1 **Population-based Carrier Screening and Prenatal Diagnosis of Fragile X**

2 **Syndrome in East Asian Populations**

3 Running head: Carrier Screening & Prenatal Diagnosis of FXS

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18 population-based carrier screening; prenatal diagnosis; cost-effectiveness analysis

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20

21 **Abstract**

22 Identification of carriers of fragile X syndrome (FXS) with the subsequent prenatal
23 diagnosis, and knowledge of FXS-associated genetic profiles are essential for
24 intervention in specific populations. We report the results of carrier screening of 39,458
25 East Asian adult women and prenatal diagnosis from 87 FXS carriers. The prevalence of
26 FXS carriers and incidence of full mutation fetuses in carrier pregnancies were found to
27 be 1/556 and 11.0%, respectively. The prevalence of FXS carriers and full mutation
28 fetuses was estimated to be 1/581 and 1/3124 in East Asian populations, respectively.
29 We confirmed the validity of the current threshold of CGG repeats for *FMRI*
30 categorization; the integral risks of full mutation expansion were approximately 6.0%,
31 43.8%, and 100% for premutation alleles with 55-74, 75-89, and ≥ 90 CGG repeats,
32 respectively. The protective effect of AGG interruption in East Asian populations was
33 validated, which is important in protecting premutation alleles with 75-89 CGG repeats
34 from full mutation expansion. Lastly, family history was shown not an effective
35 indicator for FXS carrier screening in East Asian populations and population-based
36 screening was more cost-effective. This study provides an insight into the largest carrier
37 screening and prenatal diagnosis for FXS in East Asian populations to date. The
38 FXS-associated genetic profiles of East Asian populations are delineated and
39 population-based carrier screening is shown to be promising for FXS intervention.

40 **Introduction**

41 Fragile X syndrome (FXS) (#MIM300624) is the leading monogenic cause of autism
 42 and autism spectrum disorders and the second most common cause of inherited
 43 intellectual disability ¹. FXS affects approximately 1 in 4,000 males and 1 in 7,000
 44 females worldwide ². The genetic basis of FXS is the translational silencing of FMRP
 45 translational regulator 1 (*FMR1*), an X-linked dominant gene with a reduced penetrance
 46 of 80% in males and 30-50% in females ¹. *FMR1* spans 17 exons and encodes FMRP,
 47 which plays a fundamental role in the synapses and the normal development of
 48 dendrites ³. A segment of CGG trinucleotide repeat is located in the untranslated region
 49 of the first exon of *FMR1* and the number of CGG repeats is polymorphic ⁴. According
 50 to the guidelines of American College of Medical Genetics and Genomics (ACMG),
 51 *FMR1* is categorized into four allelic forms based on the number of CGG repeat: normal,
 52 intermediate, premutation (PM), and full mutation (FM) ⁵. Specifically, normal alleles
 53 have a range of ~5 to ~44 repeats and are meiotic or mitotic stable. Intermediate alleles
 54 range from ~45 to ~54 repeats and their repeat number can slightly increase or decrease
 55 in intergenerational transmission. PM alleles range from ~55 to ~200 repeats and can
 56 expand to FM during transmission from parents (almost solely from the mother) to the
 57 child. In addition, the PM allele can cause fragile X-associated tremor ataxia syndrome,
 58 which mainly affects males, and fragile X-associated primary ovarian insufficiency,
 59 which results in reduced fertility in females. FM alleles have more than 200 repeats. The
 60 adjacent CpG islands and the repeats themselves are methylated, which block the
 61 subsequent transcription and translation, and accounts for most cases of FXS. Therefore,

asymptomatic women with PM or FM alleles are considered to be FXS carriers since their children are at risk of FXS. In addition to the CGG repeat number, other factors may be associated with the risk of CGG intergenerational expansion, although their effects require further confirmation⁶⁻⁹. Among these, AGG interruption, which refers to AGG triplet within the CGG repeats, has received the most attention^{6, 10, 11}. In many cases, CGG repeats are interrupted by one or more AGG interruptions. AGG interruption has been suggested to act as a protective factor, anchoring the region during replication to prevent strand slippage, thus decreasing the risk for intergenerational CGG expansion¹². Notably, genetic profiles, such as the prevalence of carrier and FXS, the intergenerational transmission profile of CGG repeat, and the protective effect of AGG interruption, vary widely among different populations^{5, 11, 13, 14}.

Considering the significant morbidity associated with FXS and the absence of an effective treatment, the prenatal diagnosis and intervention of FXS is warranted⁵. In this context, the identification of FXS carriers is an essential work for FXS intervention¹⁵. Moreover, evaluating the FXS-associated genetic profiles for specific populations is also fundamental to improve our knowledge for genetic counselling, revising the *FMRI* categorization, refining risk assessment, and policy-making for carrier screening strategies.

In this study, we reported the results of FXS genetic testing over a period of six years. Our analysis delineated the FXS-associated genetic profiles in East Asian populations. Furthermore, we compared the cost-effectiveness of FXS carrier screening strategies in general or targeted populations.

84 **Materials and Methods**

85 **Subjects**

86 We retrospectively analyzed the results of FXS genetic testing from January 1, 2014 to
87 December 31, 2019 in Ko's Obstetrics & Gynecology Clinic, Taiwan. We enrolled 3
88 cohorts of subjects in this study. Cohort 1 included 39,458 East Asian adult women
89 receiving testing for the first time, regardless of whether they had a family history of
90 FXS-associated conditions. Cohort 2 included 34 East Asian adult women who had been
91 diagnosed as FXS carriers in other institutes and were seeking prenatal diagnosis in our
92 clinic. Cohort 3 comprised the prenatal diagnosis cases in Cohorts 1 and 2.

93 **FXS genetic testing**

94 Pre- and pos-test counseling was offered to every participant by well-trained genetic
95 counsellors, according to the ACMG Standards and Guidelines. The CGG repeat and
96 AGG interruption status of the *FMRI* was determined using AmplideX[®] FMR1 PCR
97 reagents (Asuragen, Austin, TX) with capillary electrophoresis on ABI3730xl
98 instruments (Thermo-Fisher Scientific, Waltham, MA). To ensure the accuracy of this
99 study, all PM or FM samples were re-tested and confirmed using Biofast[®] FMR1 PCR
100 reagents (Bioson, Xiamen, China).

101 **Reference data**

102 Data from previous studies involving detection of FXS in East Asian populations were
103 combined and analyzed to estimate the FXS-associated genetic profiles.

104 **Samples**

105 For each adult woman, 2 mL of EDTA-anticoagulated peripheral blood was sampled.

106 For each prenatal diagnosis, approximately 10 mg of villus or 10 mL of amniotic fluid
107 was sampled. DNA was extracted using the MagCore Automatic Nucleic Acid Extractor
108 (RBC Bioscience, Taipei, Taiwan), according to the manufacturer's protocols. The DNA
109 concentration was determined by measuring the absorbance at 260 nm using a
110 NanoDrop 1000 spectrophotometer (Thermo Fisher, Waltham, MA)

111 **Cost-effectiveness analysis**

112 We developed a simplified decision-analytic model to compare the cost-effectiveness of
113 the FXS carrier screening strategies for adult women in cohort 1 that was based on
114 different subjects: all adult women (population-based screening), and high-risk women
115 with a family history of fragile X-related disorders or intellectual disability suggestive
116 of FXS (targeted screening)¹⁵. In this model, we assumed that the accuracy of FXS
117 genetic testing was 100% with a penetrance of *FMRI* of 60% (average of male and
118 female)¹. The cost of FXS carrier screening in Taiwan is approximately 100 USD per
119 case (including genetic testing and counseling), and the cost of prenatal diagnosis is
120 approximately 400 USD per case (including sampling, FXS genetic testing, and
121 maternal cell contamination identification). Since no data regarding the expenses of
122 FXS treatment is available in Taiwan, we conservatively assumed that the lifecycle cost
123 for an FXS patient would be approximately 500,000 USD, based on data from the
124 Europe and the United States^{16, 17}.

125 In our cost-effectiveness analysis, the cost is denoted as the sum of the estimated cost
126 for carrier screening and prenatal diagnosis. The potential cost is the estimated lifecycle
127 cost for FXS patients that would be born in a targeted screening branch. The

effectiveness of a strategy was calculated by dividing the total number of identified FM fetuses by the total number of FM fetuses that would be generated by adult women in cohort 1.

Statistical analyses

Normality testing was performed to determine whether a dataset followed a normal distribution. When a data set was non-normally distributed, a non-parametric method, Kruskal–Wallis analysis of variance (ANOVA), was used to test whether samples originated from the same distribution using OriginPro software (version 8.0, OriginLab Corp., Northampton, MA). The Chi-square test was performed using SPSS statistical software (version 20.0; IBM, Armonk, NY) to determine the differences for the frequencies between or among the different datasets.

Ethics statement

Signed informed consent was obtained from each participant. All participants agreed to the use of their data for research purposes. Except for the results of genetic testing and fetal genders, all information, including the names of the participants, were de-identified. This study was approved by the Research Ethics Committees of the Women and Children’s Hospital at the School of Medicine of Xiamen University and the Ko’s Obstetrics & Gynecology Clinic.

Results

Overview of FXS genetic testing results

An overview of the FXS genetic testing results is provided in Table 1. Cohort 1 indicates the scenario of population-based FXS carrier screening. In this cohort, we

150 identified 71 PM carriers from 39,458 adult women, revealing a 1/556 prevalence of
 151 FXS carriers in the East Asian female population. Combined with data from previous
 152 studies with relatively larger sample sizes (e.g. over 5,000)¹⁸⁻²¹, the estimated
 153 prevalence of FXS carriers was approximately 1/581 and 1/796 in the general and
 154 high-risk East Asian population, respectively (Tables 2 and 3).
 155 After genetic counselling, 53 out of 71 carriers in cohort 1 and 34 carriers in cohort 2
 156 received prenatal diagnosis in our clinic. Combining the two cohorts (i.e. in cohort 3),
 157 91 fetal *FMRI* genotypes were obtained from 87 carriers, since three carriers had two
 158 pregnancies and one carrier had a twin pregnancy (Supplemental Tables 1-3). The
 159 incidence of FM fetuses was 11.0% in all carrier pregnancies (Table 1). Compared to
 160 the carrier prevalence, the incidence of FM fetuses was more widely variable
 161 (9.1%-33.3%) among different studies¹⁹⁻²³. The average incidence was 18.6% (Table 4).
 162 Therefore, combining the 1/581 carrier prevalence and 18.6% incidence of the FM
 163 fetuses in carrier pregnancies, the estimated prevalence of FM fetuses in the East Asian
 164 population would be approximately 1/3124.
 165 Noticeably, unlike the gender distribution of normal and PM fetuses, female was the
 166 predominant gender in FM fetuses (Table 1).

167 **Intergenerational CGG repeat transmission profiles**

168 In cohort 3 (Table 1), we identified 42 PM and 10 FM fetuses that had inherited
 169 maternal PM or FM alleles. Of these 52 transmission events, 10 (19.2%) were stably
 170 transmitted, while 42 (80.8%) increased in size (Supplemental Tables 2 and 3).
 171 As shown in Fig. 1A, the extent of unstable transmissions and FM expansions increased

markedly with an increasing maternal CGG repeat size. The smallest size of PM allele associated with FM expansion was 75, while all PM alleles ≥ 90 CGG repeats expanded to FM alleles. Combined with previous studies^{19-21, 23}, the integral risk of FM expansion was determined to be 6.0%, 43.8%, and 100% for PM alleles with 55-74, 75-89, and ≥ 90 CGG repeats, respectively (Table 5).

Protective effect of AGG interruptions

In cohort 3, AGG interruption was observed in 19 PM alleles out of 87 carriers, of which 13 alleles were transmitted to the 14 fetuses (Supplemental Table 2). Of these 14 transmission events, 10 were stably transmitted, 3 were expanded with 1 CGG copy, and 1 was expanded with 2 CGG copies. In contrast, no stable transmission was observed in the PM alleles without AGG interruptions (Supplemental Tables 2 and 3).

In the subset of maternal PM alleles with 75-89 CGG repeats, 3 PM alleles with AGG interruptions were stably transmitted, while 2 of 3 PM alleles without AGG interruptions expanded to FM alleles (Fig. 1A and Supplemental Tables 2 and 3).

While no AGG interruptions were observed in the PM alleles with CGG repeat sizes ≥ 90 , 100% PM alleles in this subset expanded to FM alleles in intergenerational transmission (Fig. 1A). In terms of PM alleles with CGG repeat sizes under 90, intergenerational CGG expansion was evident in maternal alleles without AGG interruptions compared to their AGG interruption counterparts (Fig. 1B). Based on the data (Fig. 1B), Kruskal–Wallis ANOVA confirmed that the absence of AGG interruptions had a significant effect on the risk of intergenerational CGG expansion ($P < 0.001$).

194 Lastly, we compared the AGG interruption status between normal, PM, and FM alleles
 195 of 105 carriers in cohorts 1 and 2. As shown in Table 6, AGG interruptions were
 196 observed in all normal alleles, where the most prevalent number of AGG interruptions
 197 was 2. In comparison, the loss of AGG interruption began with the smallest PM alleles
 198 (55 CGG repeats), while no AGG interruptions were observed in PM alleles with over
 199 86 CGG repeats. In total, 22 PM alleles had 1 AGG interruption and 1 PM alleles had 2,
 200 while no AGG interruptions were observed in the other 82 PM alleles and 4 FM alleles.
 201 Chi-square tests confirmed that the absence of AGG interruptions was strongly
 202 correlated with PM and FM alleles ($P < 0.001$).

203 **Cost-effectiveness analysis**

204 The decision-analytic model established in this study is shown in Fig. 2. In terms of the
 205 population-based screening branch, 71 carriers were identified from 39,458 adult
 206 women based on the data of cohort 1. According to the 18.6% incidence of FM fetuses
 207 in East Asian populations (Table 4), 13 FM fetuses and 58 normal and PM fetuses were
 208 expected from these 71 carriers. In terms of the targeted screening branch, only
 209 high-risk women were screened. Based on a previous study, the prevalence of FXS
 210 carriers in Taiwanese women with FXS-associated family history was approximately
 211 100 in 725²³. The prevalence of FXS carriers in the East Asian populations without
 212 FXS-associated family history was approximately 1/796 (Table 3). Accordingly,
 213 approximately 157 women should be high-risk for FXS carriers among 39,458 adult
 214 women, with 22 carriers and 4 FM fetuses. Meanwhile, 49 carriers would be missed
 215 since the rest of the 39,301 adult women were not tested, which could result in the birth

216 of 9 FM babies. With the assumption of 60% penetrance, 5 babies would be affected
217 with FXS.

218 According to this model, population-based screening and targeted screening strategies
219 to identify a FM fetus would cost approximately 305,707.69 USD and 6125 USD
220 respectively (Table 7). However, the targeted screening strategy would miss
221 approximately 69.2% (9/13) of FM fetuses, suggesting that 1.25 equivalent FXS
222 patients would be born for every FM fetus identified (Fig. 2). Therefore, a potential cost
223 of 625,000 USD would be required due to the life-cycle burden of FXS patients. In this
224 context, population-based screening would be more cost-saving than targeted screening
225 for preventing the birth of FXS patients (Tables 7 and 8). The primary cost in
226 population-based screening is derived from the cost of screening testing, and derived
227 from the life-cycle burden of FXS patients in targeted screening (Table 8). In view of
228 the 100% effectiveness and advanced cost-effectiveness, population-based screening
229 represented a dominant strategy compared to targeted screening.

230 **Discussion**

231 Understanding the genetic profiles associated FXS is fundamental for optimizing
232 detection tests, genetic counselling, and the intervention strategies in specific
233 populations, especially in East Asian populations, which comprise more than 20% of the
234 global population. As the largest study of carrier screening and prenatal diagnosis for
235 FXS in the East Asian populations to date, our study revealed a 1/556 prevalence of
236 FXS carriers in the general population and a 11.0% incidence of FM fetuses in carrier
237 pregnancies. Several studies have previously reported various levels of FXS carrier

prevalence in East Asian populations based on different subject groups and sample sizes.

According to these studies, which had relatively large sample sizes (over 5000), the prevalence of FXS carriers in the general population is 1/530-1/788 in South Korea and 1/564 in Northern China^{18, 20, 21}, while the prevalence of FXS carriers in a population without a family history of FXS-associated conditions is 1/781-1/846 in South Korea and 1/776 in Taiwan¹⁹⁻²¹. Ma et al. reported a higher prevalence (1/410) of FXS carriers in reproductive women in Northern China²⁴. However, the cohort included more than 60% women with a history of spontaneous abortion or induced abortion due to delayed embryo growth. When excluding this subset of samples, the prevalence was adjusted to 1/756. In terms of carrier prevalence in the general population, the differences were not statistically significant among different studies (Table 2; Chi-square test, $P = 0.690$), suggesting a genetic consistency among East Asian populations. Combining this data, the estimated prevalence of FXS carriers is approximately 1/581 in the general East Asian population (Table 2), which is lower than that in Jews and Arabs in Israel²⁵, and pan-ethnic populations in Australia and the USA^{26, 27}, but higher than previous found in the same population derived from relatively small sample sizes^{23, 28}. Noticeably, the difference of carrier prevalence between the general population and populations without a family history is also statistically insignificant (1/581 vs 1/796; Chi-square test, $P = 0.073$), suggesting that the majority of carriers, at least to their knowledge, do not have a family history of FXS-associated conditions, thus minimizing the effectiveness of family history-based screening. Similarly, using the combined data, an estimated 18.6% incidence of FM fetuses in carrier pregnancies (Table 4) and an estimated 1/3124

260 prevalence of FM fetuses in general pregnancies was suggested, emphasizing the
 261 importance of FXS carrier screening.

262 In prenatal diagnosis, the PM and FM fetuses outnumbered the normal fetuses in our
 263 study (Table 1). The combined data (Table 4), which was also indicated in another
 264 population-based study²⁷, suggested that compared to normal alleles, PM alleles may
 265 be more readily transmitted to the offspring. Expanded *FMRI* alleles have been
 266 suggested to be associated with miscarriage^{24, 29}. Our finding supported the concept that
 267 miscarriages would more likely be attributed to a disrupted maternal environment rather
 268 than developmental defects caused by fetal expanded alleles^{24, 29}. However, FM alleles
 269 could be the cause of miscarriage in male fetuses. In our study, female was the
 270 predominant gender found in FM fetuses (Table 1), which agreed with a previous study
 271¹⁹. Based on the important roles of *FMRI* in fetal development, and the higher
 272 penetrance of *FMRI* in males than females, we speculate that male FM fetuses are more
 273 readily aborted. However, more data will be required to confirm this speculation.

274 The current categorization of *FMRI* alleles is used worldwide^{5, 15}. However, the
 275 thresholds between allele forms may change with increasing empirical data and research,
 276 especially in different populations⁵. In our study, the smallest repeat size of PM alleles
 277 that expanded to FM alleles was 75. However, smaller PM sizes associated with FM
 278 expansion have been reported in previous studies based on the same population, in
 279 which the smallest repeat size was 56^{19, 20, 23}. In this regard, the currently used cut-off
 280 between normal and PM alleles is valid in East Asian population. It is widely known
 281 that the number of CGG repeats is an important determinant for assessing the risk of

282 FM expansion in carriers. According to the data, the integral risk of FM expansion is
 283 6.0%, 43.8%, and 100% for PM alleles with 55-74, 75-89, and ≥ 90 CGG repeats,
 284 respectively, in preliminary assessments (Table 5). Further studies with more cases of
 285 CGG intergenerational transmission will be needed to refine the CGG repeat-associated
 286 risk assessment further.

287 As the most promising protective factor, AGG interruption has been widely confirmed
 288 to play an important role in CGG transmission stability^{6, 10, 11}. More recently, it was
 289 incorporated into the risk assessment of FXS carriers in a population-based screening
 290 clinical practice²⁶. However, the protective characteristics of AGG interruption showed
 291 differences between Israeli and USA populations¹¹. Moreover, Manor et al. showed that
 292 AGG interruptions were more likely a polymorphism rather than a protective factor in
 293 individuals of Bedouin ethnicity in Israel¹⁴. Similar to carrier prevalence, the role of
 294 AGG interruption could be ethnic-specific and will need to be comprehensively
 295 evaluated before it can be used for the clinical risk assessment of specific populations¹³.

296 Unfortunately, studies on the role of AGG interruption in East Asian populations are
 297 limited, and AGG interruption is not included in standard genetic counseling due to a
 298 lack of validation. In our study, the absence of AGG interruptions had a significant
 299 effect on the risk of intergenerational CGG expansion (Fig. 1B), and was strongly
 300 correlated with the PM and FM alleles (Table 6). Therefore, we have demonstrated that
 301 the protective effect of AGG interruption is relevant to the East Asian populations. In
 302 particular, AGG interruption seems to play an important role in protecting PM alleles
 303 from FM expansion in the subset of maternal PM alleles with 75-89 CGG repeats (Fig.

1A; statistical analysis was not performed due to the limited sample size). Moreover, the percentage of PM alleles without AGG interruptions (3/6) was comparable to the integral risk of FM expansion (43.8%) in this subset (Table 5). These results further supported that AGG interruption may play an important protective role in this PM range. However, future studies with more cases of AGG interruption with CGG transmissions will be needed to refine the AGG interruption-associated risk assessment further.

Although the benefits of the prenatal intervention of FXS are widely acknowledged, population-based FXS carrier screening has yet to be officially endorsed by professional guidelines. This strategy lacks a consensus with regards to some of the main concerns, including lack of disease education, lack of cost-benefit or cost-effectiveness advantages, difficulties in genetic counseling, and potential for psychosocial harms^{30, 31}.

Instead, targeted screening based on the family history of FXS-association conditions is currently recommended¹⁵. As mentioned above, the insignificant difference of carrier prevalence between the general population and populations without a family history of FXS suggested that family history is not an effective indicator for FXS carrier screening in East Asian populations. In our model, we calculated that targeted screening misses approximately 69% of carriers (Fig. 2). Moreover, our findings were in accordance with those reported by a study on an Australian population²⁷. Based on the genetic profiles of the East Asian population, we confirmed that population-based screening is more cost-effective than targeted screening (Tables 7 and 8). The primary cost of population-based screening is the cost of screening testing (Table 8). The cost of genetic testing had tended to decrease with rapid developments in biotechnologies, indicating

that a greater economic advantage could be expected in the near future. Furthermore, the development of novel screening methods, such as “next- and third-generation sequencing”^{32, 33}, may also improve the testing capacity, thus further advancing the implementation of population-based screening to large populations. Several issues that contribute to the cost of population-based screening, including the infrastructure and human resources needed to provide appropriate education, counselling, interventions, and follow-up, were not included in our simplified model. However, the unit cost would be limited in FXS screening due to the fact that investment is not specific for a single condition but benefits numerous genetic diseases. This is particularly pertinent in the era of expanded population-based carrier screening, where hundreds of diseases can be screened simultaneously²⁶. In addition to its cost-effectiveness, other factors point towards the expansion of FXS screening criteria^{34, 35}. In fact, several national population-based screening programs, including FXS carrier screening, have already been implemented and have obtained positive outcomes²⁵⁻²⁷. The development of an effective FXS treatment could drastically improve the health of patients and lower disease-associated expense, and could revise the strategies for FXS intervention. Nonetheless, in the current stage, population-based carrier screening is the dominant strategy for FXS intervention in East Asian populations.

In conclusion, we reported on the largest carrier screening and prenatal diagnosis for FXS in East Asian populations to date, delineating the FXS-associated genetic profiles in this population. Our findings provide evidence that supports the implementation of population-based carrier screening over family history-based targeted screening for FXS

348 intervention.

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351 **Conflict of Interest**

352 None.

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467

468 **Figure 1. Transmission of intergenerational CGG repeats.** (A) Intergenerational
 469 CGG repeat transmission profiles of 51 premutation and 2 full mutation alleles. The
 470 transmission patterns were uncertain in one mosaic mother and fetus pair. This pair was
 471 excluded (Supplemental Table 3). (B) Intergenerational CGG transmission expansion of
 472 premutation alleles with CGG repeats under 90. The colored circles denote the alleles
 473 with AGG interruptions, while the black circles denote the alleles without AGG
 474 interruptions. Alleles ≥ 200 CGG repeats are the same size to facilitate demonstration.
 475

476 **Figure 2. Decision-analytic model for strategies of FXS carrier screening based on**

477 **the general population and targeted populations.** Based on experimental and

478 hypothetical data, the number of subjects is listed.

479

480 **Table 1. Overview of the FXS genetic testing results.**

	Cohort 1	Cohort 2	Cohort 3
Population-based carrier screening			
Adult women	39458		
FXS carriers	71		
PM carriers	68		
FM carriers	2		
Mosaic carrier	1		
Prevalence of FXS carriers	1/556		
Prenatal diagnosis			
FXS carriers	53	34	87
Fetuses (male: female)	57 (25:32)	34 (20:14)	91 (45:46)
Fetal <i>FMRI</i> genotypes			
Normal (male: female)	25 (9:16)	14 (7:7)	39 (16:23)
PM (male: female)	27 (15:12)	15 (12:3)	42 (27:15)
FM (male: female)	5 (1:4)	5 (1:4)	10 (2:8)
Incidence of FM fetuses	8.8%	14.7%	11.0%

481 FXS: fragile X syndrome; PM: premutation; FM: full mutation.

482

483 **Table 2. Prevalence of FXS carriers in general East Asian populations.**

	Our study	Mol Genet Genomic Med 2020; 8:e1236.	Clin Genet 2014; 85: 441–445.	J Med Screen 2013; 20:15–20.	Total
Adult women	39458	10145	10241	5829	65673
FXS carriers	71	18	13	11	113
Prevalence	1/556	1/564	1/788	1/530	1/581

484

485

486 **Table 3. Prevalence of FXS carriers in East Asian populations without**

487 **FXS-associated family history.**

	Sci Rep 2019; 9(1): 15456.	Clin Genet 2014; 85: 441–445.	J Med Screen 2013; 20:15–20.	Total
Adult women	20188	10146	5470	35804
FXS carriers	26	12	7	45
Prevalence	1/776	1/846	1/781	1/796

488 **Table 4. Prenatal diagnosis for FXS carriers in East Asian populations.**

	Our study	Chin J Med Genet	Sci Rep 2019;	Clin Genet 2017;	Clin Genet 2014;	J Med Screen	Total
		2019; 36(9):866-869	9(1): 15456.	92:217–220.	85: 441–445.	2013; 20:15–20.	
FXS carriers	87	29	27	24	26	10	203
Fetuses (male: female)	91 (45:46)	30	31 ^a (15:10)	32	26	11	221
Fetal <i>FMRI</i> genotypes							
WT (male: female)	39 (16:23)	17	13 (8:5)	18	13	5	105
PM (male: female)	42 (27:15)	3	11 (4:7)	6	8	5	75
FM (male: female)	10 (2:8)	10	7 (2:5)	8	5	1	41
Incidence of FM fetuses	11.0%	33.3%	22.6%	25.0%	19.2%	9.1%	18.6%

489 ^aA pregnancy in which a fetus carries a *FMRI* deletion of 5' UTR and exon 1 was excluded in this cohort.

490

491 **Table 5. Risk of full mutation expansion for maternal premutation alleles with different CGG repeat lengths.**

Maternal CGG repeats	Risk					
	Our study	Sci Rep 2019; 9(1): 15456.	Clin Genet 2017; 92:217–220.	Clin Genet 2014; 85: 441–445.	J Med Screen 2013; 20:15–20.	Total
55-74	0 (0/38)	10% (1/10)	33% (2/6)	11.1% (1/9)	0 (0/4)	6.0% (4/67)
75-89	33% (2/6)	60% (3/5)	0 (0/2)	100% (2/2)	0 (0/1)	43.8% (7/16)
≥ 90	100% (8/8)	100% (3/3)	100% (6/6)	100% (2/2)	100% (1/1)	100% (20/20)

492 **Table 6. Allelic AGG interruption profiles of 105 FXS carriers.**

AGG interruption	Normal allele	PM allele	FM allele [*]
0	0	82	4
1	5	22	0
2	94	1	0
3	6	0	0

493 FXS: Fragile X syndrome; PM: pre-mutation; FM: full mutation.

494 *Four carriers carried FM alleles, two of which were size mosaics. The CGG repeat
 495 sizes of these carriers were 30/>200, 36/>200, 30/141/>200, and 29/84/111/>200,
 496 respectively.

497

498 **Table 7. Estimated cost for the identification of an FM fetus.**

Screening strategy	Cost	Identified FM fetuses	Cost / identified FM fetuses	Potential cost	Potential cost / identified FM fetuses	(Cost + potential cost) / identified FM fetuses
Population	3,974,200	13	305,707.69	0	0	305,707.69
Targeted	24,500	4	6,125	2,500,000	625,000	631,125

499

500

501 **Table 8. Cost-effectiveness of FXS carrier screening.**

Screening strategy	Cost per woman	Potential cost per woman	Effectiveness	Cost per woman / effectiveness	(Cost + potential cost) per woman / effectiveness
Population	100.72	0	1	100.72	100.72
Targeted	0.62	63.36	0.308	2.01	207.72

502

A