DISTRIBUTION OF *FMR1* ALLELES IN THE VIETNAMESE PREGNANT POPULATION: IMPLICATIONS FOR A NATIONAL SCREENING STRATEGY

Nguyen Van Truong[1], Hoang Thu Lan[1,2], Nguyen Thi Minh Ngoc[2]
Dao Thi Trang[1,2], Nguyen Huu Duc Anh[1,2], Nguyen Phuong Mai[1,2]
Doan Thi Nguyet[1], Nguyen Thi Khanh Van[1], Ho Cam Tu[1], Nguyen Thuy Thuy Ai[3]
Tang Xuan Hai[4], Nguyen Thi Hao[4], Le Hong Thinh[3], Dinh Thuy Linh[5]
Nguyen Thi Ngoc Bich[6], Vu Thi Ha[1,2], Hoang Thi Ngoc Lan[1,2]
Doan Thi Kim Phuong[1,2], and Luong Thi Lan Anh[1,2,✉]

[1]Hanoi Medical University
[2]Hanoi Medical University Hospital
[3]Can Tho Obstetrics and Gynecology Hospital
[4]Nghe An Obstetrics and Pediatrics Hospital
[5]Hanoi Obstetrics and Gynecology Hospital
[6]Thanh Nhan Hospital

The expansion of cytosine-guanine-guanine (CGG) trinucleotide repeats in the 5'-untranslated region (5'-UTR) of the FMR1 gene leads to a spectrum of multisystem conditions collectively referred to as FMR1-related disorders. This study aimed to evaluate the prevalence of FMR1 allele groups among 4,000 pregnant women, thereby elucidating the genetic landscape of FMR1 within the Vietnamese population. A multicenter cross-sectional study was conducted. Peripheral blood samples were obtained for genotyping, and data collection for analysis involving demographic, clinical, and family history. The observed frequencies of the normal, intermediate, and premutation allele carriers were 98.85%, 1.1%, and 0.05%, respectively, while no full mutation carriers were detected. A statistically significant association was observed between the distribution of FMR1 allele groups and risk stratification based on family history. The Vietnamese population may exhibit a lower risk for FMR1-related disorders. Consequently, a targeted screening strategy focusing on high-risk individuals, rather than universal screening, may be a more pragmatic and cost-effective approach within the Vietnamese healthcare context.

Keywords: *FMR1*, Fragile X syndrome, premutation, pregnancy screening, population screening, screening strategy.

I. INTRODUCTION

The *FMR1* gene (MIM 309550) is located on the long arm of the X chromosome (Xq27.3), encoding FMRP (Fragile X Mental Retardation Protein)-a selective RNA-binding protein that is widely expressed across multiple organs, especially important for central nervous system integrity and ovarian function.[1] In well over 99% of cases, the alteration results from expansion of the CGG trinucleotide repeats in the 5′-UTR of this gene.[2] Selection and interpretation of genetic tests are discussed in detail in reputable guidelines.[3-5] According to the American College of Medical Genetics and Genomics (ACMG), *FMR1* alleles are classified into four groups

Corresponding author: Luong Thi Lan Anh
Hanoi Medical University
Email: luongthilananh@hmu.edu.vn
Received: 14/04/2026
Accepted: 08/05/2026

based on the number of triplet repeats: normal (NM, <45 CGG repeats), gray zone (GZ, 45-54 CGG repeats, also known as intermediate), premutation (PM, 55-200 CGG repeats), and full mutation (FM, >200 CGG repeats).[6] FM are the large expansions, which inhibit transcription and lead to the absence of FMRP. While FM alleles result in Fragile X syndrome (FXS), the leading cause of intellectual disability and autism spectrum disorder (ASD),[7] PM alleles present a distinct clinical burden. In fact, the molecular pathogenesis of PM is not driven by DNA methylation, but rather by a toxic mechanism resulting from the elevated *FMR1*-mRNA. Beyond their ability to undergo expansion to the fetus, these alleles are associated with a multisystemic clinical spectrum encompassing Fragile X-associated primary ovarian insufficiency (FXPOI), Fragile X-associated neuropsychiatric disorders (FXAND), and Fragile X-associated tremor/ataxia syndrome (FXTAS). In addition to impacting female reproductive longevity, these conditions lead to progressive neuropsychiatric deterioration in both sexes, with clinical symptoms becoming increasingly prominent from middle age onwards. GZ alleles are generally considered clinically equivalent to NM alleles. However, recent studies are showing that they may be associated with a range of clinical manifestations, while also carrying a latent risk of expansion during meiosis.[6,8,9]

Existing evidence regarding *FMR1* in Vietnam is currently restricted to hospital-based symptomatic cohorts with intellectual disabilities. These sources do not provide accurate population-based prevalence estimates, and PM-related disorders remain under-recognized. This quantitative data gap creates a significant barrier to understanding the true genetic burden of *FMR1*-related disorders at a national scale. In this study, we provided testing for pregnant women, on one hand, to maximize the detection of *FMR1* alleles in the population, and on the other hand, to provide genetic counseling as well as establish a diagnostic framework in a timely manner for cases carrying PM or FM. We also developed cost-effectiveness models to evaluate the economic viability of screening for a relatively rare genetic disorder within the Vietnamese population. Our research represents one of the pioneering and most valuable contributions to the scientific evidence on the epidemiology of *FMR1* in the Vietnamese pregnant population. These findings enrich the ongoing discussion on the cost-effectiveness and clinical benefits of targeted screening strategies, with potential implications for public health policy and prenatal care guidelines within the Vietnamese context.[5,6,10]

II. MATERIALS AND METHODS

1. Subjects

The study population consisted of pregnant women receiving prenatal care at four tertiary hospitals representing the three geographical regions of Vietnam: Hanoi Medical University Hospital, Hanoi Obstetrics and Gynecology Hospital (Northern region), Nghe An Obstetrics and Pediatrics Hospital (Central region), and Can Tho Obstetrics and Gynecology Hospital (Southern region).

Inclusion Criteria

Childbearing women who clearly remembered their last menstrual period (LMP) and/or had an ultrasound result within the first trimester. Gestational age < 28 weeks, calculated from the first day of the LMP or based on the ultrasound estimation. In cases of discordance between LMP and ultrasound findings, the gestational age determined by ultrasound was used.

Provided informed consent to participate in the study.

Exclusion Criteria

Presence of malignant tumors.

Recent blood transfusion.

2. Methods

Study design

An analytical cross-sectional study was conducted from July 2024 to July 2025. Participants from those attending prenatal care at the participating research sites were recruited using a convenience sampling approach. This is a pragmatic choice designed to facilitate the rapid acquisition of a large-scale population sample within a defined timeframe and ensure that the study population remains diverse and reflective of the regional demographic characteristics.

Sample size

The sample size was calculated based on the prevalence of PM alleles reported by Hunter et al. (2014)[8] (P = 1/294), utilizing a relative precision (ε) of 53% at a 95% confidence level (Z = 1.96). A cohort of 4,000 participants, recruited from 54 out of 63 provinces across Vietnam, was included to ensure adequate precision for this pilot investigation of a relatively rare condition, while remaining feasible within our available resources.

Study contents

Clinical characteristics and reproductive history: maternal *age, age* at menarche*, menstrual* cycle characteristics*, gestational age, prior pregnancy loss, morphological abnormalities/soft markers on ultrasound, and maternal serum screening/non-invasive prenatal testing results at enrollment.*

Genotype classification was performed in accordance with the 2021 ACMG standards.

The study population was stratified into two groups based on clinical suspicion and/or a positive family history of *FMR1*-related conditions. A comparative analysis of allele frequencies was then conducted between these risk-stratified subgroups:

- High-risk group: these include cases where the individual or their family members exhibit one or more of the following characteristics: unexplained intellectual developmental disorders, ASD, premature ovarian failure, ataxia, or carry FMR1 premutation alleles.

- Low-risk group: those who do not exhibit any of the aforementioned features.

CGG repeats allele sizing

The *FMR1* CGG repeat sizes were determined via triplet-primed PCR (TP-PCR) followed by capillary electrophoresis (CE). Two milliliters (2 mL) of peripheral blood were collected for genotyping purposes. DNA was isolated and purified from 200 µL of whole blood using GeneAll® Exgene™ Blood SV Kit (GeneAll, Seoul, 05729 Korea) following the manufacturer's instructions. The DNA samples meeting the required purity criteria were diluted (if necessary) to a final concentration of 20 ng/µL before the PCR step. Amplification was performed with the Asuragen AmplideX® PCR/CE *FMR1* Reagents (Asuragen, Austin, TX 78744 USA) employing gene-specific primers and CGG primers according to the manufacturer's recommended thermal cycling protocol.

Following successful amplification, PCR products were then run on an ABI 3500 Genetic Analyzer to generate CE profiles with an internal size standard to ensure precise fragment sizing. The resulting amplicon lengths were subsequently converted into the *FMR1* CGG repeats by automated genotyping using the AmplideX® PCR/CE Reporter Software (Asuragen, Austin, TX 78744 USA)

with the appropriate preconfigured settings, which utilizes a proprietary signal processing algorithm for robust size calibration and artifact elimination.

As part of the quality control procedure, all allele calls were reviewed by the authors to reach a consensus. Finally, genotypes were classified based on the longer allele on the X chromosomes for each sample, in accordance with the 2021 ACMG technical standards.

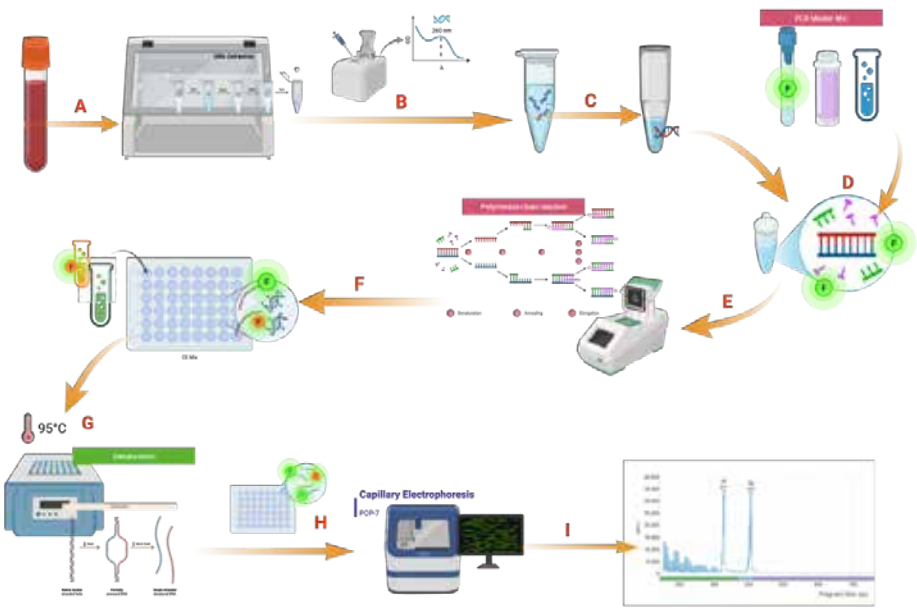

Figure 1. Molecular diagnostic workflow for *FMR1* genotyping

A. DNA extraction. B. DNA quantification and purity assessment. C. DNA samples were normalized to a standardized concentration. D. PCR Master Mix setup. E. Triplet-primed PCR amplification. F. CE Master Mix preparation. G. PCR products denaturation. H. CE and fragment sizing. I. Genotype calling.

Data processing and statistical analysis

Data management was conducted using Microsoft Excel (version 16.106.2, build 26022219), and all statistical analyses were done using RStudio (version 2026.01.1+403). Missing data were addressed via pairwise deletion to maximize the number of observations in correlation analyses. Since the CGG repeats and several clinical variables did not follow a normal distribution, we computed Spearman's rank correlation as a non-parametric method to effectively assess the associations between variables. To evaluate whether the observed correlation between the two *FMR1* allele sizes could have arisen from the random pairing of alleles within the population, a Monte Carlo permutation test was conducted. By repeatedly shuffling the allele assignments, this approach establishes a null distribution that accounts for the inherent bias of allele ordering, enabling us to assess the statistical significance of the observed association beyond mere random chance. Additionally, the Fisher-Freeman-Halton exact test was employed to compare proportions between risk groups due to the presence of small expected frequencies in certain cells.

Cost-Effectiveness Analysis

A cost-effectiveness analysis was performed using a model to evaluate three distinct strategies: (1) No screening, (2) Targeted screening, and (3) Universal screening. The model integrated probabilistic weights derived from our findings, and the missing parameters were imputed from previous studies. We constructed a decision tree model to delineate the various clinical pathways and outcomes associated with the screening process. We assumed that the frequency of PM in the Vietnamese pregnant population is 0.05%, with an amniocentesis rate of 90% when carrying PM, and a termination rate of 85% when a fetus carrying the expansion mutation is detected. The premutation expansion rate was determined as 11.3% according to Musci et al. (2005).[11-13] Associated medical expenses, encompassing amniocentesis (karyotyping and *FMR1* genotyping), termination of pregnancy, and pre- and post-procedural genetic counseling, were estimated based on prevailing healthcare costs in Vietnam. To evaluate the economic efficiency across the three screening strategies, a lifetime care cost of $320,000 per affected individual was utilized. The average cost per individual was used as a key parameter to determine the most cost-effective strategy for the Vietnamese healthcare system. To ensure long-term applicability, we also established economic thresholds for universal screening through two-way sensitivity analysis. This approach ensures that our findings remain relevant despite future reductions in testing costs or shifts in clinical evidence, thereby maintaining the robustness of the national screening strategy under varying scenarios.

3. Ethics in research

The study was approved by the Institutional Review Board (IRB) of Hanoi Medical University (Approval No. 937) on June 9, 2023. Each participant provided written informed consent, signifying their conscious decision to enroll in this project. Participants were assured that their participation was completely voluntary and were informed of their right to withdraw from the study at any point without prejudice to their ongoing medical care. Data confidentiality was strictly maintained, and all information was utilized solely for research purposes.

III. RESULTS

1. Epidemiological characteristics

A total of 4,000 childbearing women were enrolled in this study. The participants' ages ranged from 16 to 52 years old, with the majority (64%) falling within the 25-35 age bracket. The mean gestational age at the time of the visit was 12.28 ±3.59 weeks. Notably, 12 weeks of gestation was the most frequent time for consultation for 911 females, representing 22.8%. This timeframe represents a critical window for first-trimester prenatal care, as it is the optimal period for assessing nuchal translucency and performing NIPT tests (non-invasive prenatal testing). Common medical conditions seeking treatment during pregnancy included influenza (n = 12), allergy (n = 4), thyroid disorders (n = 4), threatened miscarriage (n = 4), gestational hypertension (n = 4), uterine fibroids (n = 4), gastroesophageal reflux disease (n = 2), lower genital tract infections (n = 2), gestational diabetes (n = 1), and ovarian cysts (n = 1).

Table 1. Distribution of *FMR1* alleles by geographical regions of Vietnam.

Alleles / Regions	Normal (≤44)	Intermediate (45-54)	Premutation (55-200)	Full mutation (>200)	Total	p-value
Northern	1,825 (99.40%)	9 (0.49%)	2 (0.11%)	0 (0.00%)	1,836	**0.16***
Central	3,187 (99.53%)	15 (0.47%)	0 (0.00%)	0 (0.00%)	3,202	
Southern	2,942 (99.32%)	20 (0.68%)	0 (0.00%)	0 (0.00%)	2,962	
Total	7,954 (99.42%)	44 (0.55%)	2 (0.03%)	0 (0.00%)	8,000 (100%)	

Note: Fisher's Exact Test with Monte Carlo simulation based on 20,000 replicates.*

The distribution of allele categories was highly consistent across the Northern, Central, and Southern regions of Vietnam (p-value = 0.16). The vast majority of *FMR1* alleles were within the NM range across all geographic regions of Vietnam. A total of 3,954 women (98.85%, 95% CI [98.47%, 99.16%]) carried NM alleles. FM was absent from our cohort. The GZ was observed in 44 cases (1.1%, 95% CI [0.80%, 1.47%]). Two women (0.05%, 95% CI [0.006%, 0.18%]) carried an allele in the PM range, with genotypes of 29/57 and 41/55, respectively. These two cases warranted careful consideration. The first case involved a primigravida at 14 weeks of gestation, who carried an *FMR1* allele with 55 CGG repeats and presented with an ovarian cyst; however, no immediate medical intervention was required. The second case involved a twin pregnancy at 13 weeks of gestation, with a reported family history of a male cousin with intellectual disability. Notably, this participant could not provide detailed clinical information regarding this relative at the time of the visits, primarily due to the geographical distance and limited contact, rather than any limitation in her own cognitive functioning. Both cases lacked additional clinical features suggestive of *FMR1*-related disorders. Both participants received comprehensive genetic counseling regarding the potential for allele expansion into a full mutation in the current pregnancy and future offspring, as well as the long-term health risks associated with *FMR1* PM carrier status. Following this counseling, both women made an informed decision to proceed with routine prenatal monitoring, deferring any further invasive diagnostic or intervention.

2. Characteristics and distribution of *FMR1* alleles

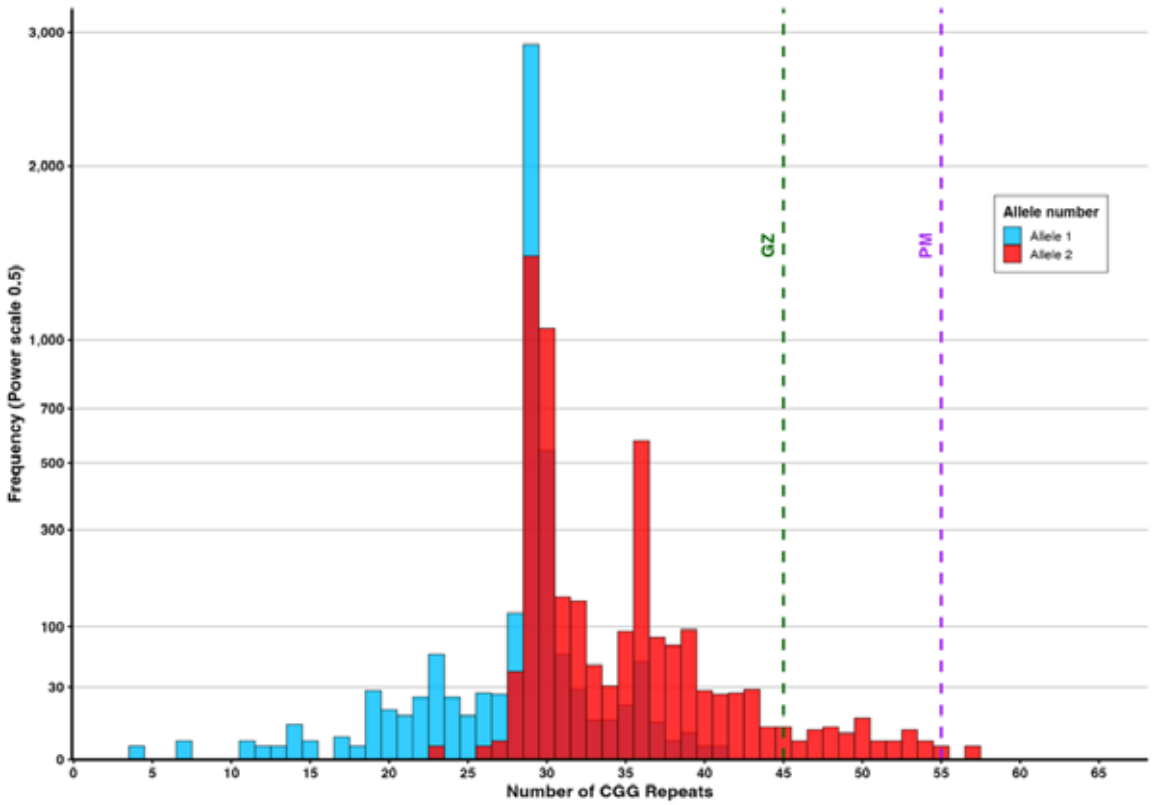

Figure 2. Spectrum of *FMR1* CGG repeat lengths

Distribution of CGG repeat lengths for Allele 1 (light blue) and Allele 2 (light red). The x-axis represents the number of CGG repeats, and the y-axis shows the frequency of each allele on a power scale (0.5) to enhance visibility across varying ranges.

Within our cohort, the most common allele is 29 CGG repeats, followed by 30, and one minor allele of 36 CGG repeats. These findings are consistent with previous reports from other Asian populations, including Taiwan, Thailand, Indonesia, and China. These values differ slightly from those reported in Japan, where the most frequent alleles are 28 and 29 CGG repeats, and in India, where the predominant values are 29 and 28. Meanwhile, the 30 CGGs allele remains dominant across most populations in the United States and Europe.[6,14-17]

3. Correlation analysis of the *FMR1* allele and *FMR1*-related features

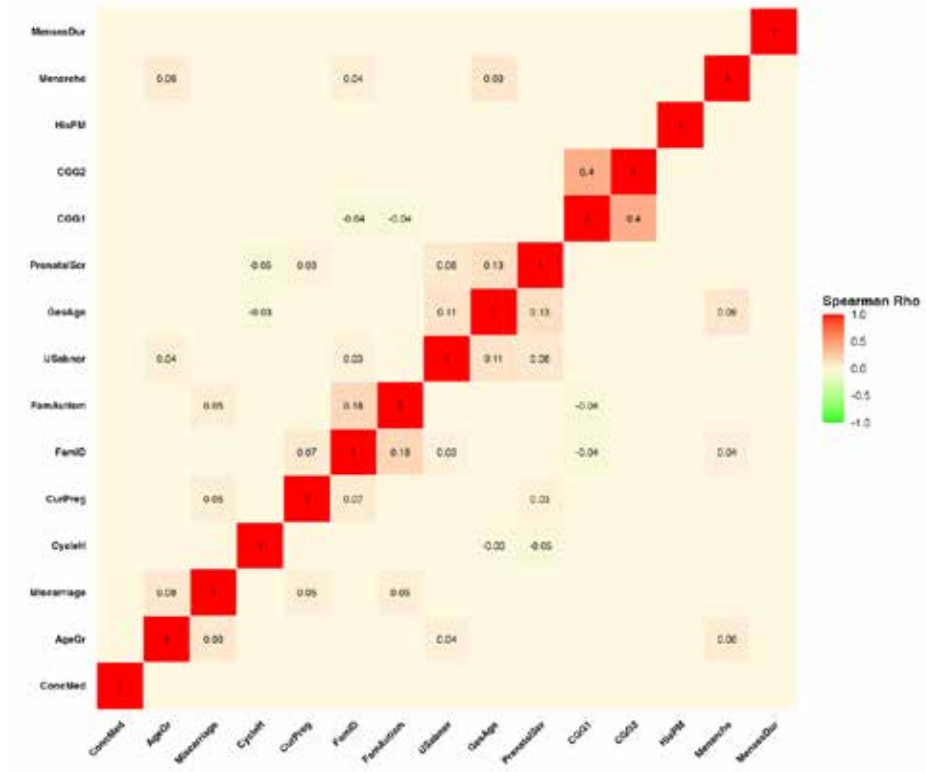

Figure 3. Spearman correlation matrix of clinical characteristics and *FMR1* alleles

The heatmap displays pairwise correlation coefficients between clinical parameters and FMR1 alleles.

Abbreviations and legends: CGG1, Allele 1; CGG2, Allele 2; AgeGr, Age group (categorized as <35 and ≥35 years); MensesDur, Menses duration (≤4 vs. >4 days); Menarche, Age at menarche; HisPM, History of premutation; PrenatalScr, Prenatal screening (risk status based on maternal serum screening/NIPT result; GesAge, Gestational age; USabnor, Ultrasound abnormality; FamAutism, Family history of ASD; FamID, Family history of intellectual disability; CurPreg, Current pregnancy symptoms; CycleH, Menstrual cycle regularity; ConcMed, Conception method (natural/assisted reproductive technology).

While the scarcity of PM carriers and the absence of FM cases limited our findings, we strived to conduct a thorough analysis to correlate allele variations with *FMR1*-related characteristics, seeking to uncover potential population-specific nuances among the Vietnamese population. Overall, most pairwise correlations were negligible, with only two specific associations emerging as statistically noteworthy.

Family history of intellectual developmental delay and family history of autism correlate at a weak borderline level ($\rho = 0.18$, $p = 3.0084 \times 10^{-31}$). Although the sample size is limited, this can also be observed in many neuropsychiatric disorders. Etyemez et al. (2022) estimated that over 70% of children with ASD have other neuropsychiatric comorbidities.[18]

The strongest correlation was observed between the repeat lengths of the two alleles

(moderate, ρ = 0.4, p-value = 1.3985 x 10^{-151}). This pattern can be explained by two primary factors. First, it arises from the size-based sorting convention used in the analysis, whereby the shorter allele in each sample is designated as allele 1 and the longer as allele 2 (2,465 of 4,000 samples, 61.6%). Second, it reflects the specific genetic architecture of the Vietnamese population. This may occur due to population stratification in a group of individuals in a certain geographical area, or a potential biological interaction between alleles due to instability. These unstable transmissions can occur even in alleles classified as "normal" due to the distinctive features of the tandem repeats.[9,19,20] To determine whether this index represents a methodological artifact or a genuine biological correlation, we performed a randomized pairing assessment by decoupling the alleles from their size-assigned designations, specifically by randomly re-pairing the entire pool of alleles regardless of their lengths. By removing constraints imposed by the conventional sorting method, we recalculated the correlation coefficient for each permutation to establish a null distribution of the index.

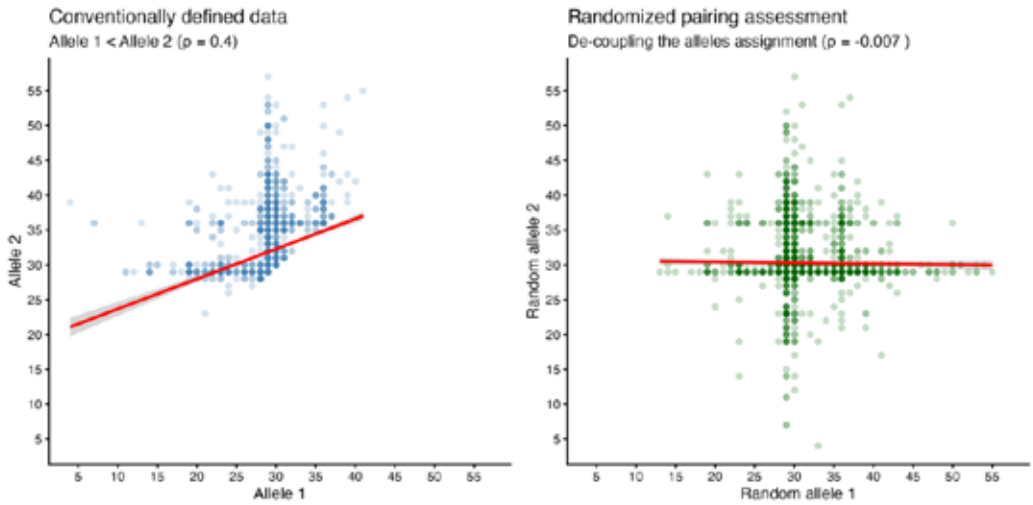

Figure 4. Pairing assessment of *FMR1* alleles

(Left) Conventionally defined pairing based on size (ρ = 0.4). (Right) Randomized pairing assessment demonstrating the effect of de-coupling allele assignment (representative iteration ρ = -0.007 falling within the 95% CI of the null distribution).

A permutation test was conducted with 10,000 iterations, which allowed us to assess the null distribution of the correlation coefficient. The horizontal orientation of the regression line (slope ≈ 0) in the randomized plot visually demonstrates the absence of a linear relationship between the alleles. This trend also confirms that the pairing is statistically independent (ρ mean = -0.0002 ± 0.0158, 95% CI [-0.0412, 0.0408], p-value = 0.4994), indicating that the association between the two alleles in the original dataset is a statistical artifact resulting from the ordering bias. Furthermore, this confirms that there is no latent biological linkage between these alleles in the Vietnamese cohort and suggests a stable gene pool maintained by random mating without evidence of significant selective pressure or population stratification.

4. Comparing findings between study groups

As outlined in the study methods, for this objective, we compared the distribution of allele categories across two risk groups with different clinical predictions. The study cohort comprised 3,959 (98.98%) low-risk and 41 (1.02%) high-risk defined pregnant women.

Table 2. *FMR1* allele distribution across clinical risk groups

Alleles category		Risk groups		p-value
		Low-risk group N = 3959	High-risk group N = 41	
Normal	n	3914	40	**0.03***
Grey zone	n	44	0	
Premutation	n	1	1	

Note: Fisher-Freeman-Halton exact test*

The Fisher-Freeman-Halton exact test indicated a statistically significant association between the distribution of *FMR1* allele categories and the risk classification based on family history (p-value = 0.03). This finding is in line with the well-established role of fragile X syndrome as a leading inherited cause of intellectual disability and ASD, particularly in males, and supports the biological plausibility of an association between *FMR1* allele status and clinical risk stratification. However, the result should be interpreted with caution. The observed statistical significance was driven by extremely sparse data, with very low expected cell counts and only a small number of premutation alleles detected in the Vietnamese cohort. Such data sparsity may inflate the apparent strength of association and limit the robustness of the exact test results. Therefore, this association should be considered exploratory rather than confirmatory, and further studies with larger cohorts are warranted to validate these findings and to more accurately characterize the relationship between *FMR1* allele distribution and clinically defined risk groups.

5. Cost-Effectiveness Analysis

We address two main objectives: (1) evaluating the effectiveness of three screening approaches, and (2) establishing a predictive framework for universal screening consistent with ACMG guidelines, contingent upon additional evidence of its feasibility in the future.

Regarding the first objective, with a PM prevalence of 0.05%, we evaluated the cost-benefit ratio of targeted versus universal screening, and given such a low observed prevalence, we also assessed whether it is necessary to implement screening for *FMR1* in Vietnam. In this model, the lifetime care cost was fixed at $320,000, while the price per test was adjusted in increments from $60 down to $10. This range accounts for the variable costs associated with utilizing commercial kits versus the potential development of in-house high-throughput assays at a significantly lower cost. When utilizing commercial kits (price at $60 per test), targeted screening proved more cost-effective than no screening, whereas universal screening exhibited the lowest economic efficiency, with average costs per individual of $11.85, $18.08, and $64.39, respectively. As testing costs decrease, the cost per individual remains constant for the no screening approach, while targeted screening shows a marginal decline. In contrast, universal screening benefits most significantly from price

reductions. In conclusion, targeted screening consistently remains the most cost-effective strategy, and it is only when the price per test drops to $13 that universal screening surpasses the efficiency of no screening, with costs per individual of $11.37 (targeted), $17.39 (universal), and $18.08 (no screening).

To address the second objective, a threshold analysis was performed using a dynamic framework of testing prices and lifetime expenses. This allowed us to assess the Net Monetary Benefit for universal screening across a range of permissible economic scenarios. With the price per test established as described above, the lifetime care cost was varied from $320,000 to $2,800,000. The minimum lifetime cost of $320,000 was established based on our estimates, which categorize the economic burden of each affected individual into three primary sources: lost productivity due to intellectual disability in FXS patients, opportunity costs for caregivers, and increased expenditures for special education. However, we hypothesize that the latter two factors are most substantial during the early stages of life and may gradually diminish as patients reach their early twenties. Beyond this milestone, many patients are expected to achieve a degree of partial independence in their daily lives. The maximum lifetime cost of $2,800,000 was derived from the study by Zhao et al. (2024), which evaluated the societal-scale economic impact of ASD with ID.[21] To ensure the applicability of these findings to the Vietnamese context, we adjusted the original estimates using Purchasing Power Parity conversion factors based on 2020 economic data.

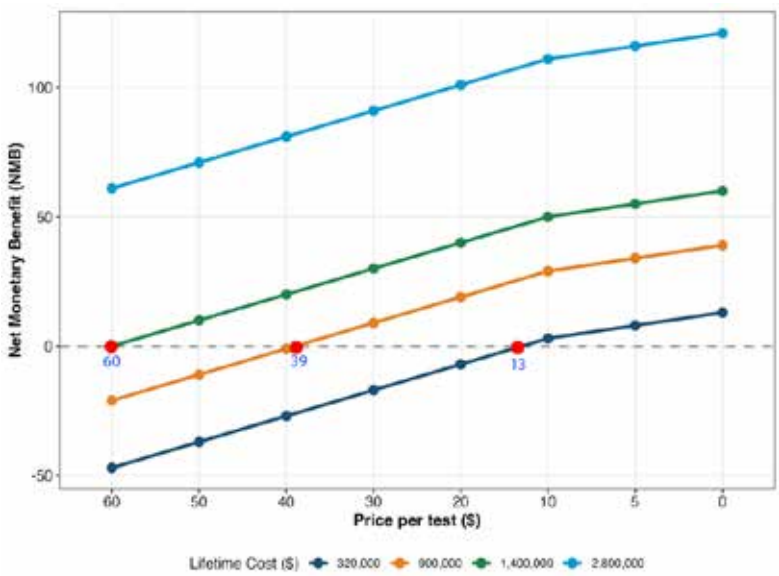

Figure 5. Two-way sensitivity analysis of price per test and lifetime costs on the Net Monetary Benefit of universal screening for *FMR1*

The intersections of the lifetime cost curves with the y=0 axis identify the specific break-even thresholds for each testing price. Lifetime costs of $320,000, $900,000, and $1,400,000 reach economic equilibrium at price-per-test values of $13, $39, and $60, respectively. The model also demonstrates that if the lifetime burden is exceptionally high ($2,800,000), universal screening remains economically advantageous even when the commercial kit price significantly exceeds the current $60 benchmark.

IV. DISCUSSION

FMR1 is one of the genes with extensive research evidence and is recommended for screening by both ACMG (the American College of Medical Genetics and Genomics) and ACOG (the American College of Obstetricians and Gynecologists).[5,22] To the best of our knowledge, this study represents the most extensive epidemiological investigation to date concerning the prevalence and distribution of *FMR1* gene alleles within the Vietnamese pregnant population. In our cohort, the prevalence of PM carriers was notably low (n = 2; 0.05%; 95% CI [0.006%, 0.18%]), and it is worth highlighting that both identified cases originated from the Northern region. However, even when considering this geographic clustering, our findings continue to underscore a notably lower frequency of *FMR1* PM compared to both regional and global epidemiological datasets. Given our large-scale sampling across diverse geographical regions, these findings may demonstrate a remarkable stability in the Vietnamese *FMR1* genetic profile. This observation potentially serves as a favorable indicator for the mental health and socio-economic well-being of the population in Vietnam.

However, we observed that the frequency of intermediate alleles in our cohort (44/4,000; 1.1%; 95% CI [0.80%, 1.47%]) was markedly higher than values reported in previous studies from some other Asian populations. While generally asymptomatic, these alleles are mildly unstable and can expand upon transmission to offspring, presenting a low-risk but long-term genetic consideration for society.[9,23] This finding further emphasizes the critical importance of maintaining a well-regulated screening strategy for this gene to prevent any clinical oversight.

Despite the valuable insights and efforts provided by this study, we must acknowledge that there are still certain limitations. The modest sample size and localized nature of the research sites may limit the generalizability of our findings to the broader Vietnamese pregnant population, particularly given the diverse ethnic composition across different regions of the country. It should also be emphasized that a comprehensive and complete classification for each allele needs to include both the detection of adenine-guanine-guanine (AGG) triplet interruptions as well as the methylation status, which is not provided in our testing kit.[6,14,19]

Based on our findings, we propose the following specific screening strategies tailored to the epidemiological landscape of *FMR1* in Vietnam:

We advocate for a dual-track strategy encompassing both pilot community-based screening and targeted carrier screening for *FMR1*-related conditions. Assays should also integrate the identification of AGG triplet interruptions to fully investigate the characteristics of the *FMR1* alleles in each population.[14,19,20,24] Expanding current screening initiatives is both timely and essential to establish a more granular understanding of the *FMR1* landscape within the Vietnamese population.

We emphasize the role of targeted screening as a strategic priority. This approach is not intended to contradict the 2021 ACMG guidelines, which recommend universal screening for all pre-conception and pregnancy cases. Rather, our proposal is a context-specific adaptation that reflects a careful consideration of the unique genetic architecture, current epidemiological landscape, and available healthcare resources within Vietnam. Prioritizing carrier screening for diseases with higher

prevalence in *FMR1* low-risk individuals reflects an appropriate clinical strategy. This approach aligns with the overarching goals of the ACMG guidelines while ensuring both evidence-based and economically sustainable within our local healthcare framework.[5]

The identification of a substantial number of GZ carriers in our cohort underscores the necessity of well-regulated surveillance, even for alleles below the formal premutation threshold. We propose conducting large-scale screening initiatives periodically (e.g., every 20 years) to re-evaluate the population's genetic structure, given the potential instability of intermediate alleles.[19,25,26]

Regarding the optimal timing for *FMR1* population-based screening, we currently do not recommend integrating *FMR1* into the newborn screening or universal national mandates. Instead, implementing pilot carrier screening for women of reproductive age represents a more feasible strategy for population-based prevention. Furthermore, although there have been significant advances in prenatal diagnosis, we believe that preconception carrier screening remains preferable to screening during pregnancy, as it broadens reproductive options, including adoption, gamete donation, and preimplantation genetic diagnosis.[27-29]

V. CONCLUSION

Notwithstanding limitations in sample size and economic variables, this study offers a pioneering perspective on establishing an evidence-based *FMR1* carrier screening framework for the Vietnamese population. As the first study to provide comprehensive insights into the distribution of *FMR1* alleles within the Vietnamese pregnant population, our findings contribute to elucidating the clinical and societal burdens associated with *FMR1*-related disorders. We maintain that a successful screening strategy must maximize carrier identification while remaining cost-effective and ensuring social equity. As genetic disorders gain increasing prominence in public health, we underscore that health equity stems from personalized care rather than uniform provision. Therefore, personalized management should be prioritized, with clinicians adapting their care strategies based on institutional resource availability and individual patient profiles. Ultimately, integrating these findings into national health policy will be crucial for alleviating the long-term socio-economic burden and improving the quality of life for individuals at risk of genetic disorders.

ACKNOWLEDGEMENTS

The research data belong to the State-level science and technology project: *"Screening and Etiologic Diagnosis of Genetic Mental Retardation in the Vietnamese people"* (Project code: ĐTĐL.CN-80/22). The authors would like to express our sincere gratitude to the Ministry of Science and Technology for financially supporting this project.

We are also grateful to the medical staff at all participating hospital sites for their invaluable support in sample collection and data acquisition, which were instrumental to the successful completion of this research.

DISCLOSURE

The authors declare no competing interests.

DATA AVAILABILITY

The data are not publicly available due to privacy and ethical restrictions.

REFERENCE

1. Malecki C, Hambly BD, Jeremy RW, et al. The RNA-binding fragile-X mental retardation protein and its role beyond the brain. *Biophys Rev*. Aug 2020; 12(4): 903-916.

2. Ciaccio C, Fontana L, Milani D, et al. Fragile X syndrome: a review of clinical and molecular diagnoses. *Ital J Pediatr*. Apr 19 2017; 43(1): 39.

3. Biancalana V, Glaeser D, McQuaid S, et al. EMQN best practice guidelines for the molecular genetic testing and reporting of fragile X syndrome and other fragile X-associated disorders. *Eur J Hum Genet*. Apr 2015; 23(4): 417-25.

4. Macpherson J, Sharif A. Practice guidelines for molecular diagnosis of Fragile X Syndrome. *Association for Clinical Genetic Science* 2014;

5. Gregg AR, Aarabi M, Klugman S, et al. Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. Oct 2021; 23(10): 1793-1806.

6. Spector E, Behlmann A, Kronquist K, et al. Laboratory testing for fragile X, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. May 2021; 23(5): 799-812.

7. Tempio A, Boulksibat A, Bardoni B, et al. Fragile X Syndrome as an interneuronopathy: a lesson for future studies and treatments. *Front Neurosci*. 2023; 17: 1171895.

8. Hunter J, Rivero-Arias O, Angelov A, et al. Epidemiology of fragile X syndrome: a systematic review and meta-analysis. *Am J Med Genet A*. Jul 2014; 164a(7): 1648-58.

9. Hall DA. In the Gray Zone in the Fragile X Gene: What are the Key Unanswered Clinical and Biological Questions? *Tremor Other Hyperkinet Mov (N Y)*. 2014; 4: 208.

10. Genereux DP, Laird CD. Why do fragile X carrier frequencies differ between Asian and non-Asian populations? *Genes & Genetic Systems*. 2013; 88(3): 211-224.

11. Musci TJ, Caughey AB. Cost-effectiveness analysis of prenatal population-based fragile X carrier screening. *Am J Obstet Gynecol*. Jun 2005; 192(6): 1905-12; discussion 1912-5.

12. Toledano-Alhadef H, Basel-Vanagaite L, Magal N, et al. Fragile-X carrier screening and the prevalence of premutation and full-mutation carriers in Israel. *Am J Hum Genet*. Aug 2001; 69(2): 351-60.

13. Guo Q, Chang YY, Huang CH, et al. Population-based carrier screening and prenatal diagnosis of fragile X syndrome in East Asian populations. *J Genet Genomics*. Dec 2021; 48(12): 1104-1110.

14. Chiu HH, Tseng YT, Hsiao HP, et al. The AGG interruption pattern within the CGG repeat of the FMR1 gene among Taiwanese population. *J Genet*. Dec 2008; 87(3): 275-7.

15. Hnoonual A, Jankittunpaiboon C, Limprasert P. Screening for FMR1 CGG Repeat Expansion in Thai Patients with Autism Spectrum Disorder. *Biomed Res Int*. 2021; 2021:4359308.

16. Hung C-C, Lee C-N, Wang Y-C, et al. Fragile X syndrome carrier screening in pregnant women in Chinese Han population. *Scientific Reports*. 2019/10/29 2019; 9(1):15456.

17. Faradz SM, Pattiiha MZ, Leigh DA, et al. Genetic diversity at the FMR1 locus in the Indonesian population. *Ann Hum Genet*. Jul 2000; 64(Pt 4): 329-39.

18. Etyemez S, Esler A, Kini A, et al. The role of intellectual disability with autism spectrum disorder and the documented cooccurring conditions: A population-based study. *Autism Res*. Dec 2022; 15(12): 2399-2408.

19. Nolin SL, Glicksman A, Tortora N, et al. Expansions and contractions of the FMR1

CGG repeat in 5,508 transmissions of normal, intermediate, and premutation alleles. *Am J Med Genet A*. Jul 2019; 179(7): 1148-1156.

20. Limprasert P, Thanakitgosate J, Jaruthamsophon K, et al. Unique AGG Interruption in the CGG Repeats of the FMR1 Gene Exclusively Found in Asians Linked to a Specific SNP Haplotype. *Genet Res Int*. 2016; 2016: 8319287.

21. Zhao Y, Lu F, Wang X, et al. The economic burden of autism spectrum disorder with and without intellectual disability in China: A nationwide cost-of-illness study. *Asian J Psychiatr*. Feb 2024; 92:103877.

22. Committee Opinion No. 691: Carrier Screening for Genetic Conditions. *Obstetrics & Gynecology*. 2017; 129(3): e41-e55.

23. Zuñiga A, Juan J, Mila M, et al. Expansion of an intermediate allele of the FMR1 gene in only two generations. *Clin Genet*. Nov 2005; 68(5): 471-3.

24. Domniz N, Ries-Levavi L, Cohen Y, et al. Absence of AGG Interruptions Is a Risk Factor for Full Mutation Expansion Among Israeli FMR1 Premutation Carriers. *Front Genet*. 2018; 9:606.

25. Horn R, Kerasidou A, Merchant J. The value of large-scale programmes in human genomics. *Eur J Hum Genet*. May 2025; 33(5): 563-569.

26. Alarcón Garavito GA, Moniz T, Déom N, et al. The implementation of large-scale genomic screening or diagnostic programmes: A rapid evidence review. *European Journal of Human Genetics*. 2023/03/01 2023; 31(3): 282-295.

27. González-Teshima LY, Payán-Gómez C, Saldarriaga W. Fragile X Syndrome Secondary to in Vitro Fertilization With a Family Egg Donor: A Case Report and Review of the Literature. *J Family Reprod Health*. Jun 2021; 15(2): 130-135.

28. Wittenberger MD, Hagerman RJ, Sherman SL, et al. The FMR1 premutation and reproduction. *Fertil Steril*. Mar 2007; 87(3): 456-65.

29. Noto V, Harrity C, Walsh D, et al. The impact of FMR1 gene mutations on human reproduction and development: a systematic review. *J Assist Reprod Genet*. Sep 2016; 33(9): 1135-47.