X
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	X
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	X
Objectives	3	State specific objectives, including any prespecified hypotheses	X
Methods			
Study design	4	Present key elements of study design early in the paper	X
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	X
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	X
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	X
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	X
Bias	9	Describe any efforts to address potential sources of bias	X
Study size	10	Explain how the study size was arrived at	X
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	not yet applicable
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	X
		(*b*) Describe any methods used to examine subgroups and interactions	X
		(*c*) Explain how missing data were addressed	not yet applicable
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods	not yet applicable

		taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	not yet applicable
Results			**Check**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	not yet applicable
		(b) Give reasons for non-participation at each stage	not yet applicable
		(c) Consider use of a flow diagram	X
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	not yet applicable
		(b) Indicate number of participants with missing data for each variable of interest	not yet applicable
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	not yet applicable
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	not yet applicable
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	not yet applicable
		(*b*) Report category boundaries when continuous variables were categorized	not yet applicable
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	not yet applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	not yet applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	not yet applicable
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	not yet applicable
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	not yet applicable
Generalisability	21	Discuss the generalisability (external validity) of the study results	not yet applicable
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	X

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

TITLE PAGE

'Offering fragile X syndrome carrier screening: a prospective mixed-methods observational study comparing carrier screening of pregnant and non-pregnant women in the general population'

Short title: The fragile X syndrome carrier screening (FaXeS) study

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ARTICLE SUMMARY

Article focus

This article is a protocol of a study that involves offering fragile X syndrome carrier screening to pregnant and non-pregnant women in the general population. We are undertaking a program evaluation approach using mixed methods to collect data about informed decision-making and predictors of test uptake, with a focus on psychosocial measures. We are also undertaking an economic appraisal.

Key messages

- Carrier screening for fragile X syndrome is the subject of debate because of concerns around education and counselling for this complex condition, and the potential for psychosocial harms.
- This study will inform policy and practice in the area of population carrier screening by examining psychosocial aspects of screening, including informed decision-making; models of screening, through antenatal care or other access points; and health economics of carrier screening for fragile X syndrome.

Strengths and limitations of this study

- This study seeks to recruit 1000 women in total. This large sample size will give us sufficient power to address the aims of the study.
- Collecting both quantitative and qualitative data will provide a more in-depth picture of screening for fragile X syndrome.
- A limitation of the study is that the data on models of screening may not be applicable to other countries that have different healthcare systems.

ABSTRACT

Fragile X syndrome (FXS), an X-linked genetic condition, is the leading cause of inherited intellectual and developmental disability. Policy development relating to carrier screening programs for FXS requires input from large scale studies examining not only test uptake but also psychosocial aspects. This study will compare carrier screening in pregnant and non-pregnant populations, examining informed decision-making, psychosocial issues and health economics.

Pregnant and non-pregnant women are being recruited from general practices and obstetric services. Women receive information about the study either in person or through clinic mail outs. Women are provided pre-test counselling by a genetic counsellor and make a decision about accepting or declining the FXS carrier test in their own time. Data are being collected from two questionnaires: one completed at the time of making the decision about FXS carrier testing, and a

second one month later. Additional data are gathered though qualitative interviews conducted at several time-points with a subset of participating women, including all women with a positive test result, and with staff from clinics involved in recruitment.

A minimum sample size of 500 women per group has been calculated to give us 88% power to detect a 10% difference in test uptake and 87% power to detect a 10% difference in informed choice between the pregnant and non-pregnant groups.

Questionnaire data will be analysed using descriptive statistics and multivariate logistic regression models. Interview data will be thematically analysed. Willingness-to-pay and cost effectiveness analyses will also be performed.

Ethics approval has been granted by the Universities of Melbourne and Western Australia and from recruiting clinics, where required. Results will be reported in peer-reviewed publications, conference and seminar presentations and via a website www.fragilexscreening.net.au. The results of this study will make a significant contribution to discussions about the wider introduction of population carrier screening for FXS.

INTRODUCTION

Population based screening programs are available for a number of genetic conditions in the newborn, prenatal and preconception settings. Several guidelines based on specific criteria exist to help assess which genetic conditions are suitable for population screening [1, 2]. Fragile X syndrome (FXS) is an X-linked condition which meets many of the criteria for population screening, as discussed in Hill et. al [3]. However, in many countries it is still not routine practice to offer carrier screening for FXS. This is because of concerns about the challenges of screening for this complex condition, including the need for genetic counselling and education and the potential psychosocial and other impacts of a positive result, discussed further in Finucane [4].

FXS is the most common inherited cause of intellectual and developmental disability. Virtually all FXS is caused by an expanded CGG trinucleotide repeat in the 5' untranslated region of the *FMR1* gene which leads to hypermethylation and silencing of the gene [5-9]. Currently, the normal range of repeats is defined as 6-44, with 45-54 repeats being considered an intermediate 'grey zone' allele (GZ), 55-200 a premutation (PM) and >200 repeats a full mutation [10, 11]. The repeats in the GZ, PM and FM ranges can expand when passed from mother to child, although not usually from father to child [8, 12, 13].

The full mutation is associated with intellectual disability, anxiety and features of autism spectrum and attention/deficit hyperactivity disorders [14]. The clinical presentation varies between individuals [15] with males usually more severely affected than females. FXS is not curable but specific treatments exist which may help a number of the physical [16-19] and behavioural symptoms [20]. Although there is currently no robust evidence to support specific pharmacological treatments for people with FXS [21], a number of new therapies are being trialled [22-25] which may lead to improved treatments in the future.

In addition to the reproductive risk of having a child with FXS, female FXS PM carriers also have personal health risks: an increased risk of fragile X associated primary ovarian insufficiency (FXPOI), with a 20% risk of premature menopause [26-29]; a higher incidence of mental health issues such as anxiety and depression [4]; a risk of developing fragile X associated tremor/ataxia syndrome (FXTAS),

a late onset neurodegenerative condition, which is more common in male PM carriers than female [29-31].

The reported prevalence of *FMR1* alleles varies. Three large studies examining *FMR1* in anonymous newborn samples [32-34] found frequencies of the *FMR1* FM in males of 1 in 2633 [33] to 1 in 6,209 [34]. Reported rates of the PM in females in four large studies [12, 34-36] range from 1 in 154 [12] in Israel to 1 in 549 [34] in Canada, with rates of 1 in 178 [35] and 1 in 209 [36] reported for the USA. Two large studies reported GZ rates of 1 in 66 [36] to 1 in 85 [34].

A number of studies have investigated carrier screening for FXS for women in the general population [12, 37-46]. Most of these studies focused on uptake of testing, *FMR1* allele sizes and expansion rates, reproductive choices and pregnancy outcomes. However, genetic population screening guidelines [1] emphasise the importance of examining the psychosocial aspects of screening, including informed decision-making. Only our pilot study [43, 47] and one other retrospective study [39] have measured the psychosocial impacts of screening for FXS and no studies to date have examined informed decision-making.

This study aims to help us better understand the psychosocial aspects of carrier screening for FXS and will:
1. Compare informed decision-making by pregnant and non-pregnant women offered carrier screening for FXS.
2. Compare uptake and predictors of uptake in pregnant and non-pregnant women offered carrier screening for FXS.
3. Undertake an economic appraisal of FXS population carrier screening.

Informed decision making is complex and involves many factors [48]. One measure used in population carrier screening for Down syndrome to estimate informed decision making is the multidimensional model of informed choice (MMIC) [49], which describes an informed choice as a decision made with sufficient knowledge that is value consistent. Our study will measure informed choice using MMIC and will also collect additional information on factors involved in informed decision making in the two study questionnaires and through qualitative interviews.

Our study will also provide information on when to offer population carrier screening for FXS by comparing screening in non-pregnant and pregnant women. Population carrier screening guidelines recommend pre-conception carrier screening [1] but such screening is often embedded in antenatal care, as this provides a convenient (from the perspective of the service provider) point of access, although may be a more anxious time for women. Research on informed decision-making in prenatal screening, primarily for Down syndrome, has shown that decisions about testing are often not informed [50-53]. Our study will be the first to investigate whether rates of informed choice and uptake differ between pregnant and non-pregnant women.

We are testing two hypotheses:
1. A lower proportion of pregnant women will make an informed decision about carrier screening compared with non-pregnant women.
2. Carrier screening for FXS will result in a higher uptake of testing by pregnant women compared with non-pregnant women.

The findings of this study will contribute valuable data to inform debate on policy and approaches to population carrier screening for FXS.

METHODS AND ANALYSIS

Key elements of study design

Study design

The development and implementation of an effective carrier screening program is a multi-step process requiring a clear theoretical framework. We have developed a program evaluation model (see Figure One) to investigate FXS carrier screening incorporating 5 stages: (1) negotiation and planning; (2) program development; (3) program implementation; (4) short-term outcomes; and (5) long-term outcomes. The results of our qualitative needs assessment and pilot study, representing stages 1 and 2, have previously been published [43, 47, 54, 55].

The current study covers stages 3 and 4 and uses a mixed-methods approach to data collection to investigate the short-term outcomes of implementing an FXS carrier screening program. Figure Two provides an overview of the study design. Specifically, we will investigate test uptake, informed decision-making, predictors of test uptake, psychosocial outcomes (depression, anxiety, stress, decisional conflict and decisional regret) and health economic factors (willingness-to-pay).

The key elements of the study are that all women will receive a purpose-made brochure and genetic counselling before making a decision about testing, the test is optional, convenient and non-invasive and offered at no charge to the participants. Genetic counselling and the field-tested brochure is included in the protocol, as participants in our pilot study and needs assessment indicated that having sufficient information and the chance to discuss it is important in making an informed decision [43, 54]. Offering a test that can be performed at home after sufficient time for decision-making is important, as we found in our pilot study that having to return to the clinic for an invasive test was identified as a barrier to testing, although did allow some time for deliberation [43]. Recruiting pregnant and non-pregnant women will allow us to examine if there are any differences in test uptake, informed choice or psychosocial measures between these groups. Our economic appraisal will provide important information to guide policy on offering carrier screening for FXS.

Figure One: Program evaluation model to investigate FXS carrier screening

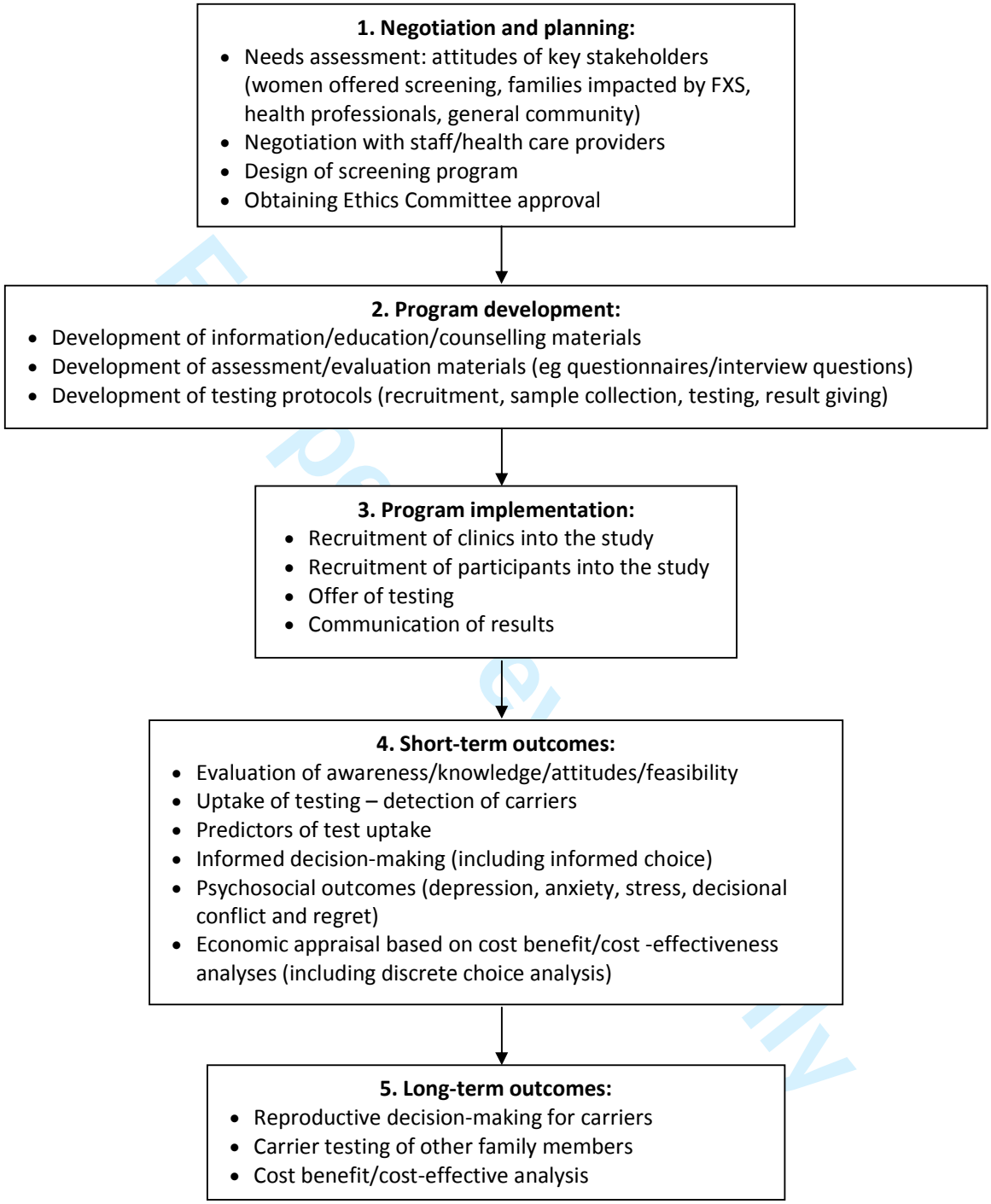
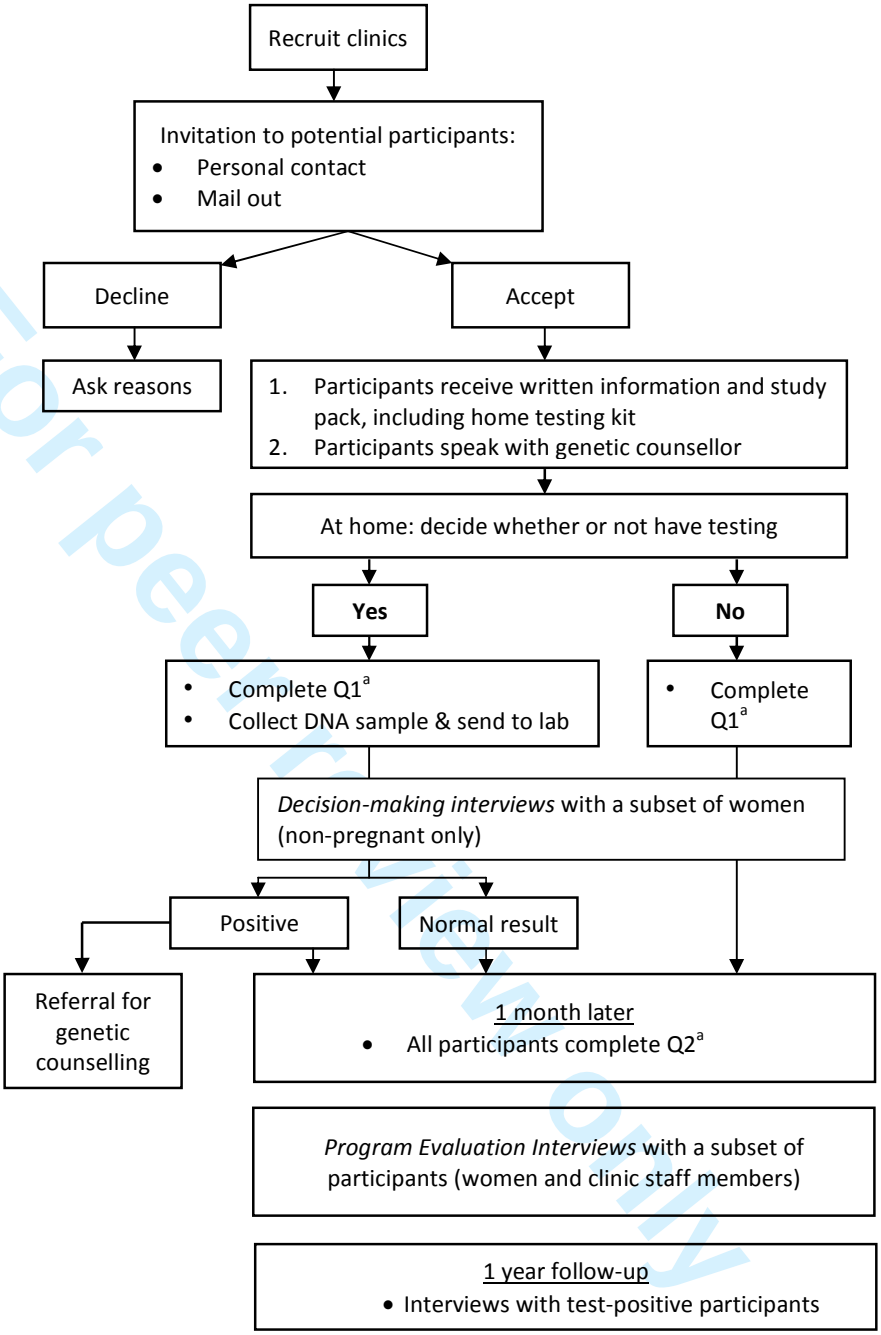


Figure Two: Overview of study protocol



[a] See table 1 for details of measures included in questionnaires 1 and 2

Settings

The study is being conducted in general practices, public and private obstetric clinics and through private obstetric ultrasound services in Melbourne, Victoria and Perth, Western Australia.

General Practice

In Australia, women may attend any general practice of their choice, and may attend more than one practice. General practitioners (GPs) are the gatekeepers to access secondary and tertiary care services. About 88% of the Australian population visit a GP at least once a year [56]. Most GP clinics also operate a reminder system for the National Cervical Screening Program, which offers women between the ages of 18 and 69 a cervical (Pap) smear test every two years. Thus most GP clinics have a mail-out system in place to send a reminder letter to their female patients every 2 years. This provides one approach to inviting non-pregnant women into the study and could act as a future service model for population carrier screening.

Obstetrics

A range of maternity care models exist in Australia but they can be broadly divided into private maternity care, public hospital maternity care and shared local health practitioner/ public hospital maternity care. The first step in accessing maternity care is to attend a GP in early pregnancy to obtain a referral to a private obstetrician or public hospital. The timing of the first appointment with the maternity care provider varies, but in the public hospital system women are often not seen until the second trimester of pregnancy. In 2009, the majority (96.9%) of Australian women gave birth in hospitals and of these, 69.9% (150,157 women) were in the public system and 30.1% (64,771 women) were in the private system [57].

Obstetric ultrasound – first trimester combined screening

Provision of antenatal screening varies across Australia. In Victoria and Western Australia, first trimester combined screening is available through private pathology laboratories and private ultrasound clinics with some rebate available from the government funded Medicare system, while second trimester screening is state funded. General practitioners or private obstetricians refer women to the private ultrasound clinic for a first trimester nuchal fold thickness scan. In Victoria, about 70% of pregnant women have first trimester combined screening (personal communication, L Bonacquisto, 2013) [58, 59] and so would be expected to attend a private ultrasound practice. In addition to offering testing at initial presentation in primary care, linking FXS carrier screening to first trimester screening is another potential service model.

Participants

Enrolling women in the study

Women are eligible to enter the study if they are 18 or over and either not pregnant or up to 12 weeks + 6 days pregnant at the time of recruitment. For non-pregnant women the upper age limit is 70, the age at which participation in the National Cervical Screening Program ends. Women who are unable to speak read and write English are not eligible to enter the study.

Recruitment is occurring in a number of different ways according to the preferences of individual clinics. Non-pregnant women are being recruited from general practice clinics. Women are provided with information about the study either personally (by a researcher, GP, practice nurse or receptionist) or they receive the information through the mail. Study information is not being provided by researchers to women attending general practice clinics who are obviously ill. Pregnant women are being recruited from general practice, private ultrasound and private or public obstetric and ultrasound clinics. In general practice, women are provided with information about the study by the GP when they attend for their pregnancy confirmation appointment. In private ultrasound clinics, study information is provided by clinic reception staff when women attend for their 12 week

scan. In private and public obstetric clinics, women are sent the study information in the mail prior to their first appointment, or are given the information personally by an obstetrician or midwife. Women who receive information about the study are asked to complete an expression of interest which is faxed to the research team, either indicating why they do not wish to take part, or providing their contact details so they can be recruited by a researcher. All recruitment is completed by the research team and all women speak with a research genetic counsellor.

Enrolling clinics in the study

General practice clinics located across the metropolitan areas of Melbourne and Perth are being targeted to try and achieve a geographical spread and a broad representation of different socioeconomic areas. General practices with established shared-care programs are being identified using registered shared care provider lists. Professional networks and an in-house database of GPs and obstetricians who have previously ordered prenatal carrier testing for FXS or cystic fibrosis in Victoria is also being used to identify practices that might be interested in participating. We anticipate requiring 5 general practice, 5 private obstetric and 1 obstetric ultrasound clinic to recruit the 1000 women needed for the study.

Members of the project team are providing academic detailing to clinics involved in recruitment. Academic detailing covers background information on FXS, the aims of the project and what the study involves for participants. It is emphasised that the aim of the study is not to test as many women as possible, but rather to understand what factors influence a woman's decision to accept or decline carrier testing for FXS. Clinics are provided with project resources, including study brochures and expression of interest forms.

Australian GPs are primarily funded by a fee for service system and receive no government funding (personal or infrastructure) for involvement in research. Private obstetricians and ultrasound clinics also receive no government funding for involvement in research. All clinics are being offered a small amount of remuneration to cover their costs of involvement in the study, depending on the number of women recruited from their clinic.

Data collection

This research protocol will use mixed-methods data collection that includes genetic testing uptake and outcomes, questionnaires and interviews.

Questionnaires

The questionnaires use validated and psychometrically robust self-reported scales. Table 1 shows which scales are used in questionnaire 1 (Q1), completed after making a decision about carrier testing for FXS, and questionnaire 2 (Q2), completed one month after returning Q1.

Table One: Questionnaire Measures and Scales

Measure / Scale	Description	Q1	Q2
Knowledge	10 item scale containing questions on FXS (True/False/Unsure). A score of 7 or higher is classified as 'good' knowledge [55]	√	√
Attitudes	5 item scale (0-4) used to assess a woman's attitude to screening (beneficial/harmful; important/unimportant, bad thing/good thing, pleasant/unpleasant, worrying/not worrying). Dichotomous scale: women are classified as having a positive (11-20) or a negative (0-10) attitude toward screening [49].	√	
Multi-dimensional Model of Informed Choice (MMIC)	Defines an informed choice as a decision made with 'good' knowledge which is consistent with a person's values. Incorporates three dimensions: knowledge, attitudes and uptake. Dichotomous scale: 'informed choice' or 'not informed choice' [49].	√	
Deliberation	6 item scale measuring the extent to which a decision is deliberated on a 5 point Likert scale (0 = strongly agree – 4 = strongly disagree). Dichotomous scale: responses below the midpoint (11 or under) classified as not deliberated and those at or above the midpoint as deliberated [53].	√	
Decisional Conflict Scale	16 item scale measuring uncertainty about a course of action on a 5 point Likert scale (0 = strongly agree – 4 = strongly disagree). Mean scores are reported with higher scores indicating higher decisional conflict. Scores range from 0 to 100 with scores over 37.5 associated with decision delay or uncertainty about implementation [60].	√	
Depression Anxiety Stress Scale, short form (DASS-21)	21 item scale divided into 3 subscales measuring depression, anxiety and stress. Responses are classified into 5 categories: 1 (normal) to 5 (extremely severe) [61, 62].	√	√
State Trait Anxiety Index, short form (STAI-6)	6 item scale measuring state anxiety. The maximum score is 80 with scores 31-49 considered average and scores over 50 indicating elevated state anxiety [59].	√	√
Health Belief	16 items measuring the importance of a range of factors which may influence decision-making: perceived benefits; perceived susceptibility; perceived severity; and perceived barriers; in a woman's decision to accept or decline testing for FXS [47, 63]	√	
Decisional Regret	5 item scale measuring distress or remorse after a health care decision using a 5 point Likert scale (0-4). Scores range from 0-100 with higher scores indicating a higher level of regret [64].		√
Willingness-to-Pay	2 questions (piloted) that address WTP and gross family income. Income question has 6 income ranges with tick box. WTP question has 11 item income values with tick box and sub-questions that address: i) utility of test (information only or information plus decision-making); and ii) who receives test result (recipient only or recipient plus shared with health share professionals).	√	
Socio- demographics	Marital status, age, parity, reproductive life-stage, education, occupation, postcode	√	

Interviews

To provide in-depth data on participants' experiences, semi-structured qualitative interviews are being conducted with participants at a number of time-points (See Table Two).

Table Two: Overview of Interview Schedule

Time-point	Interview type	Interview description	Selection
After return of Q1, before Q2 and result sent (if tested)	Decision-making interviews	Knowledge, attitudes, factors influencing decision-making, the decision-making process, and perspectives on decisions	Non-pregnant women only; mix of tested and untested women
1 month after return of Q2	Program evaluation interviews (women)	Motivations for participating, factors influencing decision-making, experience of participating in the study including genetic counselling, reflections on decision and views on screening	Mix of tested and untested women from each clinic, including all women with positive test results. Socio-demographic data examined to ensure selected women are representative of the overall sample
After completion of recruitment at any given clinic	Program evaluation interviews (clinic staff)	Attitudes to population carrier screening for FXS, knowledge of FXS, reflections on offering FXS carrier screening at their clinic, and feedback on the study.	Mix of staff from each clinic involved in recruitment
1 year after return of Q2	1 year follow-up	Motivations for screening, interpretation of result, perceived value of result, impact of result and reflections on decision	All women with a test-positive result (i.e. GZ, PM or FM)

Data entry quality control

To ensure accuracy of the questionnaire data, every 20th questionnaire entered is being checked prior to analysis. The rate of accuracy will be calculated as the number of errors per number of data items entered. To ensure rigour in the qualitative data analysis, transcripts will be independently coded.

Testing

One of the aims of our study is to evaluate the performance of a new innovative assay specifically designed for population screening for FXS [65]. Therefore, for the first part of the study, we collected DNA from a saliva sample (Oragene- DNA collection kit) and carried out the gold standard two step diagnostic test [8, 66] in parallel with the innovative screening test. The routine FXS diagnostic test may involve Southern blotting and so can take up to 4 weeks [43]. This is performed

by the Victorian Clinical Genetic Service laboratory. Refinements to the innovative screening assay [67] mean that we are now able to collect DNA from cheek brush samples and have results available in one week. This screening assay, marketed by Asuragen, is being performed by Healthscope Pathology.

All women who choose to have carrier testing are being given information about their result based on current best practice. Women with a result in the normal range receive a letter that includes an offer to speak to a genetic counsellor at their local clinical service should they require further information. Women with a test-positive result (GZ, PM or FM) are telephoned and offered face-to-face genetic counselling at their local clinical genetics service. Genetic counselling for women with test-positive results follows usual clinical practice [4, 68]. Any pregnant woman found to have a PM or FM is given her result and, as part of genetic counselling, is offered prenatal diagnostic testing of the fetus, due to the risk of having a child with FXS. An important outcome of receiving an FXS carrier result is that relatives can access genetic testing, which may lead to identification of other carriers and/or the diagnosis of fragile X related disorders in other family members. Genetic testing is discussed as part of the genetic counselling process and family members are offered genetic counselling and testing where appropriate.

Outcomes

The primary outcomes for the study are test uptake and informed choice. Study participants (denominator) are defined as the number of women recruited into the study who do not actively withdraw at any point. Test uptake is defined as the number of women accepting testing (numerator) divided by the number of study participants and will be reported as a percentage. Informed choice will be reported as the percentage of women in each group (pregnant and non-pregnant, tested and untested) making an informed choice as measured using the Multi-dimensional Measure of Informed Choice (MMIC) [62]. MMIC will be measured in Q1 at the time closest to decision-making. Knowledge, a component of the MMIC, will be measured in Q1 and Q2 and mean knowledge scores will be reported for each time-point.

The study will also examine predictors of test uptake. These multivariate analyses will make use of socio-demographic, family history, health belief and psychosocial items included in Q1.

Psychosocial factors will be examined as secondary measures in this study, including anxiety, depression and stress. These will be administered in both questionnaires to allow them to be measured at the time of decision-making and 1 month later. Decisional conflict will be measured in Q1 and decisional regret in Q2.

State anxiety will be reported as the difference in the mean STAI-6 item short form score of women in each group (pregnant and non-pregnant, tested and untested, normal result versus test positive). Depression, anxiety and stress will be reported as the mean score of women in each group. Decisional conflict and decisional regret will be reported as mean scores.

In the willingness-to-pay (WTP) literature there is keen interest in how WTP dollar values for information may vary in accordance with intended use, who receives the information and capacity-to-pay. Our questions have been designed to address these key issues. Accordingly, WTP data will be reported in a number of ways: i) intended use ('information only' and/or 'decision-making – personal or medical'); ii) by recipients of information ('women only' or 'women plus health care professionals'); iii) for women in the trial as a whole and for each group (pregnant and non-pregnant, tested and untested, normal result versus test positive); as mean dollar values together with associated ranges around each mean to facilitate sensitivity testing.

Sample size

In our pilot study, in which women were required to return on a separate occasion to give a blood sample, test uptake in non-pregnant women was 20%, although 50% indicated they intended to be tested [43]. Based on the relevance to reproductive life-stage, we expect test uptake in the pregnant group to be greater than in the non-pregnant group. Our minimum sample size of 500 women per group will give us 88% power to detect a difference of 10% in test uptake between groups (50% v 40% or 50% vs 60%). We have less information about the likely percentage of women making an informed choice. If the percentage is 50%, with a minimum sample size of 500 per group an unadjusted analysis would have 87% power to detect a difference of 10% (i.e. 50% vs 40% or 505 vs 60%) between groups. If the base rate is greater than or less than 50% we would have >87% power to detect a difference of 10%. The study will therefore be sufficiently powered to exclude anything other than small percentage differences between groups.

Proposed analysis

Descriptive statistics will be used to describe the socio-demographic, knowledge, attitudes and psychological characteristics of the sample. To compare uptake of testing by pregnant and non-pregnant women, a multivariate logistic regression model with uptake as the dependent variable, and socio-demographic variables such as age, education and parity, together with pregnant/non-pregnant status and mode of recruitment as the independent variables, will be estimated. This will ensure that a difference in uptake is not due to differences in socio-economic composition of the pregnant and non-pregnant samples. Robust standard errors will be estimated to take into account the possible effect of clustering due to recruitment methods. Odds ratios will be transformed back to percentage differences [69]. A similar analysis will be performed to compare informed choice. To investigate predictors of uptake of testing, a multivariate logistic regression model will be estimated with independent variables including: informed choice, attitudes, number of children, prior awareness of FXS, psychosocial variables, family history of intellectual disability, age and education. Interactions between predictors and pregnancy/non-pregnancy will be examined, and if necessary, separate models will be estimated for pregnant and non-pregnant women.

Interviews are transcribed verbatim and NVivo 10 (QSR International, Australia) is being used to manage the data and facilitate coding. Coding is being done by at least two independent researchers to provide rigour of analysis. The decision-making interviews are being examined using content and thematic analysis. These interviews occur between the return of Q1 and the issuing of results (for tested women) and Q2. As such they involve only non-pregnant women, as we were concerned that an interview at this time before receiving a result, or needing to delay sending out the result prior to the interview, could be distressing for pregnant women at a time when they might be vulnerable. Data from the post-Q2 interviews are being analysed using directed content analysis [70]. The coding framework has been developed using data from the needs assessment phase of the study [43, 47, 54]. As little prior research has explored the experiences of women identified as carrying GZ, PM or FM alleles through population-based carrier screening, or the experiences of staff in clinics offering population carrier screening, the interviews will be analysed thematically. This will involve an iterative process where data are coded, compared, contrasted and refined to generate emergent themes [71] using an approach we have described previously [54].

The economic analysis is matched to the stages of FXS carrier screening described in our program evaulation model (Figure One). At this stage the analysis is concerned with examining stage 3 (program implementation) and stage 4 (short-term outcomes). Placing a dollar value on the health and non-health outcomes of FXS screening is complex. The immediate result of FXS screening is information. That information might be about a risk to a foetus the women is carrying, implications for the women's future health, or implications for the woman's future reproductive health and reproductive choices. It is for this reason that we have started with willingness-to-pay (WTP)

methods to explore the value that individuals place on the information provided. The WTP data will be analysed in accordance with the intervention design and policy issues set out above. The WTP data will also be analysed to see if there is an association between the dollar values and preparedness to undergo testing. Similarly, to the extent feasible, the relationship between socio-demographic variables and WTP will be analysed to see if these variables impact on WTP.

Longer-term economic modelling using a surrogate is planned for Stage 5. We aim to go on to record the actions that the women undertake as a result of their test results and the incidence of births of babies with FXS to women in the study, discussion of test results with family and identification of carriers/affected individuals with cascade testing. This will facilitate full economic appraisal using a range of methods, including discrete choice experiments (DCE). DCE has applicability to this field because non-health outcomes and process attributes are also important, and DCE is a logical extension to the WTP for inclusion in Stage 5.

ETHICS AND DISSEMINATION

Ethics

Ethics approval to conduct this study has been granted by the Human Research Ethics Committees of the Universities of Melbourne (HREC 0830733) and Western Australia (RA/4/1/4028). Additionally, approval has been granted by the ethics committees of the following recruitment sites: Family Planning Victoria (09/2); Women's and Newborn Health Service and Charles Gardiner Hospital – King Edward Memorial Hospital (1925/EW); Swan Kalamunda Health Service (2012-160). This project is being carried out according to the National Statement on Ethical Conduct in Human Research (2007) and the Australian Code for the Responsible Conduct of Research (2007) produced by the National Health and Medical Research Council of Australia. A plain language statement is provided to all women and to clinics and health professionals involved in recruiting women for the study and a signed consent form is obtained from all participants at the time of recruitment.

Steering group and advisory committee

This study has a designated research team and an advisory group. The advisory group includes representation from the Victorian Department of Health, the Fragile X Association of Australia and clinicians involved in the study. This group meets annually. The research team includes expertise in population health, genetics, primary care, epidemiology, FXS, health economics, pathology and psychology, with the full team meeting quarterly.

Dissemination

This study will be the first of its kind worldwide to address informed decision-making in carrier screening for FXS and to compare screening in pregnant and non-pregnant women. It will inform appropriate clinic service models for offering FXS screening and will provide important exploratory health economic data. We expect to publish one main trial outcome paper and a number of additional papers exploring aspects of the data in more detail. We will also present our findings at a number of international conferences. A report outlining the main findings of the study will also be made available on the study website www.fragilexscreening.net.au on completion. The findings of this study will inform policy development about when and how to offer population carrier screening for FXS.

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Offering fragile X syndrome carrier screening: a prospective mixed-methods observational study comparing carrier screening of pregnant and non-pregnant women in the general population

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TITLE PAGE

'Offering fragile X syndrome carrier screening: a prospective mixed-methods observational study comparing carrier screening of pregnant and non-pregnant women in the general population'

Short title: Fragile X syndrome carrier screening in the general population

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ARTICLE SUMMARY

Article focus

This article is a protocol of a study that involves offering fragile X syndrome carrier screening to pregnant and non-pregnant women in the general population. We are undertaking a program evaluation approach using mixed methods to collect data about informed decision-making and predictors of test uptake, with a focus on psychosocial measures. We are also undertaking an economic appraisal.

Key messages

- Carrier screening for fragile X syndrome is the subject of debate because of concerns around education and counselling for this complex condition, and the potential for psychosocial harms.
- This study will inform policy and practice in the area of population carrier screening by examining psychosocial aspects of screening, including informed decision-making; models of screening, through antenatal care or other access points; and health economics of carrier screening for fragile X syndrome.

Strengths and limitations of this study

- This study seeks to recruit 1000 women in total. This large sample size will give us sufficient power to address the aims of the study.
- Collecting both quantitative and qualitative data will provide a more in-depth picture of screening for fragile X syndrome.
- A limitation of the study is that the data on models of screening may not be applicable to other countries that have different healthcare systems.

ABSTRACT

Introduction

Fragile X syndrome (FXS), an X-linked genetic condition, is the leading cause of inherited intellectual and developmental disability. Policy development relating to carrier screening programs for FXS requires input from large scale studies examining not only test uptake but also psychosocial aspects. This study will compare carrier screening in pregnant and non-pregnant populations, examining informed decision-making, psychosocial issues and health economics.

Methods and Analysis

Pregnant and non-pregnant women are being recruited from general practices and obstetric services. Women receive information about the study either in person or through clinic mail outs. Women are provided pre-test counselling by a genetic counsellor and make a decision about accepting or declining the FXS carrier test in their own time. Data are being collected from two questionnaires: one completed at the time of making the decision about FXS carrier testing, and a second one month later. Additional data are gathered though qualitative interviews conducted at several time-points with a subset of participating women, including all women with a positive test result, and with staff from clinics involved in recruitment.

A minimum sample size of 500 women per group has been calculated to give us 88% power to detect a 10% difference in test uptake and 87% power to detect a 10% difference in informed choice between the pregnant and non-pregnant groups.

Questionnaire data will be analysed using descriptive statistics and multivariate logistic regression models. Interview data will be thematically analysed. Willingness-to-pay and cost effectiveness analyses will also be performed.

Recruitment commenced in July 2009 and data collection will be completed by December 2013.

Ethics and Dissemination

Ethics approval has been granted by the Universities of Melbourne and Western Australia and from recruiting clinics, where required. Results will be reported in peer-reviewed publications, conference and seminar presentations and via a website www.fragilexscreening.net.au. The results of this study will make a significant contribution to discussions about the wider introduction of population carrier screening for FXS.

INTRODUCTION

Population based screening programs are available for a number of genetic conditions in the newborn, prenatal and preconception settings. Several guidelines based on specific criteria exist to help assess which genetic conditions are suitable for population screening [1, 2]. Fragile X syndrome (FXS) is an X-linked condition which meets many of the criteria for population screening, as discussed in Hill et. al [3]. However, in many countries it is still not routine practice to offer carrier screening for FXS. This is because of concerns about the challenges of screening for this complex condition, including the need for genetic counselling and education and the potential psychosocial and other impacts of a positive result, discussed further in Finucane [4].

FXS is the most common inherited cause of intellectual and developmental disability. Virtually all FXS is caused by an expanded CGG trinucleotide repeat in the 5' untranslated region of the *FMR1* gene which leads to hypermethylation and silencing of the gene [5-9]. Currently, the normal range of repeats is defined as 6-44, with 45-54 repeats being considered an intermediate 'grey zone' allele (GZ), 55-200 a premutation (PM) and >200 repeats a full mutation [10, 11]. The repeats in the GZ, PM and FM ranges can expand when passed from mother to child, although not usually from father to child [8, 12, 13].

The full mutation is associated with intellectual disability, anxiety and features of autism spectrum and attention/deficit hyperactivity disorders [14]. The clinical presentation varies between individuals [15] with males usually more severely affected than females. FXS is not curable but specific treatments exist which may help a number of the physical [16-19] and behavioural symptoms [20]. Although there is currently no robust evidence to support specific pharmacological treatments for people with FXS [21], a number of new therapies are being trialled [22-25] which may lead to improved treatments in the future.

In addition to the reproductive risk of having a child with FXS, female FXS PM carriers also have personal health risks: an increased risk of fragile X associated primary ovarian insufficiency (FXPOI), with a 20% risk of premature menopause [26-29]; a higher incidence of mental health issues such as anxiety and depression [4]; a risk of developing fragile X associated tremor/ataxia syndrome (FXTAS),

a late onset neurodegenerative condition, which is more common in male PM carriers than female [29-31].

The reported prevalence of *FMR1* alleles varies. Three large studies examining *FMR1* in anonymous newborn samples [32-34] found frequencies of the *FMR1* FM in males of 1 in 2633 [33] to 1 in 6,209 [34]. Reported rates of the PM in females in four large studies [12, 34-36] range from 1 in 154 [12] in Israel to 1 in 549 [34] in Canada, with rates of 1 in 178 [35] and 1 in 209 [36] reported for the USA. Two large studies reported GZ rates of 1 in 66 [36] to 1 in 85 [34].

A number of studies have investigated carrier screening for FXS for women in the general population [12, 37-46]. Most of these studies focused on uptake of testing, *FMR1* allele sizes and expansion rates, reproductive choices and pregnancy outcomes. However, genetic population screening guidelines [1] emphasise the importance of examining the psychosocial aspects of screening, including informed decision-making. Only our pilot study [43, 47] and one other retrospective study [39] have measured the psychosocial impacts of screening for FXS and no studies to date have examined informed decision-making.

This study aims to help us better understand the psychosocial aspects of carrier screening for FXS and will:
1. Compare informed decision-making by pregnant and non-pregnant women offered carrier screening for FXS.
2. Compare uptake and predictors of uptake in pregnant and non-pregnant women offered carrier screening for FXS.
3. Undertake an economic appraisal of FXS population carrier screening.

Informed decision making is complex and involves many factors [48]. One measure used in population carrier screening for Down syndrome to estimate informed decision making is the multidimensional model of informed choice (MMIC) [49], which describes an informed choice as a decision made with sufficient knowledge that is value consistent. Our study will measure informed choice using MMIC and will also collect additional information on factors involved in informed decision making in the two study questionnaires and through qualitative interviews.

Our study will also provide information on when to offer population carrier screening for FXS by comparing screening in non-pregnant and pregnant women. Population carrier screening guidelines recommend pre-conception carrier screening [1] but such screening is often embedded in antenatal care, as this provides a convenient (from the perspective of the service provider) point of access, although may be a more anxious time for women. Research on informed decision-making in prenatal screening, primarily for Down syndrome, has shown that decisions about testing are often not informed [50-53]. Our study will be the first to investigate whether rates of informed choice and uptake differ between pregnant and non-pregnant women.

We are testing two hypotheses:
1. A lower proportion of pregnant women will make an informed decision about carrier screening compared with non-pregnant women.
2. Carrier screening for FXS will result in a higher uptake of testing by pregnant women compared with non-pregnant women.

The findings of this study will contribute valuable data to inform debate on policy and approaches to population carrier screening for FXS.

METHODS AND ANALYSIS

Key elements of study design

Study design

The development and implementation of an effective carrier screening program is a multi-step process requiring a clear theoretical framework. We have developed a program logic model (see Figure One) to investigate FXS carrier screening incorporating 5 stages: (1) negotiation and planning; (2) program development; (3) program implementation; (4) short-term outcomes; and (5) long-term outcomes. The results of our qualitative needs assessment and pilot study, representing stages 1 and 2, have previously been published [43, 47, 54, 55].

The current study covers stages 3 and 4 and uses a mixed-methods approach to data collection to investigate the short-term outcomes of implementing an FXS carrier screening program. Figure Two provides an overview of the study design. Specifically, we will investigate test uptake, informed decision-making, predictors of test uptake, psychosocial outcomes (depression, anxiety, stress, decisional conflict and decisional regret) and health economic factors (willingness-to-pay).

The key elements of the study are that all women will receive a purpose-made brochure and genetic counselling before making a decision about testing, the test is optional, convenient and non-invasive and offered at no charge to the participants. Genetic counselling and the field-tested brochure is included in the protocol, as participants in our pilot study and needs assessment indicated that having sufficient information and the chance to discuss it is important in making an informed decision [43, 54]. Offering a test that can be performed at home after sufficient time for decision-making is important, as we found in our pilot study that having to return to the clinic for an invasive test was identified as a barrier to testing, although did allow some time for deliberation [43]. Recruiting pregnant and non-pregnant women will allow us to examine if there are any differences in test uptake, informed choice or psychosocial measures between these groups. Our economic appraisal will provide important information to guide policy on offering carrier screening for FXS.

Figure One: Program logic model to investigate FXS carrier screening

Figure Two: Overview of study protocol

[a] See table 1 for details of measures included in questionnaires 1 and 2

Settings

The study is being conducted in general practices, public and private obstetric clinics and through private obstetric ultrasound services in Melbourne, Victoria and Perth, Western Australia.

General Practice

In Australia, women may attend any general practice of their choice, and may attend more than one practice. General practitioners (GPs) are the gatekeepers to access secondary and tertiary care services. About 88% of the Australian population visit a GP at least once a year [56]. Most GP clinics also operate a reminder system for the National Cervical Screening Program, which offers women between the ages of 18 and 69 a cervical (Pap) smear test every two years. Thus most GP clinics have a mail-out system in place to send a reminder letter to their female patients every 2 years. This provides one approach to inviting non-pregnant women into the study and could act as a future service model for population carrier screening.

Obstetrics

A range of maternity care models exist in Australia but they can be broadly divided into private maternity care, public hospital maternity care and shared local health practitioner/ public hospital maternity care. The first step in accessing maternity care is to attend a GP in early pregnancy to obtain a referral to a private obstetrician or public hospital. The timing of the first appointment with the maternity care provider varies, but in the public hospital system women are often not seen until the second trimester of pregnancy. In 2009, the majority (96.9%) of Australian women gave birth in hospitals and of these, 69.9% (150,157 women) were in the public system and 30.1% (64,771 women) were in the private system [57].

Obstetric ultrasound – first trimester combined screening

Provision of antenatal screening varies across Australia. In Victoria and Western Australia, first trimester combined screening is available through private pathology laboratories and private ultrasound clinics with some rebate available from the government funded Medicare system, while second trimester screening is state funded. General practitioners or private obstetricians refer women to the private ultrasound clinic for a first trimester nuchal fold thickness scan. In Victoria, about 70% of pregnant women have first trimester combined screening (personal communication, L Bonacquisto, 2013) [58, 59] and so would be expected to attend a private ultrasound practice. In addition to offering testing at initial presentation in primary care, linking FXS carrier screening to first trimester screening is another potential service model.

Participants

Enrolling women in the study

Women are eligible to enter the study if they are 18 or over and either not pregnant or up to 12 weeks + 6 days pregnant at the time of recruitment. For non-pregnant women the upper age limit is 70, the age at which participation in the National Cervical Screening Program ends. Women who are unable to speak read and write English are not eligible to enter the study.

Recruitment is occurring in a number of different ways according to the preferences of individual clinics. Non-pregnant women are being recruited from general practice clinics. Women are provided with information about the study either personally (by a researcher, GP, practice nurse or receptionist) or they receive the information through the mail. Study information is not being provided by researchers to women attending general practice clinics who are obviously ill. Pregnant women are being recruited from general practice, private ultrasound and private or public obstetric and ultrasound clinics. In general practice, women are provided with information about the study by the GP when they attend for their pregnancy confirmation appointment. In private ultrasound

clinics, study information is provided by clinic reception staff when women attend for their 12 week scan. In private and public obstetric clinics, women are sent the study information in the mail prior to their first appointment, or are given the information personally by an obstetrician or midwife. Women who receive information about the study are asked to complete an expression of interest which is faxed to the research team, either indicating why they do not wish to take part, or providing their contact details so they can be recruited by a researcher. All recruitment is completed by the research team and all women speak with a research genetic counsellor.

Enrolling clinics in the study

General practice clinics located across the metropolitan areas of Melbourne and Perth are being targeted to try and achieve a geographical spread and a broad representation of different socioeconomic areas. General practices with established shared-care programs are being identified using registered shared care provider lists. Professional networks and an in-house database of GPs and obstetricians who have previously ordered prenatal carrier testing for FXS or cystic fibrosis in Victoria is also being used to identify practices that might be interested in participating. We anticipate requiring 5 general practice, 5 private obstetric and 1 obstetric ultrasound clinic to recruit the 1000 women needed for the study.

Members of the project team are providing academic detailing to clinics involved in recruitment. Academic detailing covers background information on FXS, the aims of the project and what the study involves for participants. It is emphasised that the aim of the study is not to test as many women as possible, but rather to understand what factors influence a woman's decision to accept or decline carrier testing for FXS. Clinics are provided with project resources, including study brochures and expression of interest forms.

Australian GPs are primarily funded by a fee for service system and receive no government funding (personal or infrastructure) for involvement in research. Private obstetricians and ultrasound clinics also receive no government funding for involvement in research. All clinics are being offered a small amount of remuneration to cover their costs of involvement in the study, depending on the number of women recruited from their clinic.

Data collection

This research protocol will use mixed-methods data collection that includes genetic testing uptake and outcomes, questionnaires and interviews.

Questionnaires

The questionnaires use validated and psychometrically robust self-reported scales. Table 1 shows which scales are used in questionnaire 1 (Q1), completed after making a decision about carrier testing for FXS, and questionnaire 2 (Q2), completed one month after returning Q1.

Table One: Questionnaire Measures and Scales

Measure / Scale	Description	Q1	Q2
Knowledge	10 item scale containing questions on FXS (True/False/Unsure). A score of 7 or higher is classified as 'good' knowledge [55]	√	√
Attitudes	5 item scale (0-4) used to assess a woman's attitude to screening (beneficial/harmful; important/unimportant, bad thing/good thing, pleasant/unpleasant, worrying/not worrying). Dichotomous scale: women are classified as having a positive (11-20) or a negative (0-10) attitude toward screening [49].	√	
Multi-dimensional Model of Informed Choice (MMIC)	Defines an informed choice as a decision made with 'good' knowledge which is consistent with a person's values. Incorporates three dimensions: knowledge, attitudes and uptake. Dichotomous scale: 'informed choice' or 'not informed choice' [49].	√	
Deliberation	6 item scale measuring the extent to which a decision is deliberated on a 5 point Likert scale (0 = strongly agree – 4 = strongly disagree). Dichotomous scale: responses below the midpoint (11 or under) classified as not deliberated and those at or above the midpoint as deliberated [53].	√	
Decisional Conflict Scale	16 item scale measuring uncertainty about a course of action on a 5 point Likert scale (0 = strongly agree – 4 = strongly disagree). Mean scores are reported with higher scores indicating higher decisional conflict. Scores range from 0 to 100 with scores over 37.5 associated with decision delay or uncertainty about implementation [60].	√	
Depression Anxiety Stress Scale, short form (DASS-21)	21 item scale divided into 3 subscales measuring depression, anxiety and stress. Responses are classified into 5 categories: 1 (normal) to 5 (extremely severe) [61, 62].	√	√
State Trait Anxiety Index, short form (STAI-6)	6 item scale measuring state anxiety. The maximum score is 80 with scores 31-49 considered average and scores over 50 indicating elevated state anxiety [59].	√	√
Health Belief	16 items measuring the importance of a range of factors which may influence decision-making: perceived benefits; perceived susceptibility; perceived severity; and perceived barriers; in a woman's decision to accept or decline testing for FXS [47, 63]	√	
Decisional Regret	5 item scale measuring distress or remorse after a health care decision using a 5 point Likert scale (0-4). Scores range from 0-100 with higher scores indicating a higher level of regret [64].		√
Willingness-to-Pay	2 questions (piloted) that address WTP and gross family income. Income question has 6 income ranges with tick box. WTP question has 11 item income values with tick box and sub-questions that address: i) utility of test (information only or information plus decision-making); and ii) who receives test result (recipient only or recipient plus shared with health share professionals).	√	
Socio- demographics	Marital status, age, parity, reproductive life-stage, education, occupation, postcode	√	

Interviews

To provide in-depth data on participants' experiences, semi-structured qualitative interviews are being conducted with participants at a number of time-points (See Table Two). Interviews are being conducted by two members of the research team with genetic counselling and qualitative research skills.

Table Two: Overview of Interview Schedule

Time-point	Interview type	Interview description	Selection
After return of Q1, before Q2 and result sent (if tested)	Decision-making interviews	Knowledge, attitudes, factors influencing decision-making, the decision-making process, and perspectives on decisions	Non-pregnant women only; mix of tested and untested women
1 month after return of Q2	Program evaluation interviews (women)	Motivations for participating, factors influencing decision-making, experience of participating in the study including genetic counselling, reflections on decision and views on screening	Mix of tested and untested women from each clinic, including all women with positive test results. Socio-demographic data examined to ensure selected women are representative of the overall sample
After completion of recruitment at any given clinic	Program evaluation interviews (clinic staff)	Attitudes to population carrier screening for FXS, knowledge of FXS, reflections on offering FXS carrier screening at their clinic, and feedback on the study.	Mix of staff from each clinic involved in recruitment
1 year after return of Q2	1 year follow-up	Motivations for screening, interpretation of result, perceived value of result, impact of result and reflections on decision	All women with a test-positive result (i.e. GZ, PM or FM)

Data entry quality control

To ensure accuracy of the questionnaire data, every 20th questionnaire entered is being checked prior to analysis. The rate of accuracy will be calculated as the number of errors per number of data items entered. To ensure rigour in the qualitative data analysis, transcripts will be independently coded.

Testing

One of the aims of our study is to evaluate the performance of a new innovative assay specifically designed for population screening for FXS [65]. Therefore, for the first part of the study, we collected DNA from a saliva sample (Oragene- DNA collection kit) and carried out the gold standard two step diagnostic test [8, 66] in parallel with the innovative screening test. The routine FXS diagnostic test may involve Southern blotting and so can take up to 4 weeks [43]. This is performed by the Victorian Clinical Genetic Service laboratory. Refinements to the innovative screening assay [67] mean that we are now able to collect DNA from cheek brush samples and have results available in one week. This screening assay, marketed by Asuragen, is being performed by Healthscope Pathology.

All women who choose to have carrier testing are being given information about their result based on current best practice. Women with a result in the normal range receive a letter that includes an offer to speak to a genetic counsellor at their local clinical service should they require further information. Women with a test-positive result (GZ, PM or FM) are telephoned and offered face-to-face genetic counselling at their local clinical genetics service. Genetic counselling for women with test-positive results follows usual clinical practice [4, 68]. Any pregnant woman found to have a PM or FM is given her result and, as part of genetic counselling, is offered prenatal diagnostic testing of the fetus, due to the risk of having a child with FXS. An important outcome of receiving an FXS carrier result is that relatives can access genetic testing, which may lead to identification of other carriers and/or the diagnosis of fragile X related disorders in other family members. Genetic testing is discussed as part of the genetic counselling process and family members are offered genetic counselling and testing where appropriate.

Outcomes

The primary outcomes for the study are test uptake and informed choice. Study participants (denominator) are defined as the number of women recruited into the study who do not actively withdraw at any point. Test uptake is defined as the number of women accepting testing (numerator) divided by the number of study participants and will be reported as a percentage. Informed choice will be reported as the percentage of women in each group (pregnant and non-pregnant, tested and untested) making an informed choice as measured using the Multi-dimensional Measure of Informed Choice (MMIC) [62]. MMIC will be measured in Q1 at the time closest to decision-making. Knowledge, a component of the MMIC, will be measured in Q1 and Q2 and mean knowledge scores will be reported for each time-point.

The study will also examine predictors of test uptake. These multivariate analyses will make use of socio-demographic, family history, health belief and psychosocial items included in Q1.

Psychosocial factors will be examined as secondary measures in this study, including anxiety, depression and stress. These will be administered in both questionnaires to allow them to be measured at the time of decision-making and 1 month later. Decisional conflict will be measured in Q1 and decisional regret in Q2.

State anxiety will be reported as the difference in the mean STAI-6 item short form score of women in each group (pregnant and non-pregnant, tested and untested, normal result versus test positive). Depression, anxiety and stress will be reported as the mean score of women in each group. Decisional conflict and decisional regret will be reported as mean scores.

In the willingness-to-pay (WTP) literature there is keen interest in how WTP dollar values for information may vary in accordance with intended use, who receives the information and capacity-to-pay. Our questions have been designed to address these key issues. Accordingly, WTP data will be reported in a number of ways: i) intended use ('information only' and/or 'decision-making –

personal or medical'); ii) by recipients of information ('women only' or 'women plus health care professionals'); iii) for women in the trial as a whole and for each group (pregnant and non-pregnant, tested and untested, normal result versus test positive); as mean dollar values together with associated ranges around each mean to facilitate sensitivity testing.

Sample size

In our pilot study, in which women were required to return on a separate occasion to give a blood sample, test uptake in non-pregnant women was 20%, although 50% indicated they intended to be tested [43]. Based on the relevance to reproductive life-stage, we expect test uptake in the pregnant group to be greater than in the non-pregnant group. Our minimum sample size of 500 women per group will give us 88% power to detect a difference of 10% in test uptake between groups (50% v 40% or 50% vs 60%). We have less information about the likely percentage of women making an informed choice. If the percentage is 50%, with a minimum sample size of 500 per group an unadjusted analysis would have 87% power to detect a difference of 10% (i.e. 50% vs 40% or 505 vs 60%) between groups. If the base rate is greater than or less than 50% we would have >87% power to detect a difference of 10%. The study will therefore be sufficiently powered to exclude anything other than small percentage differences between groups.

Proposed analysis

Descriptive statistics will be used to describe the socio-demographic, knowledge, attitudes and psychological characteristics of the sample. To compare uptake of testing by pregnant and non-pregnant women, a multivariate logistic regression model with uptake as the dependent variable, and socio-demographic variables such as age, education and parity, together with pregnant/non-pregnant status and mode of recruitment as the independent variables, will be estimated. This will ensure that a difference in uptake is not due to differences in socio-economic composition of the pregnant and non-pregnant samples. Robust standard errors will be estimated to take into account the possible effect of clustering due to recruitment methods. Odds ratios will be transformed back to percentage differences [69]. A similar analysis will be performed to compare informed choice. To investigate predictors of uptake of testing, a multivariate logistic regression model will be estimated with independent variables including: informed choice, attitudes, number of children, prior awareness of FXS, psychosocial variables, family history of intellectual disability, age and education. Interactions between predictors and pregnancy/non-pregnancy will be examined, and if necessary, separate models will be estimated for pregnant and non-pregnant women.

Interviews are transcribed verbatim and NVivo 10 (QSR International, Australia) is being used to manage the data and facilitate coding. Coding is being done by at least two independent researchers to provide rigour of analysis. The decision-making interviews are being examined using content and thematic analysis. These interviews occur between the return of Q1 and the issuing of results (for tested women) and Q2. As such they involve only non-pregnant women, as we were concerned that an interview at this time before receiving a result, or needing to delay sending out the result prior to the interview, could be distressing for pregnant women at a time when they might be vulnerable. Data from the post-Q2 interviews are being analysed using directed content analysis [70]. The coding framework has been developed using data from the needs assessment phase of the study [43, 47, 54]. As little prior research has explored the experiences of women identified as carrying GZ, PM or FM alleles through population-based carrier screening, or the experiences of staff in clinics offering population carrier screening, the interviews will be analysed thematically. This will involve an iterative process where data are coded, compared, contrasted and refined to generate emergent themes [71] using an approach we have described previously [54].

The economic analysis is matched to the stages of FXS carrier screening described in our program logic model (Figure One). At this stage the analysis is concerned with examining stage 3 (program

implementation) and stage 4 (short-term outcomes). Placing a dollar value on the health and non-health outcomes of FXS screening is complex. The immediate result of FXS screening is information. That information might be about a risk to a fetus the women is carrying, implications for the women's future health, or implications for the woman's future reproductive health and reproductive choices. It is for this reason that we have started with willingness-to-pay (WTP) methods to explore the value that individuals place on the information provided. The WTP data will be analysed in accordance with the intervention design and policy issues set out above. The WTP data will also be analysed to see if there is an association between the dollar values and preparedness to undergo testing. Similarly, to the extent feasible, the relationship between socio-demographic variables and WTP will be analysed to see if these variables impact on WTP.

Longer-term economic modelling using a surrogate is planned for Stage 5. We aim to go on to record the actions that the women undertake as a result of their test results and the incidence of births of babies with FXS to women in the study, discussion of test results with family and identification of carriers/affected individuals with cascade testing. This will facilitate full economic appraisal using a range of methods, including discrete choice experiments (DCE). DCE has applicability to this field because non-health outcomes and process attributes are also important, and DCE is a logical extension to the WTP for inclusion in Stage 5.

ETHICS AND DISSEMINATION

Ethics

Ethics approval to conduct this study has been granted by the Human Research Ethics Committees of the Universities of Melbourne (HREC 0830733) and Western Australia (RA/4/1/4028). Additionally, approval has been granted by the ethics committees of the following recruitment sites: Family Planning Victoria (09/2); Women's and Newborn Health Service and Charles Gardiner Hospital – King Edward Memorial Hospital (1925/EW); Swan Kalamunda Health Service (2012-160). This project is being carried out according to the National Statement on Ethical Conduct in Human Research (2007) and the Australian Code for the Responsible Conduct of Research (2007) produced by the National Health and Medical Research Council of Australia. A plain language statement is provided to all women and to clinics and health professionals involved in recruiting women for the study and a signed consent form is obtained from all participants at the time of recruitment.

Steering group and advisory committee

This study has a designated research team and an advisory group. The advisory group includes representation from the Victorian Department of Health, the Fragile X Association of Australia and clinicians involved in the study. This group meets annually. The research team includes expertise in population health, genetics, primary care, epidemiology, FXS, health economics, pathology and psychology, with the full team meeting quarterly.

Dissemination

This study will be the first of its kind worldwide to address informed decision-making in carrier screening for FXS and to compare screening in pregnant and non-pregnant women. It will inform appropriate clinic service models for offering FXS screening and will provide important exploratory health economic data. We expect to publish one main trial outcome paper and a number of additional papers exploring aspects of the data in more detail. We will also present our findings at a number of international conferences. A report outlining the main findings of the study will also be made available on the study website www.fragilexscreening.net.au on completion. The findings of this study will inform policy development about when and how to offer population carrier screening for FXS.

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Contributorship statement

Dr M Martyn led the writing of the manuscript and co-ordinates the study.

Dr A Archibald contributed to study design, ethics application, data collection and drafting of manuscript.

Prof V Anderson contributed to the design of the study and advised on management of women with high DASS and STAI scores

Prof R Carter and Ms S Younie were the health economists involved in the NHMRC project grant, prepared the text on the WTP analysis and associated economic appraisal and read/approved the manuscript.

Dr J Cohen contributed to the initial development of the project concept, provided input to the development of tools used in project, assisted with site selection, and provided relevant input to the manuscript.

Prof M Delatycki contributed to the design of the study

A/Prof Donath was involved in the study design and is responsible for the sample size calculations and the statistical analysis.

Prof J Emery contributed to study design, conduct of study in WA and drafting of this manuscript.

A/Prof J Halliday was involved in the study design and contributed to the drafting of the manuscript.

Dr M Hill provided critical input into the design and set-up of the study, as well as study materials.

Dr L Sheffield contributed to the initial development of the project concept and was involved in the design of the study.

A/Prof H Slater contributed to the design of the study and provided oversight of the diagnostic FXS testing.

Prof F Tassone designed a test for FXS which makes it possible to offer population carrier screening .

Prof S Metcalfe was responsible for the overall design of the study, study materials and contributed to the drafting of this manuscript.

Competing interest
There are no competing interests.

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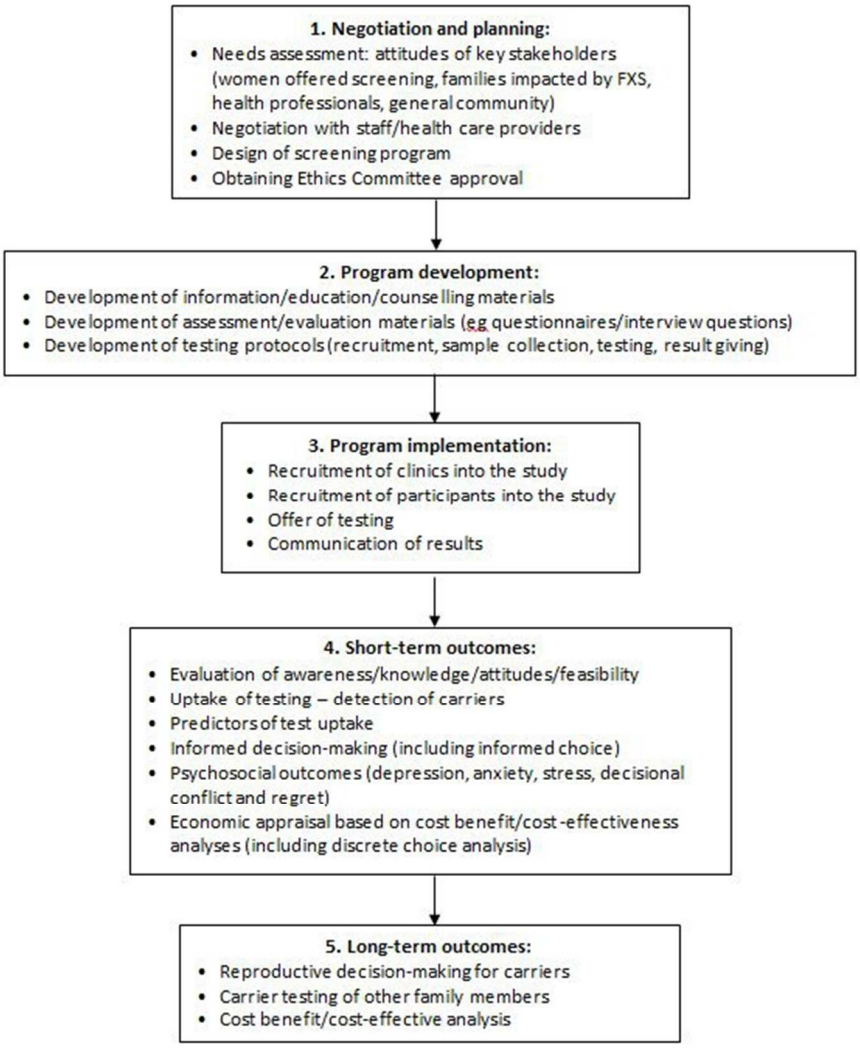

Figure One: Program logic model to investigate FXS carrier screening
170x190mm (96 x 96 DPI)

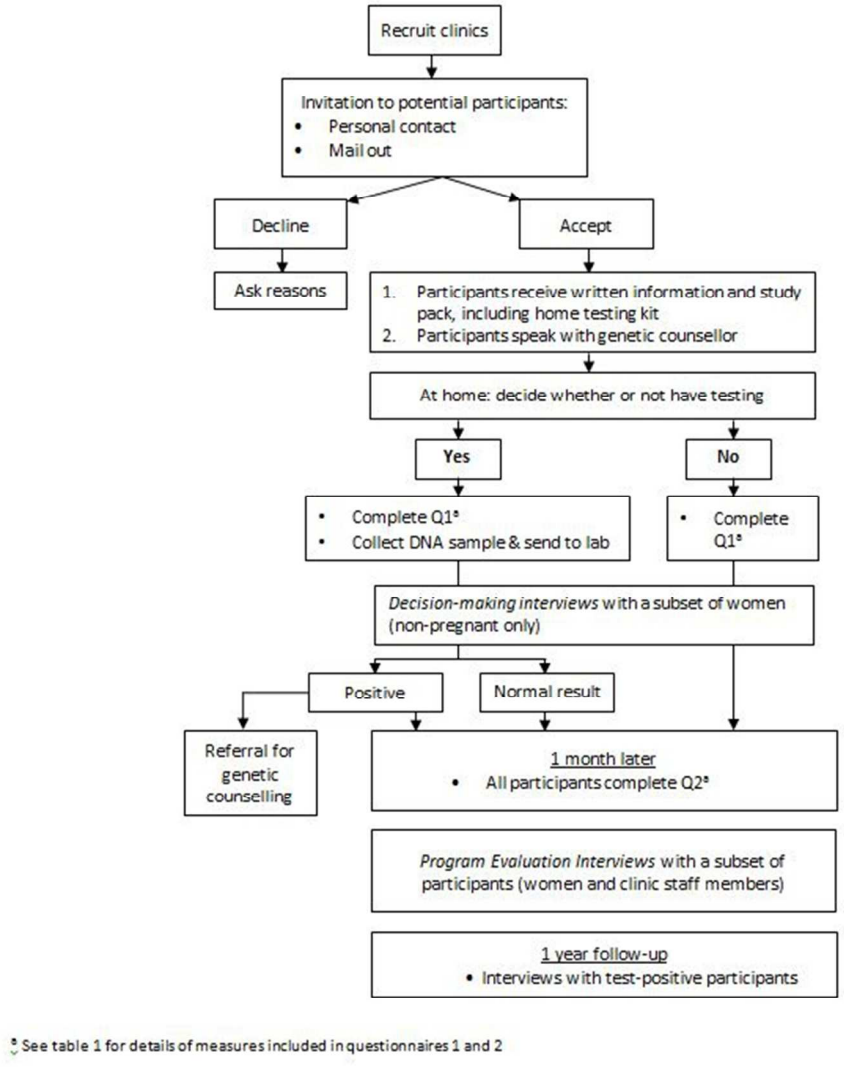

Figure Two: Overview of study protocol
168x185mm (96 x 96 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Check
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	X
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	X
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	X
Objectives	3	State specific objectives, including any prespecified hypotheses	X
Methods			
Study design	4	Present key elements of study design early in the paper	X
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	X
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	X
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	X
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	X
Bias	9	Describe any efforts to address potential sources of bias	X
Study size	10	Explain how the study size was arrived at	X
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	not yet applicable
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	X
		(*b*) Describe any methods used to examine subgroups and interactions	X
		(*c*) Explain how missing data were addressed	not yet applicable
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods	not yet applicable

		taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	not yet applicable
Results			**Check**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	not yet applicable
		(b) Give reasons for non-participation at each stage	not yet applicable
		(c) Consider use of a flow diagram	X
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	not yet applicable
		(b) Indicate number of participants with missing data for each variable of interest	not yet applicable
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	not yet applicable
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	not yet applicable
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	not yet applicable
		(*b*) Report category boundaries when continuous variables were categorized	not yet applicable
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	not yet applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	not yet applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	not yet applicable
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	not yet applicable
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	not yet applicable
Generalisability	21	Discuss the generalisability (external validity) of the study results	not yet applicable
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	X

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

TITLE PAGE

'Offering fragile X syndrome carrier screening: a prospective mixed-methods observational study comparing carrier screening of pregnant and non-pregnant women in the general population'

Short title: ~~F~~The ~~f~~ragile X syndrome carrier screening ~~(FaXeS) study~~in the general population

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~~Primary subject heading: Genetics and Genomics~~
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~~Keywords: Genetics, Public Health, Health policy: health administration and management~~

~~Word Count: 4129~~

ARTICLE SUMMARY

Article focus

This article is a protocol of a study that involves offering fragile X syndrome carrier screening to pregnant and non-pregnant women in the general population. We are undertaking a program evaluation approach using mixed methods to collect data about informed decision-making and predictors of test uptake, with a focus on psychosocial measures. We are also undertaking an economic appraisal.

Key messages

- Carrier screening for fragile X syndrome is the subject of debate because of concerns around education and counselling for this complex condition, and the potential for psychosocial harms.
- This study will inform policy and practice in the area of population carrier screening by examining psychosocial aspects of screening, including informed decision-making; models of screening, through antenatal care or other access points; and health economics of carrier screening for fragile X syndrome.

Strengths and limitations of this study

- This study seeks to recruit 1000 women in total. This large sample size will give us sufficient power to address the aims of the study.
- Collecting both quantitative and qualitative data will provide a more in-depth picture of screening for fragile X syndrome.
- A limitation of the study is that the data on models of screening may not be applicable to other countries that have different healthcare systems.

ABSTRACT

Introduction

Fragile X syndrome (FXS), an X-linked genetic condition, is the leading cause of inherited intellectual and developmental disability. Policy development relating to carrier screening programs for FXS requires input from large scale studies examining not only test uptake but also psychosocial aspects. This study will compare carrier screening in pregnant and non-pregnant populations, examining informed decision-making, psychosocial issues and health economics.

Methods and Analysis

Pregnant and non-pregnant women are being recruited from general practices and obstetric services. Women receive information about the study either in person or through clinic mail outs. Women are provided pre-test counselling by a genetic counsellor and make a decision about

accepting or declining the FXS carrier test in their own time. Data are being collected from two questionnaires: one completed at the time of making the decision about FXS carrier testing, and a second one month later. Additional data are gathered though qualitative interviews conducted at several time-points with a subset of participating women, including all women with a positive test result, and with staff from clinics involved in recruitment.

A minimum sample size of 500 women per group has been calculated to give us 88% power to detect a 10% difference in test uptake and 87% power to detect a 10% difference in informed choice between the pregnant and non-pregnant groups.

Questionnaire data will be analysed using descriptive statistics and multivariate logistic regression models. Interview data will be thematically analysed. Willingness-to-pay and cost effectiveness analyses will also be performed.

Recruitment commenced in July 2009 and data collection will be completed by December 2013.

Ethics and Dissemination

Ethics approval has been granted by the Universities of Melbourne and Western Australia and from recruiting clinics, where required. Results will be reported in peer-reviewed publications, conference and seminar presentations and via a website www.fragilexscreening.net.au. The results of this study will make a significant contribution to discussions about the wider introduction of population carrier screening for FXS.

INTRODUCTION

Population based screening programs are available for a number of genetic conditions in the newborn, prenatal and preconception settings. Several guidelines based on specific criteria exist to help assess which genetic conditions are suitable for population screening [1, 2]. Fragile X syndrome (FXS) is an X-linked condition which meets many of the criteria for population screening, as discussed in Hill et. al [3]. However, in many countries it is still not routine practice to offer carrier screening for FXS. This is because of concerns about the challenges of screening for this complex condition, including the need for genetic counselling and education and the potential psychosocial and other impacts of a positive result, discussed further in Finucane [4].

FXS is the most common inherited cause of intellectual and developmental disability. Virtually all FXS is caused by an expanded CGG trinucleotide repeat in the 5' untranslated region of the *FMR1* gene which leads to hypermethylation and silencing of the gene [5-9]. Currently, the normal range of repeats is defined as 6-44, with 45-54 repeats being considered an intermediate 'grey zone' allele (GZ), 55-200 a premutation (PM) and >200 repeats a full mutation [10, 11]. The repeats in the GZ, PM and FM ranges can expand when passed from mother to child, although not usually from father to child [8, 12, 13].

The full mutation is associated with intellectual disability, anxiety and features of autism spectrum and attention/deficit hyperactivity disorders [14]. The clinical presentation varies between individuals [15] with males usually more severely affected than females. FXS is not curable but specific treatments exist which may help a number of the physical [16-19] and behavioural symptoms [20]. Although there is currently no robust evidence to support specific pharmacological treatments for people with FXS [21], a number of new therapies are being trialled [22-25] which may lead to improved treatments in the future.

In addition to the reproductive risk of having a child with FXS, female FXS PM carriers also have personal health risks: an increased risk of fragile X associated primary ovarian insufficiency (FXPOI), with a 20% risk of premature menopause [26-29]; a higher incidence of mental health issues such as anxiety and depression [4]; a risk of developing fragile X associated tremor/ataxia syndrome (FXTAS), a late onset neurodegenerative condition, which is more common in male PM carriers than female [29-31].

The reported prevalence of *FMR1* alleles varies. Three large studies examining *FMR1* in anonymous newborn samples [32-34] found frequencies of the *FMR1* FM in males of 1 in 2633 [33] to 1 in 6,209 [34]. Reported rates of the PM in females in four large studies [12, 34-36] range from 1 in 154 [12] in Israel to 1 in 549 [34] in Canada, with rates of 1 in 178 [35] and 1 in 209 [36] reported for the USA. Two large studies reported GZ rates of 1 in 66 [36] to 1 in 85 [34].

A number of studies have investigated carrier screening for FXS for women in the general population [12, 37-46]. Most of these studies focused on uptake of testing, *FMR1* allele sizes and expansion rates, reproductive choices and pregnancy outcomes. However, genetic population screening guidelines [1] emphasise the importance of examining the psychosocial aspects of screening, including informed decision-making. Only our pilot study [43, 47] and one other retrospective study [39] have measured the psychosocial impacts of screening for FXS and no studies to date have examined informed decision-making.

This study aims to help us better understand the psychosocial aspects of carrier screening for FXS and will:
1. Compare informed decision-making by pregnant and non-pregnant women offered carrier screening for FXS.
2. Compare uptake and predictors of uptake in pregnant and non-pregnant women offered carrier screening for FXS.
3. Undertake an economic appraisal of FXS population carrier screening.

Informed decision making is complex and involves many factors [48]. One measure used in population carrier screening for Down syndrome to estimate informed decision making is the multidimensional model of informed choice (MMIC) [49], which describes an informed choice as a decision made with sufficient knowledge that is value consistent. Our study will measure informed choice using MMIC and will also collect additional information on factors involved in informed decision making in the two study questionnaires and through qualitative interviews.

Our study will also provide information on when to offer population carrier screening for FXS by comparing screening in non-pregnant and pregnant women. Population carrier screening guidelines recommend pre-conception carrier screening [1] but such screening is often embedded in antenatal care, as this provides a convenient (from the perspective of the service provider) point of access, although may be a more anxious time for women. Research on informed decision-making in prenatal screening, primarily for Down syndrome, has shown that decisions about testing are often not informed [50-53]. Our study will be the first to investigate whether rates of informed choice and uptake differ between pregnant and non-pregnant women.

We are testing two hypotheses:
1. A lower proportion of pregnant women will make an informed decision about carrier screening compared with non-pregnant women.
2. Carrier screening for FXS will result in a higher uptake of testing by pregnant women compared with non-pregnant women.

The findings of this study will contribute valuable data to inform debate on policy and approaches to population carrier screening for FXS.

METHODS AND ANALYSIS

Key elements of study design

Study design

The development and implementation of an effective carrier screening program is a multi-step process requiring a clear theoretical framework. We have developed a program logic model (see Figure One) to investigate FXS carrier screening incorporating 5 stages: (1) negotiation and planning; (2) program development; (3) program implementation; (4) short-term outcomes; and (5) long-term outcomes. The results of our qualitative needs assessment and pilot study, representing stages 1 and 2, have previously been published [43, 47, 54, 55].

The current study covers stages 3 and 4 and uses a mixed-methods approach to data collection to investigate the short-term outcomes of implementing an FXS carrier screening program. Figure Two provides an overview of the study design. Specifically, we will investigate test uptake, informed decision-making, predictors of test uptake, psychosocial outcomes (depression, anxiety, stress, decisional conflict and decisional regret) and health economic factors (willingness-to-pay).

The key elements of the study are that all women will receive a purpose-made brochure and genetic counselling before making a decision about testing, the test is optional, convenient and non-invasive and offered at no charge to the participants. Genetic counselling and the field-tested brochure is included in the protocol, as participants in our pilot study and needs assessment indicated that having sufficient information and the chance to discuss it is important in making an informed decision [43, 54]. Offering a test that can be performed at home after sufficient time for decision-making is important, as we found in our pilot study that having to return to the clinic for an invasive test was identified as a barrier to testing, although did allow some time for deliberation [43]. Recruiting pregnant and non-pregnant women will allow us to examine if there are any differences in test uptake, informed choice or psychosocial measures between these groups. Our economic appraisal will provide important information to guide policy on offering carrier screening for FXS.

Figure One: Program logic model to investigate FXS carrier screening

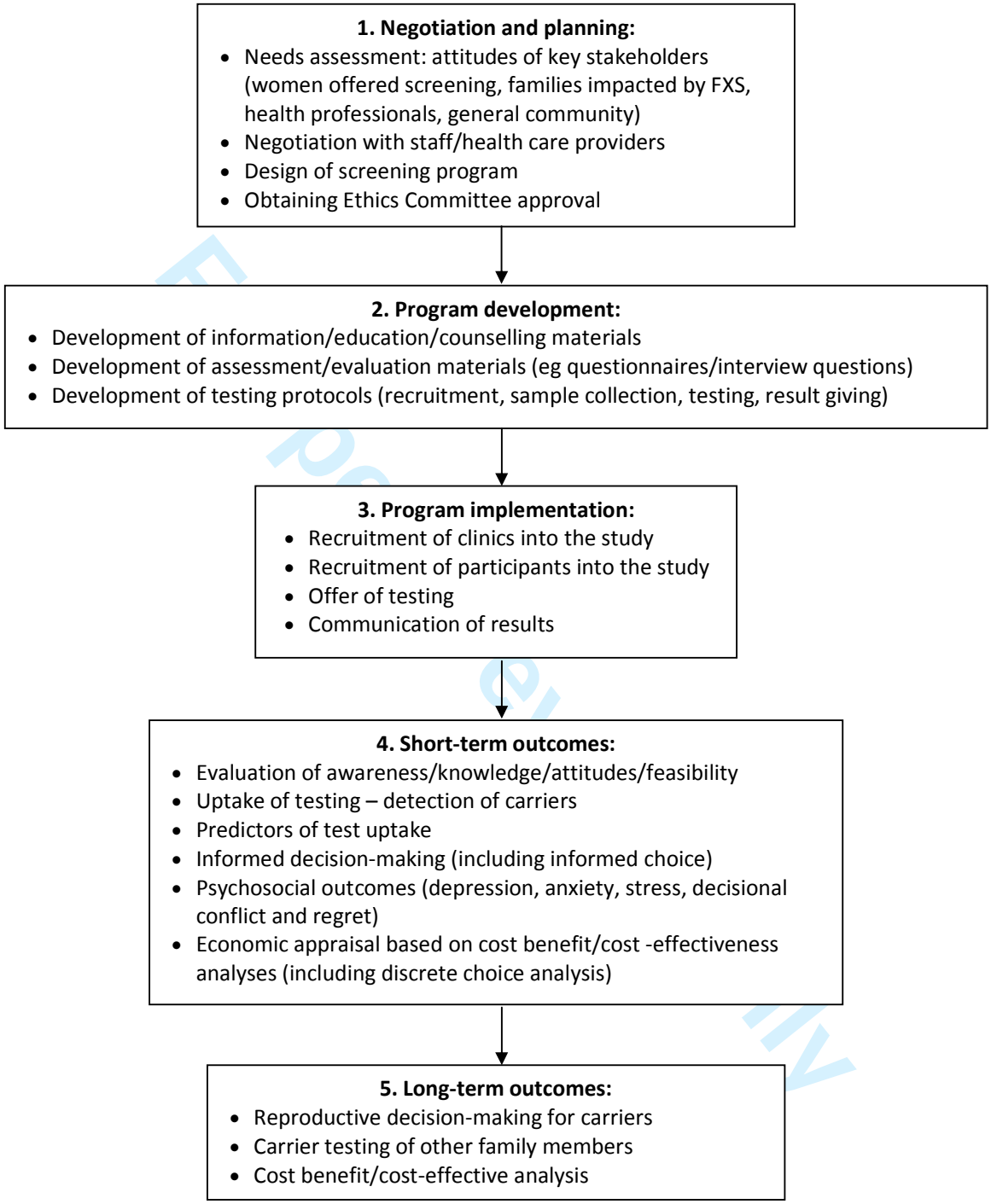
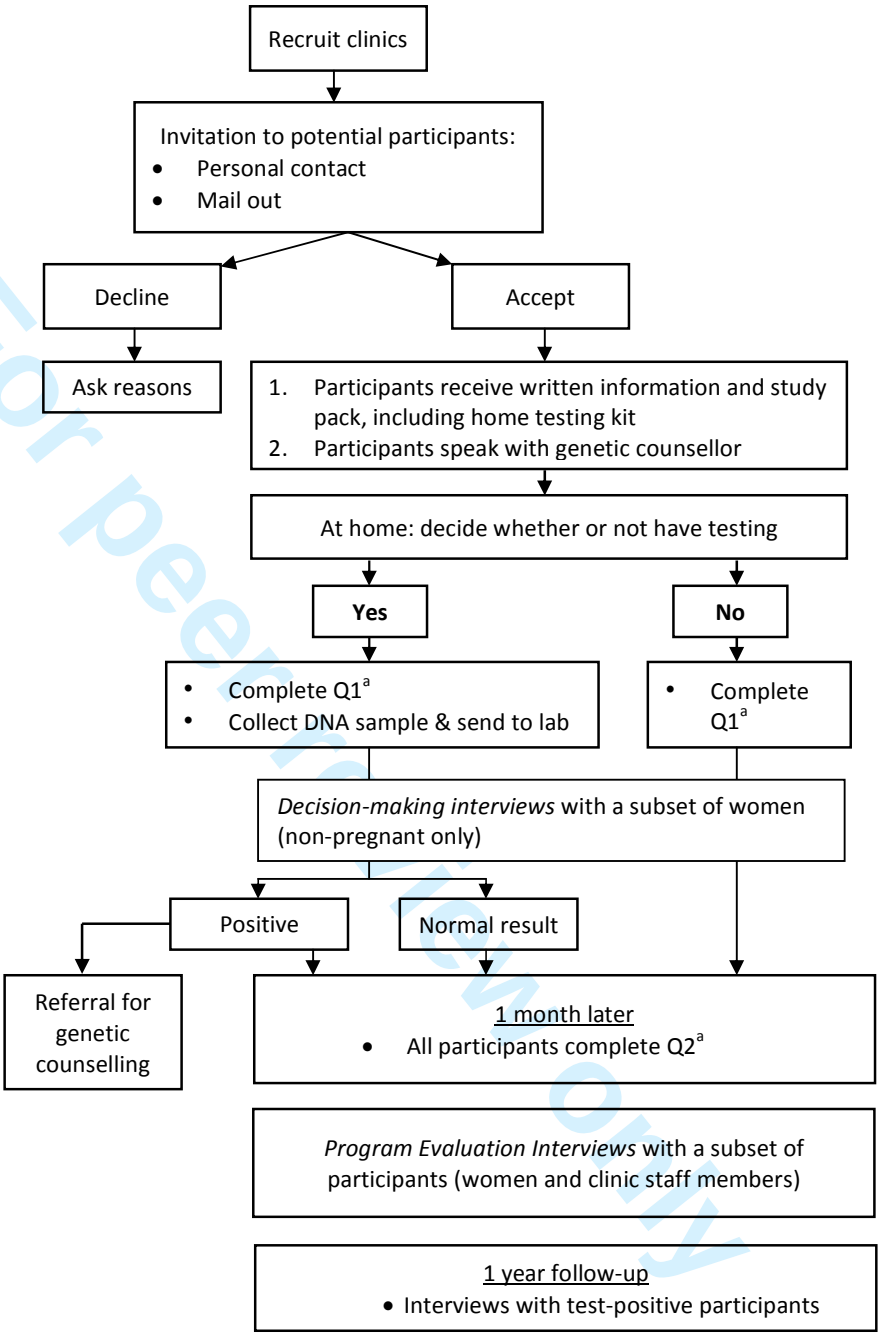


Figure Two: Overview of study protocol



[a] See table 1 for details of measures included in questionnaires 1 and 2

Settings

The study is being conducted in general practices, public and private obstetric clinics and through private obstetric ultrasound services in Melbourne, Victoria and Perth, Western Australia.

General Practice

In Australia, women may attend any general practice of their choice, and may attend more than one practice. General practitioners (GPs) are the gatekeepers to access secondary and tertiary care services. About 88% of the Australian population visit a GP at least once a year [56]. Most GP clinics also operate a reminder system for the National Cervical Screening Program, which offers women between the ages of 18 and 69 a cervical (Pap) smear test every two years. Thus most GP clinics have a mail-out system in place to send a reminder letter to their female patients every 2 years. This provides one approach to inviting non-pregnant women into the study and could act as a future service model for population carrier screening.

Obstetrics

A range of maternity care models exist in Australia but they can be broadly divided into private maternity care, public hospital maternity care and shared local health practitioner/ public hospital maternity care. The first step in accessing maternity care is to attend a GP in early pregnancy to obtain a referral to a private obstetrician or public hospital. The timing of the first appointment with the maternity care provider varies, but in the public hospital system women are often not seen until the second trimester of pregnancy. In 2009, the majority (96.9%) of Australian women gave birth in hospitals and of these, 69.9% (150,157 women) were in the public system and 30.1% (64,771 women) were in the private system [57].

Obstetric ultrasound – first trimester combined screening

Provision of antenatal screening varies across Australia. In Victoria and Western Australia, first trimester combined screening is available through private pathology laboratories and private ultrasound clinics with some rebate available from the government funded Medicare system, while second trimester screening is state funded. General practitioners or private obstetricians refer women to the private ultrasound clinic for a first trimester nuchal fold thickness scan. In Victoria, about 70% of pregnant women have first trimester combined screening (personal communication, L Bonacquisto, 2013) [58, 59] and so would be expected to attend a private ultrasound practice. In addition to offering testing at initial presentation in primary care, linking FXS carrier screening to first trimester screening is another potential service model.

Participants

Enrolling women in the study

Women are eligible to enter the study if they are 18 or over and either not pregnant or up to 12 weeks + 6 days pregnant at the time of recruitment. For non-pregnant women the upper age limit is 70, the age at which participation in the National Cervical Screening Program ends. Women who are unable to speak read and write English are not eligible to enter the study.

Recruitment is occurring in a number of different ways according to the preferences of individual clinics. Non-pregnant women are being recruited from general practice clinics. Women are provided with information about the study either personally (by a researcher, GP, practice nurse or receptionist) or they receive the information through the mail. Study information is not being provided by researchers to women attending general practice clinics who are obviously ill. Pregnant women are being recruited from general practice, private ultrasound and private or public obstetric and ultrasound clinics. In general practice, women are provided with information about the study by the GP when they attend for their pregnancy confirmation appointment. In private ultrasound clinics, study information is provided by clinic reception staff when women attend for their 12 week

scan. In private and public obstetric clinics, women are sent the study information in the mail prior to their first appointment, or are given the information personally by an obstetrician or midwife. Women who receive information about the study are asked to complete an expression of interest which is faxed to the research team, either indicating why they do not wish to take part, or providing their contact details so they can be recruited by a researcher. All recruitment is completed by the research team and all women speak with a research genetic counsellor.

Enrolling clinics in the study

General practice clinics located across the metropolitan areas of Melbourne and Perth are being targeted to try and achieve a geographical spread and a broad representation of different socioeconomic areas. General practices with established shared-care programs are being identified using registered shared care provider lists. Professional networks and an in-house database of GPs and obstetricians who have previously ordered prenatal carrier testing for FXS or cystic fibrosis in Victoria is also being used to identify practices that might be interested in participating. We anticipate requiring 5 general practice, 5 private obstetric and 1 obstetric ultrasound clinic to recruit the 1000 women needed for the study.

Members of the project team are providing academic detailing to clinics involved in recruitment. Academic detailing covers background information on FXS, the aims of the project and what the study involves for participants. It is emphasised that the aim of the study is not to test as many women as possible, but rather to understand what factors influence a woman's decision to accept or decline carrier testing for FXS. Clinics are provided with project resources, including study brochures and expression of interest forms.

Australian GPs are primarily funded by a fee for service system and receive no government funding (personal or infrastructure) for involvement in research. Private obstetricians and ultrasound clinics also receive no government funding for involvement in research. All clinics are being offered a small amount of remuneration to cover their costs of involvement in the study, depending on the number of women recruited from their clinic.

Data collection

This research protocol will use mixed-methods data collection that includes genetic testing uptake and outcomes, questionnaires and interviews.

Questionnaires

The questionnaires use validated and psychometrically robust self-reported scales. Table 1 shows which scales are used in questionnaire 1 (Q1), completed after making a decision about carrier testing for FXS, and questionnaire 2 (Q2), completed one month after returning Q1.

Table One: Questionnaire Measures and Scales

Measure / Scale	Description	Q1	Q2
Knowledge	10 item scale containing questions on FXS (True/False/Unsure). A score of 7 or higher is classified as 'good' knowledge [55]	√	√
Attitudes	5 item scale (0-4) used to assess a woman's attitude to screening (beneficial/harmful; important/unimportant, bad thing/good thing, pleasant/unpleasant, worrying/not worrying). Dichotomous scale: women are classified as having a positive (11-20) or a negative (0-10) attitude toward screening [49].	√	
Multi-dimensional Model of Informed Choice (MMIC)	Defines an informed choice as a decision made with 'good' knowledge which is consistent with a person's values. Incorporates three dimensions: knowledge, attitudes and uptake. Dichotomous scale: 'informed choice' or 'not informed choice' [49].	√	
Deliberation	6 item scale measuring the extent to which a decision is deliberated on a 5 point Likert scale (0 = strongly agree – 4 = strongly disagree). Dichotomous scale: responses below the midpoint (11 or under) classified as not deliberated and those at or above the midpoint as deliberated [53].	√	
Decisional Conflict Scale	16 item scale measuring uncertainty about a course of action on a 5 point Likert scale (0 = strongly agree – 4 = strongly disagree). Mean scores are reported with higher scores indicating higher decisional conflict. Scores range from 0 to 100 with scores over 37.5 associated with decision delay or uncertainty about implementation [60].	√	
Depression Anxiety Stress Scale, short form (DASS-21)	21 item scale divided into 3 subscales measuring depression, anxiety and stress. Responses are classified into 5 categories: 1 (normal) to 5 (extremely severe) [61, 62].	√	√
State Trait Anxiety Index, short form (STAI-6)	6 item scale measuring state anxiety. The maximum score is 80 with scores 31-49 considered average and scores over 50 indicating elevated state anxiety [59].	√	√
Health Belief	16 items measuring the importance of a range of factors which may influence decision-making: perceived benefits; perceived susceptibility; perceived severity; and perceived barriers; in a woman's decision to accept or decline testing for FXS [47, 63]	√	
Decisional Regret	5 item scale measuring distress or remorse after a health care decision using a 5 point Likert scale (0-4). Scores range from 0-100 with higher scores indicating a higher level of regret [64].		√
Willingness-to-Pay	2 questions (piloted) that address WTP and gross family income. Income question has 6 income ranges with tick box. WTP question has 11 item income values with tick box and sub-questions that address: i) utility of test (information only or information plus decision-making); and ii) who receives test result (recipient only or recipient plus shared with health share professionals).	√	
Socio- demographics	Marital status, age, parity, reproductive life-stage, education, occupation, postcode	√	

Interviews

To provide in-depth data on participants' experiences, semi-structured qualitative interviews are being conducted with participants at a number of time-points (See Table Two). Interviews are being conducted by two members of the research team with genetic counselling and qualitative research skills.

Table Two: Overview of Interview Schedule

Time-point	Interview type	Interview description	Selection
After return of Q1, before Q2 and result sent (if tested)	Decision-making interviews	Knowledge, attitudes, factors influencing decision-making, the decision-making process, and perspectives on decisions	Non-pregnant women only; mix of tested and untested women
1 month after return of Q2	Program evaluation interviews (women)	Motivations for participating, factors influencing decision-making, experience of participating in the study including genetic counselling, reflections on decision and views on screening	Mix of tested and untested women from each clinic, including all women with positive test results. Socio-demographic data examined to ensure selected women are representative of the overall sample
After completion of recruitment at any given clinic	Program evaluation interviews (clinic staff)	Attitudes to population carrier screening for FXS, knowledge of FXS, reflections on offering FXS carrier screening at their clinic, and feedback on the study.	Mix of staff from each clinic involved in recruitment
1 year after return of Q2	1 year follow-up	Motivations for screening, interpretation of result, perceived value of result, impact of result and reflections on decision	All women with a test-positive result (i.e. GZ, PM or FM)

Data entry quality control

To ensure accuracy of the questionnaire data, every 20th questionnaire entered is being checked prior to analysis. The rate of accuracy will be calculated as the number of errors per number of data items entered. To ensure rigour in the qualitative data analysis, transcripts will be independently coded.

Testing

One of the aims of our study is to evaluate the performance of a new innovative assay specifically designed for population screening for FXS [65]. Therefore, for the first part of the study, we collected DNA from a saliva sample (Oragene- DNA collection kit) and carried out the gold standard

two step diagnostic test [8, 66] in parallel with the innovative screening test. The routine FXS diagnostic test may involve Southern blotting and so can take up to 4 weeks [43]. This is performed by the Victorian Clinical Genetic Service laboratory. Refinements to the innovative screening assay [67] mean that we are now able to collect DNA from cheek brush samples and have results available in one week. This screening assay, marketed by Asuragen, is being performed by Healthscope Pathology.

All women who choose to have carrier testing are being given information about their result based on current best practice. Women with a result in the normal range receive a letter that includes an offer to speak to a genetic counsellor at their local clinical service should they require further information. Women with a test-positive result (GZ, PM or FM) are telephoned and offered face-to-face genetic counselling at their local clinical genetics service. Genetic counselling for women with test-positive results follows usual clinical practice [4, 68]. Any pregnant woman found to have a PM or FM is given her result and, as part of genetic counselling, is offered prenatal diagnostic testing of the fetus, due to the risk of having a child with FXS. An important outcome of receiving an FXS carrier result is that relatives can access genetic testing, which may lead to identification of other carriers and/or the diagnosis of fragile X related disorders in other family members. Genetic testing is discussed as part of the genetic counselling process and family members are offered genetic counselling and testing where appropriate.

Outcomes

The primary outcomes for the study are test uptake and informed choice. Study participants (denominator) are defined as the number of women recruited into the study who do not actively withdraw at any point. Test uptake is defined as the number of women accepting testing (numerator) divided by the number of study participants and will be reported as a percentage. Informed choice will be reported as the percentage of women in each group (pregnant and non-pregnant, tested and untested) making an informed choice as measured using the Multi-dimensional Measure of Informed Choice (MMIC) [62]. MMIC will be measured in Q1 at the time closest to decision-making. Knowledge, a component of the MMIC, will be measured in Q1 and Q2 and mean knowledge scores will be reported for each time-point.

The study will also examine predictors of test uptake. These multivariate analyses will make use of socio-demographic, family history, health belief and psychosocial items included in Q1.

Psychosocial factors will be examined as secondary measures in this study, including anxiety, depression and stress. These will be administered in both questionnaires to allow them to be measured at the time of decision-making and 1 month later. Decisional conflict will be measured in Q1 and decisional regret in Q2.

State anxiety will be reported as the difference in the mean STAI-6 item short form score of women in each group (pregnant and non-pregnant, tested and untested, normal result versus test positive). Depression, anxiety and stress will be reported as the mean score of women in each group. Decisional conflict and decisional regret will be reported as mean scores.

In the willingness-to-pay (WTP) literature there is keen interest in how WTP dollar values for information may vary in accordance with intended use, who receives the information and capacity-to-pay. Our questions have been designed to address these key issues. Accordingly, WTP data will be reported in a number of ways: i) intended use ('information only' and/or 'decision-making – personal or medical'); ii) by recipients of information ('women only' or 'women plus health care professionals'); iii) for women in the trial as a whole and for each group (pregnant and non-

pregnant, tested and untested, normal result versus test positive); as mean dollar values together with associated ranges around each mean to facilitate sensitivity testing.

Sample size

In our pilot study, in which women were required to return on a separate occasion to give a blood sample, test uptake in non-pregnant women was 20%, although 50% indicated they intended to be tested [43]. Based on the relevance to reproductive life-stage, we expect test uptake in the pregnant group to be greater than in the non-pregnant group. Our minimum sample size of 500 women per group will give us 88% power to detect a difference of 10% in test uptake between groups (50% v 40% or 50% vs 60%). We have less information about the likely percentage of women making an informed choice. If the percentage is 50%, with a minimum sample size of 500 per group an unadjusted analysis would have 87% power to detect a difference of 10% (i.e. 50% vs 40% or 505 vs 60%) between groups. If the base rate is greater than or less than 50% we would have >87% power to detect a difference of 10%. The study will therefore be sufficiently powered to exclude anything other than small percentage differences between groups.

Proposed analysis

Descriptive statistics will be used to describe the socio-demographic, knowledge, attitudes and psychological characteristics of the sample. To compare uptake of testing by pregnant and non-pregnant women, a multivariate logistic regression model with uptake as the dependent variable, and socio-demographic variables such as age, education and parity, together with pregnant/non-pregnant status and mode of recruitment as the independent variables, will be estimated. This will ensure that a difference in uptake is not due to differences in socio-economic composition of the pregnant and non-pregnant samples. Robust standard errors will be estimated to take into account the possible effect of clustering due to recruitment methods. Odds ratios will be transformed back to percentage differences [69]. A similar analysis will be performed to compare informed choice. To investigate predictors of uptake of testing, a multivariate logistic regression model will be estimated with independent variables including: informed choice, attitudes, number of children, prior awareness of FXS, psychosocial variables, family history of intellectual disability, age and education. Interactions between predictors and pregnancy/non-pregnancy will be examined, and if necessary, separate models will be estimated for pregnant and non-pregnant women.

Interviews are transcribed verbatim and NVivo 10 (QSR International, Australia) is being used to manage the data and facilitate coding. Coding is being done by at least two independent researchers to provide rigour of analysis. The decision-making interviews are being examined using content and thematic analysis. These interviews occur between the return of Q1 and the issuing of results (for tested women) and Q2. As such they involve only non-pregnant women, as we were concerned that an interview at this time before receiving a result, or needing to delay sending out the result prior to the interview, could be distressing for pregnant women at a time when they might be vulnerable. Data from the post-Q2 interviews are being analysed using directed content analysis [70]. The coding framework has been developed using data from the needs assessment phase of the study [43, 47, 54]. As little prior research has explored the experiences of women identified as carrying GZ, PM or FM alleles through population-based carrier screening, or the experiences of staff in clinics offering population carrier screening, the interviews will be analysed thematically. This will involve an iterative process where data are coded, compared, contrasted and refined to generate emergent themes [71] using an approach we have described previously [54].

The economic analysis is matched to the stages of FXS carrier screening described in our program logic model (Figure One). At this stage the analysis is concerned with examining stage 3 (program implementation) and stage 4 (short-term outcomes). Placing a dollar value on the health and non-health outcomes of FXS screening is complex. The immediate result of FXS screening is information.

That information might be about a risk to a fetus the women is carrying, implications for the women's future health, or implications for the woman's future reproductive health and reproductive choices. It is for this reason that we have started with willingness-to-pay (WTP) methods to explore the value that individuals place on the information provided. The WTP data will be analysed in accordance with the intervention design and policy issues set out above. The WTP data will also be analysed to see if there is an association between the dollar values and preparedness to undergo testing. Similarly, to the extent feasible, the relationship between socio-demographic variables and WTP will be analysed to see if these variables impact on WTP.

Longer-term economic modelling using a surrogate is planned for Stage 5. We aim to go on to record the actions that the women undertake as a result of their test results and the incidence of births of babies with FXS to women in the study, discussion of test results with family and identification of carriers/affected individuals with cascade testing. This will facilitate full economic appraisal using a range of methods, including discrete choice experiments (DCE). DCE has applicability to this field because non-health outcomes and process attributes are also important, and DCE is a logical extension to the WTP for inclusion in Stage 5.

ETHICS AND DISSEMINATION

Ethics

Ethics approval to conduct this study has been granted by the Human Research Ethics Committees of the Universities of Melbourne (HREC 0830733) and Western Australia (RA/4/1/4028). Additionally, approval has been granted by the ethics committees of the following recruitment sites: Family Planning Victoria (09/2); Women's and Newborn Health Service and Charles Gardiner Hospital – King Edward Memorial Hospital (1925/EW); Swan Kalamunda Health Service (2012-160). This project is being carried out according to the National Statement on Ethical Conduct in Human Research (2007) and the Australian Code for the Responsible Conduct of Research (2007) produced by the National Health and Medical Research Council of Australia. A plain language statement is provided to all women and to clinics and health professionals involved in recruiting women for the study and a signed consent form is obtained from all participants at the time of recruitment.

Steering group and advisory committee

This study has a designated research team and an advisory group. The advisory group includes representation from the Victorian Department of Health, the Fragile X Association of Australia and clinicians involved in the study. This group meets annually. The research team includes expertise in population health, genetics, primary care, epidemiology, FXS, health economics, pathology and psychology, with the full team meeting quarterly.

Dissemination

This study will be the first of its kind worldwide to address informed decision-making in carrier screening for FXS and to compare screening in pregnant and non-pregnant women. It will inform appropriate clinic service models for offering FXS screening and will provide important exploratory health economic data. We expect to publish one main trial outcome paper and a number of additional papers exploring aspects of the data in more detail. We will also present our findings at a number of international conferences. A report outlining the main findings of the study will also be made available on the study website www.fragilexscreening.net.au on completion. The findings of this study will inform policy development about when and how to offer population carrier screening for FXS.

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