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Characterization of *FMR1* CGG Repeat Structure and AGG Interruption Patterns in Turkish and Syrian Individuals

Türk ve Suriyeli Bireylerde *FMR1* CGG Tekrar Yapısı ve AGG Kesinti Paternlerinin Karakterizasyonu

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ABSTRACT

Objective: This study aimed to characterize *FMR1* CGG repeat length, AGG interruption number and patterns, and uninterrupted CGG tract architecture in Turkish and Syrian individuals referred for *FMR1*-associated phenotypes and evaluate their implications for repeat instability.

Methods: This retrospective study analysed 513 alleles from 351 unrelated individuals (162 females, 189 males), including 476 Turkish and 37 Syrian alleles. CGG repeat length, AGG interruption count and interspersion patterns, and the length and positional distribution of uninterrupted CGG tracts were assessed. Allele classifications and family transmission data were evaluated when available.

Results: Alleles with two AGG interruptions were the most prevalent configuration in both cohorts (70% in Turkish and 58% in Syrian alleles). In the Turkish cohort, 117 patterns were observed, including 56 population-specific variants; in the Syrian cohort, 23 distinct patterns were detected, of which seven were unique. The most common CGG repeat size was 30 in both cohorts. The most frequent AGG interspersion patterns were (CGG)9-AGG-(CGG)9-AGG-(CGG)10 and (CGG)9-AGG-(CGG)9-AGG-(CGG)9. Among 53 normal alleles with uninterrupted CGG tracts of >20 repeats, 94.3% were located at the 5′ end and were significantly associated with a positive family history ($p = 0.004$). All alleles that expanded to full mutations lacked AGG interruptions.

CONCLUSION: These findings demonstrate marked variability in *FMR1*

ÖZ

Amaç: Bu çalışmanın amacı, *FMR1* ile ilişkili fenotipler nedeniyle yönlendirilen Türk ve Suriyeli bireylerde *FMR1* CGG tekrar uzunluğunu, AGG kesinti sayısı ve paternlerini ve kesintisiz CGG tekrar bölgelerinin mimarisini karakterize etmek ve bu özelliklerin tekrar instabilitesi üzerindeki etkilerini değerlendirmektir.

Yöntemler: Bu retrospektif çalışmada, 351 akraba olmayan bireye (162 kadın, 189 erkek) ait toplam 513 alel analiz edildi; bunların 476'sı Türk, 37'si Suriyeli bireylere aitti. CGG tekrar uzunluğu, AGG kesinti sayısı ve dağılım paternleri ile kesintisiz CGG tekrar bölgelerinin uzunluğu ve pozisyonel dağılımı değerlendirildi. Uygun olan olgularda alel sınıflandırmaları ve aile içi geçiş verileri incelendi.

Bulgular: İki AGG kesintisi içeren aleller her iki kohortta da en sık görülen yapıydı (Türk alellerinde %70, Suriyeli alellerinde %58). Türk kohortunda 56'sı popülasyona özgü olmak üzere toplam 117 patern saptanırken, Suriyeli kohortta yedisi özgün olmak üzere 23 farklı patern belirlendi. En sık gözlenen CGG tekrar sayısı her iki kohortta da 30 idi. En yaygın AGG dağılım paternleri (CGG)9-AGG-(CGG)9-AGG-(CGG)10 ve (CGG)9-AGG-(CGG)9-AGG-(CGG)9 olarak saptandı. Yirmiden fazla kesintisiz CGG tekrarına sahip 53 normal alelin %94,3'ünde bu bölgelerin 5′ uçta yer aldığı ve pozitif aile öyküsü ile anlamlı ilişkili olduğu gösterildi ($p = 0,004$). Tam mutasyona genişleyen tüm alellerin AGG kesintisi içermediği belirlendi.

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ABSTRACT

CGG repeat length and AGG interruption architecture in Turkish and Syrian populations, although the small sample size of the Syrian cohort warrants cautious interpretation. The data further suggest that repeat instability is associated not only with repeat number but also with repeat structure and positional context. Incorporating AGG interruption analysis into routine *FMR1* testing may improve genetic counseling and risk stratification.

Keywords: AGG interruption, *FMR1* gene, genetic variation, population genetics, trinucleotide repeat expansion, uninterrupted CGG tract

ÖZ

Sonuç: Bu bulgular, Türk ve Suriyeli popülasyonlarda *FMR1* CGG tekrar uzunluğu ve AGG kesinti mimarisinin belirgin değişkenlik gösterdiğini ortaya koymaktadır; ancak Suriye kohortunun küçük örneklem büyüklüğü, bu bulguların dikkatli yorumlanmasını gerektirmektedir. Veriler ayrıca tekrar instabilitesinin yalnızca tekrar sayısından değil, aynı zamanda tekrar yapısı ve pozisyonel bağlamdan da etkilendiğini düşündürmektedir. Rutin *FMR1* testlerine AGG kesinti analizinin eklenmesi, özellikle ailesel ve kuşaklar arası değerlendirmelerde genetik danışmanlık ve risk sınıflandırmasını iyileştirebilir.

Anahtar Sözcükler: AGG kesintisi, *FMR1* geni, genetik değişkenlik, popülasyon genetiği, trinükleotid tekrar genişlemesi, kesintisiz CGG dizisi

INTRODUCTION

Fragile X syndrome is the leading inherited cause of intellectual disability and a primary monogenic contributor to autism spectrum disorder. This condition arises from the expansion of a CGG trinucleotide repeat within the 5' untranslated region of the *FMR1* gene on the X chromosome (1). Alleles harboring ≥200 CGG repeats typically exhibit hypermethylation and transcriptional silencing of the *FMR1* gene, leading to a deficiency in fragile X mental retardation protein and the characteristic FXS phenotype. Whereas premutation alleles are associated with diverse *FMR1*-associated disorders, including fragile X–associated primary ovarian insufficiency and fragile X–associated tremor/ataxia syndrome (2).

While the total CGG repeat length constitutes the cornerstone of *FMR1* allele classification, the repeat tract architecture—particularly the presence and distribution of AGG interruptions within the CGG array—exerts a pivotal influence on repeat stability (3,4). AGG interruptions are hypothesized to stabilize the CGG repeat tract by interrupting extended runs of pure CGG repeats, thereby preventing DNA slippage during replication and reducing the risk of expansion (3-5). Normal *FMR1* alleles typically contain two or three AGG interruptions, whereas premutation and full mutation alleles frequently exhibit fewer interruptions or are entirely devoid of AGGs (4,6,7).

Beyond the number of AGG interruptions, recent studies suggest that the spatial arrangement of uninterrupted CGG tracts exerts a substantial influence on *FMR1* repeat instability. In intermediate and premutation alleles, AGG interruptions tend to cluster toward the 5' end of the repeat tract, positioning the longest contiguous CGG stretch at the 3' end (8,9). Both the length and the position of these uninterrupted CGG tracts have been associated with an increased risk of repeat expansion, underscoring the importance of evaluating repeat tract architecture in addition to total CGG repeat length (5,6,8).

Population studies have identified significant geographic and ethnic differences in CGG repeat length distributions and AGG interruption patterns (5,10-12). Although the most common normal *FMR1* alleles worldwide typically comprise 29–30 CGG repeats, the relative frequencies of specific AGG interruption patterns —e.g., $(CGG)_9$-AGG-$(CGG)_9$-AGG-$(CGG)_9$, $(CGG)_{10}$-AGG-$(CGG)_9$-AGG-$(CGG)_9$—differ considerably between populations, owing to variations in evolutionary history, and population admixture (4,12). Despite the expanding body of literature on this topic, data from Türkiye remain sparse, and in-depth analyses of AGG interruption numbers and configurations are lacking. Furthermore, AGG interruption profiles have not yet been documented in Syrian populations.

Accordingly, this study sought *to* characterize thoroughly FMR1 CGG repeat lengths, AGG interruption counts, and detailed interruption patterns among Turkish and Syrian individuals referred for FMR1-associated phenotypes. By examining 513 FMR1 alleles spanning the normal, intermediate, premutation, and full-mutation categories, this investigation aimed to characterize population-specific AGG interruption patterns, evaluate the distribution and positional characteristics of extended uninterrupted CGG tracts, and investigate the relationships between repeat architecture and family histories of FMR1-related phenotypes. This study contributes to a deeper understanding of FMR1 repeat instability and supports the incorporation of AGG-interruption profiling into genetic counseling and risk assessment.

MATERIALS AND METHODS

Study Population

This multicenter retrospective study evaluated CGG trinucleotide repeat lengths and AGG interruption patterns in 513 *FMR1* alleles obtained from patients who presented to the Medical Genetics Outpatient Clinics of Konya City Hospital and Uşak Training and Research Hospital between January 2021 and December 2024. Individuals were referred because of clinical suspicion of Fragile X syndrome or Fragile X–associated disorders, a positive family history, or both.

A total of 351 unrelated individuals (162 females and 189 males) with normal karyotypes were included. Clinical and family histories were obtained directly from participants or, when necessary, from their parents or legal guardians. Relevant laboratory data and ancillary clinical information were retrieved from existing medical records.

The study protocol, covering both participating centers, was approved by the Uşak University Non-Interventional Clinical Research Ethics Committee (decision number: 710-710-14, dated 12.06.2025). Informed consent was waived due to the retrospective design.

FMR1 Repeat and AGG Interruption Profiling

Genomic DNA was extracted from peripheral blood samples of all participants using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The extracted DNA underwent polymerase chain reaction (PCR) amplification with specific primers. PCR products were subsequently analyzed by fragment analysis on an ABI 3500 Genetic Analyzer System with the Adellgene Fragile X Screening Kit (BDR, Zaragoza, Spain). The resulting data were interpreted using Gene Mapper software. AGG interruption genotyping was performed to determine both the number of AGG interruptions and the precise CGG repeat configuration. An AGG interruption pattern was defined as the number of uninterrupted CGG repeats between consecutive AGG interruptions, ordered from the 5′ to the 3′ end of the FMR1 CGG repeat tract. In female subjects, when multiple patterns were observed, these were clarified by evaluating family studies when available.

The classification of *FMR1* CGG repeat sizes was performed based on established criteria from previous studies and guidelines from the National Center for Biotechnology Information (13). Alleles containing 5-44 CGG repeats were categorized as normal, 45-54 repeats as intermediate, 55-199 repeats as premutation, and ≥200 repeats as full mutation. The association between categorical variables, including the presence of long uninterrupted CGG tracts and family history status, was evaluated using the chi-square test.

Statistical Analysis

Statistical evaluation of the data was performed using descriptive statistics, including frequencies and percentages. To examine the relationships between categorical variables, Fisher's exact test was used for comparisons involving small sample sizes. All statistical analyses were carried out using SPSS version 27.0 (IBM Corp., Armonk, NY, USA), and a p-value of <0.05 was considered statistically significant.

RESULTS

A total of 513 *FMR1* alleles (n = 476 Turkish and n = 37 Syrian) were analyzed. Their classification and distribution by AGG count in both populations are presented in Supplementary Table 1. In the Turkish cohort, alleles were classified as normal (93.9%), intermediate (1.7%), premutation (1.7%), and full mutation (2.7%) alleles, whereas the Syrian cohort had only normal (97.3%) and intermediate (2.7%) alleles (Table 1). Forty-eight distinct repeat sizes (5 to >200 repeats) were detected in the Turkish cohort, whereas 16 distinct CGG repeat sizes (17–45 repeats) were identified in the Syrian cohort (Supplementary Table 1). In both cohorts, alleles with two AGG interruptions were the most prevalent configuration, accounting for 70% and 58% of alleles in the Turkish and Syrian cohorts, respectively. These were followed, in decreasing order of frequency, by alleles with one, zero, and three AGG interruptions. Notably, a configuration with four AGG interruptions was observed in a single allele exclusively within the Turkish cohort (Figure 1, Supplementary Table 2).

Analysis of FMR1 AGG-interruption patterns in 513 alleles revealed 125 distinct configurations, indicating a high level of allelic diversity (Supplementary Table 2). Of these, five patterns were exclusive to the combined Turkish and Syrian datasets and have not been reported in other populations (Table 2). In the Turkish cohort, 117 AGG interruption patterns were identified across 476 alleles, including 56 population-specific variants. In the Syrian cohort, 23 distinct patterns were observed among 37 alleles, seven of which were unique to this population (Table 2). Supplementary Table 2 presents the distribution of AGG interruption patterns according to allele classification.

Normal alleles were analyzed to assess the distributions of CGG repeat size and AGG configuration in both cohorts. The most common allele was the 30-CGG-repeat allele (34% in the Turkish cohort and 33% in the Syrian cohort), followed by the 29-CGG-repeat allele (28% and 19%, respectively) (Figure 2). In the Turkish cohort, the most common AGG interspersion pattern was $(CGG)_9$-AGG-$(CGG)_9$-AGG-$(CGG)_{10}$ (27.7%), followed by $(CGG)_9$-AGG-$(CGG)_9$-AGG-$(CGG)_9$ (25.1%), whereas in the Syrian cohort, these two patterns were observed with equal frequency (16.7% each) (Supplementary Table 2).

Normal alleles were also examined for uninterrupted CGG segments longer than 20. A total of 53 alleles met this criterion, including 18 pure CGG alleles and 35 alleles containing at least one AGG interruption (Supplementary Table 1). Among alleles with AGG interruptions, uninterrupted CGG tracts were predominantly located at the 5′ end of the *FMR1* gene (94.3%, 33/35), whereas only two alleles showed localization at the 3′ end.

Uninterrupted CGG repeats located at the 5′ end showed a significant association with family history status. While this pattern in normal alleles was observed in both family-history–positive and -negative individuals, all intermediate and premutation alleles with uninterrupted CGG repeats at the 5′ end were detected exclusively in individuals with a positive family history (n = 8 alleles; Fisher's exact test, p = 0.004) (Table 3).

In family screenings of 19 index cases, 57 individuals were analyzed. Among these alleles, those that expanded to full mutations ranged from 56 to 159 CGG repeats, and none contained AGG interruptions. No contractions in the total CGG repeat number were observed

Table 1. Comparative distribution of FMR1 alleles between Turkish and Syrian cohorts according to AGG interruptions and allele classification.

Number of alleles in Turkish patients					**AGG number**	**Number of alleles in Syrian patients**				
Normal	**Intermediate**	**Premutant**	**Mutant**	**Total**		**Total**	**Normal**	**Intermediate**	**Premutant**	**Mutant**
21	1	7	13	**42**	0	**4**	3	1	0	0
101	1	0	0	**102**	1	**11**	11	0	0	0
312	5	0	0	**317**	2	**21**	21	0	0	0
12	1	1	0	**14**	3	**1**	1	0	0	0
1	0	0	0	**1**	4	**0**	0	0	0	0
447	**8**	**8**	**13**	**476**		**37**	**36**	**1**	**0**	**0**

Table 2. Distribution of AGG interruption patterns according to allele classification in Turkish and Syrian populations.

Classification	AGG patterns	In Turkish cohorts	In Syrian cohorts	Shared patterns
Normal	**106**	**99**	**22**	**15**
Intermediate	**9**	**8**	**1**	**0**
Premutant	**7**	**7**	**0**	**0**
Mutant	**3**	**3**	**0**	**0**
Total	125	117	23	15

Table 3. Association between family history and uninterrupted CGG repeat patterns (>20 repeats) located at the 5′ end according to allele classification.

Uninterrupted CGG repeat pattern	Family history (+) number (%)	Family history (–) number (%)	Total
Normal alleles (5′ end)	14 (42.4)	19 (57.6)	33
Intermediate and Premutant alleles (5′ end)	8 (100.0)	0 (0.0)	8

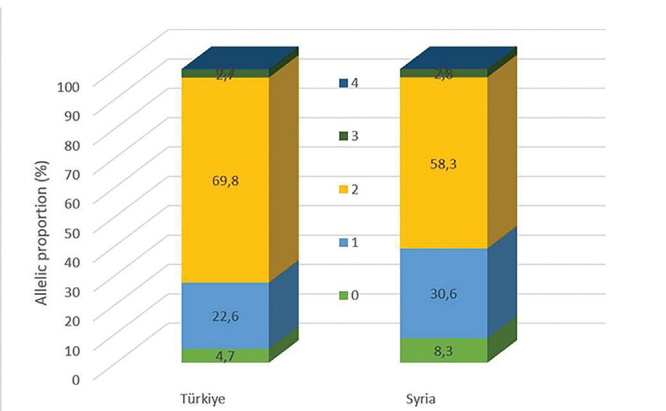

Figure 1. AGG interruption number distribution in *FMR1* normal alleles in Turkish and Syrian populations.

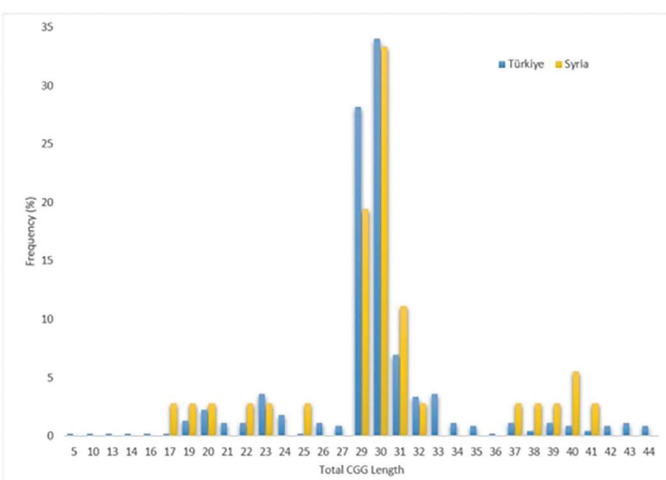

Figure 2. Distribution of *FMR1* CGG repeat lengths in normal alleles from Turkish and Syrian populations.

across generations. Alleles within the normal range containing AGG interruptions demonstrated stable transmission in both repeat size and structure across generations. However, a single exceptional transmission event was identified, in which an allele carrying one AGG interruption (29 + 9) was inherited in the subsequent generation as a pure 33 CGG allele lacking AGG interruptions (Supplementary Table 1).

DISCUSSION

Previous studies have reported on the total number of CGG repeats in the *FMR1* gene, as well as the number and patterns of AGG interruptions and their geographic and ethnic distributions (5,10-12). Although a limited number of studies from Türkiye have reported CGG repeat length distributions and *FMR1* mutation profiles (14-16), comprehensive analyses of AGG interruption number and patterning remain scarce. In addition, AGG interruption data specific to Syrian individuals have not yet been reported in the literature. To address this deficiency, 513 FMR1 alleles from Turkish and Syrian individuals were genotyped, and the total CGG length and the number and patterns of AGG interruptions were evaluated. The findings were compared with those reported in the literature for different populations.

The fact that the most common total CGG repeat length in the Turkish cohort is 30, followed by 29 mirrors the global pattern (5,11). However, it has been shown that the most common allele is 29 repeats in Indonesian, African American, Asian populations, and Malay, Borneo, and Tibetan communities (5,17,18). This consistency supports the evolutionary stability of FMR1 CGG repeat alleles within the common normal range across different populations.

Both the Turkish and Syrian cohorts showed a predominance of alleles carrying two AGG interruptions, followed by alleles with a single AGG interruption, a distribution that is consistent with previously reported population-based studies (5,11). Notably, the identification of an allele harboring four AGG interruptions in the Turkish cohort is of particular interest, as alleles with four AGG interruptions have been reported only in a limited number of populations, including Australia, the United Arab Emirates, Indonesia, and Spain (5). Further studies incorporating comprehensive characterization of such rare AGG patterns in global databases will be required to determine whether these alleles reflect independent mutational events or a shared ancestral origin. In our cohort, 55% (n = 5) of the intermediate alleles carried two AGG interruptions, whereas the vast majority of premutation alleles (87.5%, n = 7) and all full mutation alleles (n = 13) lacked AGG interruptions. These observations align with the established literature indicating that premutation alleles typically harbor zero to two AGG interruptions, while normal alleles most commonly contain two to three AGG interruptions (4,7). Pure CGG repeats lacking AGG interruptions are generally considered uncommon among normal *FMR1* alleles (10,19). Nevertheless, in our cohort, such alleles were identified in 4.7% of cases (n = 21), indicating that fully uninterrupted CGG tracts may not be as exceptional as previously assumed, at least within certain populations or ascertainment contexts. Although these alleles fall within the normal repeat size range, their structural configuration is noteworthy, given the well-established stabilizing role of AGG interruptions. The presence of a measurable proportion of pure alleles in the normal range may, therefore, represent a latent source of repeat instability, potentially predisposing these alleles to expansion upon intergenerational transmission. This observation underscores the importance of considering not only CGG repeat length but also repeat architecture when evaluating *FMR1* allele stability.

The 125 different AGG interruption patterns identified in this study highlight the high mutational and structural variability of the *FMR1* CGG repeat region in Turkish and Syrian cohorts. Notably, 56 of the 117 AGG interruption patterns observed in the Turkish cohort were exclusive to this population, suggesting a highly heterogeneous genetic structure. Türkiye's historical role as a geographical bridge between Asia and Europe and substantial migration from diverse regions can be considered among the possible reasons for this diversity. The presence of shared AGG configurations between Turkish and Syrian individuals points to a partial overlap in ancestral or regional genetic backgrounds, while distinct population-specific signatures are retained. The limited sample size of the Syrian cohort represents an important constraint in the interpretation of these findings. Nonetheless, the most frequent total CGG repeat lengths observed (29 and 30 repeats) were consistent with existing literature, and the identification of seven population-specific AGG-interruption patterns among a total of 23 distinct patterns suggests the presence of cohort-specific AGG architectures despite the smaller sample size.

With respect to AGG interruption patterns, the Turkish cohort most frequently exhibited the 9 + 9 + 10 pattern, followed closely by the 9 + 9 + 9 pattern. In contrast, most studies report 10 + 9 + 9 as the predominant AGG pattern (17%–53% across studies), with 9 + 9 + 9 ranking second (1–32% across studies) (5,11). However, the opposite of this ordering of patterns has been documented in several populations, including Indonesian (35%–21%), African American (19%–18%), Asian (61%–9%), Malay, Borneo, and Tibetan groups (48%–29%) (17,18). Notably, in African Americans, the 9 + 9 + 9 pattern has been reported at only 1% higher frequency than the 10 + 9 + 9 pattern, highlighting that relatively small frequency shifts can distinguish population-specific AGG architectures (17,18). In the Syrian cohort, the 9 + 9 + 10 and 9 + 9 + 9 AGG interruption patterns were observed at equal frequencies. While this finding should be interpreted cautiously due to the limited sample size, it may suggest a balanced distribution of prevalent AGG configurations in this population. The 9 + 9 + 10 pattern has been reported at its highest relative frequency in African populations (9%), followed by African Americans (4%) and Caucasian and Italian populations (3%) (5,11). However, these proportions remain low in absolute terms, indicating that this AGG configuration is not dominant in any population. The relatively higher frequency of the 9 + 9 + 10 pattern in the Turkish cohort suggests that this AGG configuration is a prominent feature of this population and may reflect regional trends rather than being strictly population-specific. In contrast to most populations in which the 10 + 9 + 9 pattern is reported as the predominant AGG configuration, this pattern was observed in only three alleles (<1%) in the Turkish cohort and was not detected in the Syrian cohort. Following the two most frequent patterns, additional AGG interruption configurations were observed among normal alleles, including 9 + 13 (2.7%), 9 + 10 (2.2%), 19 + 9 (2.2%), and 9 + 10 + 9 (2.2%). Notably, these configurations have been reported in other populations worldwide at comparable frequencies. Specifically, the 9 + 13 pattern has been most frequently reported in Indonesian (3%) and Asian (2%) populations; the 9 + 10 pattern in Basque (2%) and Indian (2%) populations; the 19 + 9 pattern in Guatemalan (3%) and African American (2%) populations; and the 9 + 10 + 9 pattern in Emirati (7%) and Jewish Arabic (4%) populations (5,10,12,20). The presence of these shared patterns across geographically and ethnically distinct populations suggests that certain AGG configurations may be evolutionarily conserved, potentially due to their stabilizing effect on the CGG repeat tract.

Most studies to date investigating AGG interruption patterns in the *FMR1* gene have focused primarily on describing AGG distributions in normal alleles (5,21). In contrast, studies addressing premutation alleles have generally focused on total CGG repeat length and/or AGG interruption number, examining their association with clinical outcomes such as primary ovarian insufficiency, as well as with *FMR1* gene stability (2,6,9,22). However, systematic analyses of AGG interruption patterns specifically within premutation alleles remain scarce (5,10,11). In this context, our study extends the existing literature by characterizing AGG patterns and interruption numbers not only in normal alleles but also in intermediate, premutation, and full mutation alleles (Supplementary Tables 1 and 2), thereby providing a more comprehensive view of AGG architecture across the full spectrum of FMR1 allele classes. In our cohort, the proportion of AGG-less premutation alleles was markedly higher (87.5%) than that reported by Rodrigues et al. (11) (50.3%), whose study population consisted predominantly of Caucasian individuals. Given the established association between the absence of AGG interruptions and increased CGG repeat instability, this difference may provide a biological context for the relatively higher frequencies of premutation (1.7%) and full mutation (2.7%) alleles observed in our clinically selected cohort compared with population-based estimates from Caucasian populations (23,24). However, because both studies used non-population-based samples, these findings should not be interpreted as reflecting true population-level differences. Rather, they highlight potential population-specific variability in AGG interspersion patterns and underscore the need for population-based studies to clarify the contribution of AGG structure to Fragile X–related allele distributions.

Previous studies have reported that, in intermediate and premutation *FMR1* alleles, AGG interruptions preferentially cluster toward the 5′ end of the locus, whereas the longest uninterrupted CGG stretch is typically located at the 3′ end (8,9). By contrast, the present study found that all intermediate and premutation alleles containing AGG interruptions displayed uninterrupted CGG tracts exceeding 20 repeats exclusively at the 5′ end. Notably, the occurrence of this repeat configuration in cases with a positive family history suggests an association with familial transmission and increased allelic instability of FMR1. However, given the limited number of intermediate and premutation alleles analyzed, this finding should be interpreted with caution.

Supportive evidence for this interpretation is provided by an intergenerational transmission observed in a single family, in which a maternal allele that harbored a long uninterrupted CGG segment at the 5′ end (29 + 9) lost its AGG interruption and expanded to a pure 33 CGG repeat in the offspring. Although anecdotal and not intended to imply causality, this observation is consistent with the hypothesis that uninterrupted CGG segments at the 5′ end may constitute a structurally unstable configuration during transmission, predisposing alleles to AGG loss and early repeat expansion. Taken together with the group-level association observed in the present study, these findings suggest that the contribution of uninterrupted CGG tracts to *FMR1* instability may depend not only on repeat length but also on their positional context within the repeat array.

Although the loss of AGG interruptions during transmission is considered a rare event, it has been previously documented in the literature (25,26). In this context, our observation of an intergenerational AGG loss followed by repeat expansion is consistent with previous reports. Furthermore, none of the alleles that expanded to full mutations during family screening in the present study contained AGG interruptions, which is consistent with a potential stabilizing role of AGG interruptions against large CGG expansions. No contractions in total CGG repeat number were observed across generations, in keeping with the well-established bias of *FMR1* alleles toward repeat expansion rather than contraction (9).

Study Limitations

This study has several limitations. Its retrospective design and the inclusion of a clinically referred, potentially high-risk cohort limit the generalizability of the findings to the general population. In addition, the relatively small sample size reduces statistical power, particularly for the evaluation of rare AGG interruption patterns. This limitation is especially relevant for subgroup analyses, in which the Fisher's exact test is based on very small samples, and for the Syrian cohort; it may limit the robustness and generalizability of the findings. Furthermore, familial data were available only for a subset of cases, and systematic intergenerational assessment of CGG repeat expansion was not feasible across all allele categories. In particular, normal alleles with longer CGG repeat tracts are not routinely subjected to family-based analyses in clinical practice, limiting the ability to directly evaluate transmission dynamics in this group. In addition, AGG interruption patterns cannot be reliably determined in alleles within the full mutation range due to technical limitations in the analysis of large CGG repeat expansions. Moreover, data on the clinical relevance of AGG interruption patterns that deviate from the evolutionarily conserved 9–10 uninterrupted CGG units remain limited. Consequently, the relationship between different repeat architectures and clinical outcomes is not yet fully understood. Prospective, population-based studies with larger cohorts are needed to clarify the clinical and biological significance of distinct AGG interruption patterns, and further investigations addressing this question are currently being planned by our group.

CONCLUSION

This study demonstrates marked variability in *FMR1* CGG repeat length and AGG interruption architecture in Turkish and Syrian cohorts, although the small sample size of the Syrian cohort warrants cautious interpretation. The data further suggest that repeat instability is associated not only with repeat number but also with repeat structure and positional context. The identification of population-specific AGG patterns and long uninterrupted CGG tracts, particularly at the 5′ end, supports the concept that repeat architecture and positional context contribute to *FMR1* instability. Our findings are also consistent with previous studies suggesting that AGG interruptions may contribute to the stabilization of CGG repeat tracts; however, given the limited scope of familial analyses in this study, this interpretation should be viewed with caution. Integrating AGG interruption patterns into routine FMR1 testing may enhance genetic counseling and risk stratification, particularly in families undergoing carrier screening or intergenerational follow-up. However, larger, multicenter studies will be required to confirm these findings and to define their broader clinical significance.

Ethics

Ethics Committee Approval: The study protocol, covering both participating centers, was approved by the Uşak University Non-Interventional Clinical Research Ethics Committee (decision number: 710-710-14, dated 12.06.2025).

Informed Consent: Informed consent was waived due to the retrospective design.

Footnotes

Authorship Contributions

Surgical and Medical Practices: H.K.E., F.D., B.E.C., E.T., Ö.B., M.B., T.A.D., L.Ş., Concept: H.K.E., F.D., Design: H.K.E., S.K., F.D., Data Collection or Processing: H.K.E., S.K., B.E.C., Ö.B., M.B., Analysis or Interpretation: H.K.E., S.K., E.T., T.A.D., Literature Search: H.K.E., B.E.C., L.Ş., Writing: H.K.E., L.Ş.

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Supplementary Tables: https://d2v96fxpocvxx.cloudfront.net/7a593d95-86ec-4d2b-8dd3-26b0d0b79ea4/content-images/e8b95427-9d1a-46ae-b2c0-e41fa768d1f0.pdf

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