

## PATIENT INFORMATION LEAFLET FOR FMR1 GENETIC TESTING

### Fragile X Syndrome and Other FMR1-Associated Conditions

#### Purpose of this information sheet

This information sheet describes the main features of the genetic conditions associated with the **FMR1** gene, the purpose of molecular testing, the possible test results and the limitations of the analysis.

The information provided is intended to support both patients and healthcare professionals in making informed decisions regarding genetic testing and in understanding the test results.

This information sheet does not replace medical or genetic counselling, which may be recommended before testing and, in particular, whenever an intermediate allele, a premutation, a full mutation or an inconclusive result is identified.

#### What is the FMR1 gene?

The **FMR1** (*Fragile X Messenger Ribonucleoprotein 1*) gene is located on the long arm of the X chromosome, at **Xq27.3**.

Within the 5' untranslated region (5' UTR) of the gene there is a sequence composed of repeated **CGG trinucleotides**. The number of CGG repeats normally varies among individuals.

When the number of repeats exceeds specific thresholds, the sequence may become unstable and be associated with different clinical conditions.

The main conditions associated with **FMR1** repeat expansions include:

- **Fragile X Syndrome (FXS);**
- **Fragile X-associated Primary Ovarian Insufficiency (FXPOI);**
- **Fragile X-associated Tremor/Ataxia Syndrome (FXTAS);**
- **other clinical manifestations associated with the FMR1 premutation, collectively referred to as Fragile X Premutation-Associated Conditions (FXPAC).**

FMR1-associated conditions may differ considerably according to the number of CGG repeats, biological sex, age and other individual factors.

#### Why may this test be recommended?

Analysis of the **FMR1** gene may be indicated in different clinical and reproductive settings.

| Clinical setting          | When testing may be indicated                                                                                                                                                                                                                                                                    |
|---------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Clinical diagnosis</b> | In individuals presenting with clinical features suggestive of an FMR1-associated condition, such as intellectual disability, developmental or speech delay, autism spectrum disorder, primary ovarian insufficiency, adult-onset tremor/ataxia syndrome, or other suggestive clinical findings. |
| <b>Family history</b>     | In individuals with a family history of Fragile X syndrome or other FMR1-associated conditions, or for the evaluation of relatives of a person in whom an FMR1 expansion has already been identified..                                                                                           |

|                                                                 |                |                                                                                                                                                                                                                                               |
|-----------------------------------------------------------------|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Preconception screening and reproductive risk assessment</b> | <b>carrier</b> | In couples wishing to assess the risk of transmitting an FMR1 expansion to their offspring before a natural pregnancy or as part of an Assisted Reproductive Technology (ART) programme, including the evaluation of gamete donor candidates. |
|-----------------------------------------------------------------|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

The indication for testing and the interpretation of the results may vary according to the clinical reason for which the analysis has been requested.

For this reason, it is important to **provide the laboratory with complete clinical and family information**, including any genetic test results already available for family members.

### How are FMR1 alterations inherited?

The **FMR1** gene is located on the **X chromosome**.

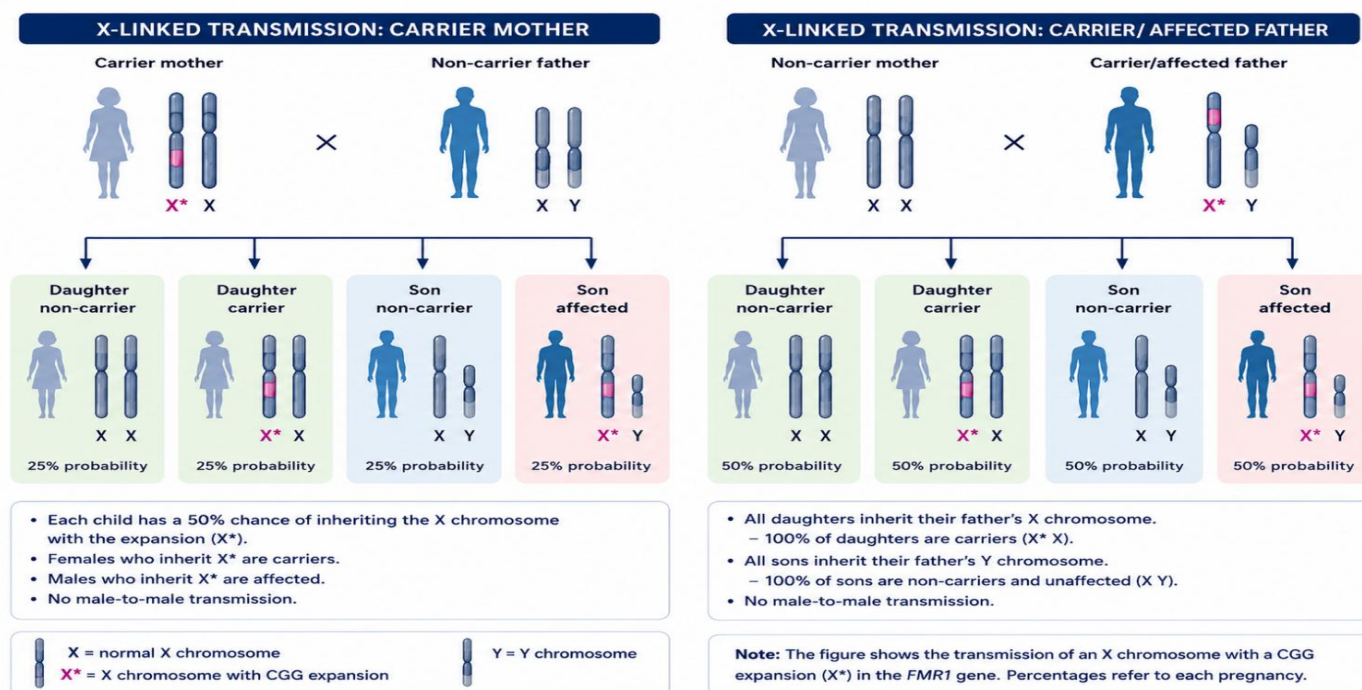
A woman carrying an expanded **FMR1** allele has, in each pregnancy:

- a **50% chance** of transmitting the X chromosome carrying the expanded allele;
- a **50% chance** of transmitting the X chromosome carrying the normal allele.

These probabilities are independent of the sex of the fetus; however, the clinical consequences may differ between males and females.

A man transmits his single X chromosome to all of his daughters and his Y chromosome to all of his sons. Therefore:

- **all daughters** of a man carrying an FMR1 premutation will inherit the paternal expanded allele and will be **obligate premutation carriers**;
- **none of his sons** will inherit the paternal X chromosome carrying the FMR1 allele.



When a premutation is transmitted by a woman, the number of CGG repeats may increase. This phenomenon is known as **dynamic repeat expansion**.

In some cases, the transmitted allele may expand into the **full mutation** range, defined as **more than 200 CGG repeats**.

The risk of expansion depends primarily on the number of CGG repeats in the maternal allele and on the presence and distribution of **AGG interruptions** within the repeat sequence.

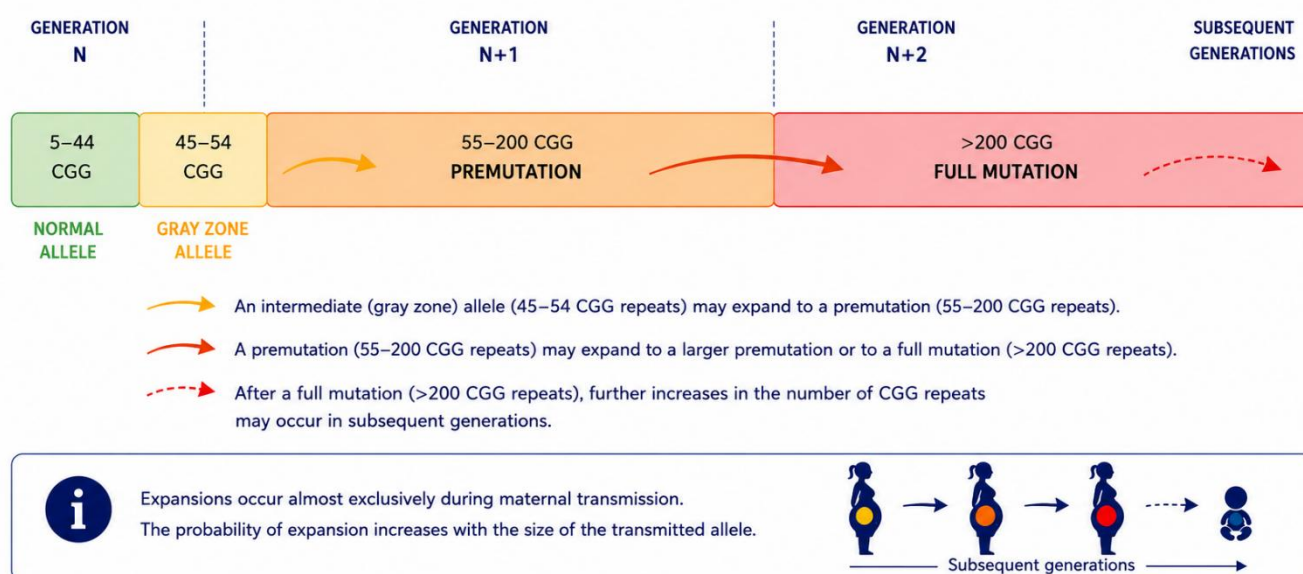
Transmission of a premutation from a father to his daughters may be associated with small changes in repeat size but does **not generally** result in expansion to a full mutation.

The actual risk for relatives and future offspring should always be assessed on the basis of the specific genetic result and discussed within the context of **genetic counselling**.

## DYNAMIC EXPANSION OF CGG REPEATS IN THE *FMR1* GENE

During maternal transmission, the number of CGG repeats can increase in size from one generation to the next.

The larger the allele, the greater the risk of further expansion.



### What does the test analyse?

The test determines the number of **CGG repeats** within the **5' untranslated region (5' UTR)** of the **FMR1** gene.

Based on the number of CGG repeats, alleles are classified into different categories, each associated with distinct clinical and reproductive implications, as described below.

#### Normal allele: 5–44 CGG repeats

Alleles containing **5 to 44 CGG repeats** are considered **normal**.

These alleles are generally stable during transmission from one generation to the next and are not associated with **Fragile X syndrome** or other clinical conditions related to **FMR1** repeat expansions.

In individuals undergoing **preconception carrier screening**, a normal result significantly reduces the likelihood of carrying a clinically significant **FMR1** expansion.

However, a negative result does not exclude the presence of other alterations of the **FMR1** gene that are not investigated by this analysis, nor does it exclude other genetic causes of similar clinical manifestations.

#### Intermediate (Gray Zone) allele: 45–54 CGG repeats

Alleles containing **45–54 CGG repeats** are classified as **intermediate (Gray Zone) alleles**.

According to current knowledge, these alleles are **not associated with Fragile X syndrome or other clinical manifestations related to FMR1 repeat expansions**, nor do they indicate carrier status for Fragile X syndrome.

Intermediate alleles may, however, show a limited degree of instability during transmission from one generation to the next, particularly when inherited through the maternal line.

Approximately **14%** of intermediate alleles transmitted by women may undergo a small increase in repeat size and expand into the **premutation** range.

Intermediate alleles are **not known to expand directly to a full mutation within a single generation**. Therefore, their identification does not increase the risk of having a child affected by Fragile X syndrome in the current pregnancy or in the immediately following generation.

Nevertheless, expansion into the premutation range may become relevant for future generations.

When there is a suggestive family history or within the context of preconception evaluation, **genetic counselling** may be recommended.

#### Premutation: 55–200 CGG repeats

Alleles containing **55–200 CGG repeats** are classified as **premutations**.

Individuals carrying a premutation are **not affected by Fragile X syndrome**. However, a premutation is associated with an increased risk of developing a number of clinical conditions related to the **FMR1** gene, collectively referred to as **Fragile X Premutation-Associated Conditions (FXPAC)**.

It is important to note that **carrying a premutation does not necessarily result in the development of clinical symptoms**.

The likelihood of developing a premutation-associated condition, as well as its severity, may vary according to biological sex, age, CGG repeat size, X-chromosome inactivation in females, and other genetic, clinical and environmental factors.

#### Fragile X-associated Primary Ovarian Insufficiency (FXPOI)

Women carrying an **FMR1 premutation** have an increased risk of developing **Fragile X-associated Primary Ovarian Insufficiency (FXPOI)**, defined as reduced or absent ovarian function before the age of 40 years.

Approximately **20%** of women carrying a premutation develop FXPOI, compared with about **1%** of women in the general population.

The risk is not the same for all premutation sizes and appears to be highest among women carrying **mid-range premutation alleles**.

A diagnosis of primary ovarian insufficiency does not completely exclude the possibility of occasional ovulation or spontaneous pregnancy.

When a premutation is identified in a woman of reproductive age, referral to a gynaecologist or a reproductive medicine specialist may be appropriate.

#### Fragile X-associated Tremor/Ataxia Syndrome (FXTAS)

An FMR1 premutation **may also be associated with Fragile X-associated Tremor/Ataxia Syndrome (FXTAS)**, a progressive neurodegenerative disorder that typically develops later in life.

FXTAS may occur in both male and female premutation carriers, although the risk is substantially higher in men and increases with both age and CGG repeat size.

In women, FXTAS is less common and generally presents with milder or incomplete manifestations, partly because of the presence of a second X chromosome.

Nevertheless, most premutation carriers never develop FXTAS.

#### Other premutation-associated conditions

Additional neurological, neuropsychiatric, endocrine, autoimmune and chronic pain manifestations have been described in individuals carrying an **FMR1 premutation**.

Some of these manifestations are collectively referred to as **Fragile X Premutation-Associated Conditions (FXPAC)** or, when limited to neuropsychiatric features, as **Fragile X-associated Neuropsychiatric Disorders (FXAND)**.

The associations with these conditions show variable penetrance and severity, and for some manifestations the available scientific evidence remains inconclusive.

Therefore, the presence of non-specific symptoms alone is not sufficient to establish a causal relationship with the premutation.

Test results should always be interpreted in the context of the individual's clinical history.

#### Reproductive implications of the premutation

A woman carrying an **FMR1 premutation** has a **50% chance** of transmitting the expanded allele in each pregnancy.

During maternal transmission, the premutation may increase in size and expand into the **full mutation** range.

The risk of expansion is not the same for all premutations. In general, it increases with the number of CGG repeats and is influenced by the presence of **AGG interruptions** within the repeat sequence.

A man carrying a premutation transmits his X chromosome to **all daughters**, who will inherit the premutation, and to **none of his sons**.

Paternally transmitted premutations do **not generally expand** to a full mutation.

### Full mutation: more than 200 CGG repeats

Alleles containing **more than 200 CGG repeats** are classified as **full mutations**.

In most cases, a full mutation is associated with **abnormal methylation** of the regulatory region of the **FMR1** gene. This results in markedly reduced or absent production of the **Fragile X Messenger Ribonucleoprotein (FMRP)**, a protein that plays an essential role in normal brain development and function.

A full mutation is responsible for **Fragile X syndrome**.

In individuals with a **single X chromosome**, a full mutation generally results in the typical clinical features of Fragile X syndrome.

In individuals with **two X chromosomes**, clinical manifestations are considerably more variable. Some women may present with intellectual disability or learning and behavioural difficulties, whereas others may have normal cognitive function.

This variability is influenced, among other factors, by the pattern of **X-chromosome inactivation**.

Cases of **mosaicism** may also be identified, in which different cell populations carry alleles of different sizes and/or methylation status. Mosaicism may contribute to variability in the clinical presentation.

Identification of a **full mutation** should always be followed by **genetic counselling** to discuss the clinical, reproductive and familial implications of the result.

| Category                                                                                                            | CGG Repeats | Fragile X Syndrome | Personal Risk                                  | Reproductive Risk                                                         |
|---------------------------------------------------------------------------------------------------------------------|-------------|--------------------|------------------------------------------------|---------------------------------------------------------------------------|
|  <b>Normal</b>                   | 5–44        | No                 | None                                           | None                                                                      |
|  <b>Intermediate (Gray Zone)</b> | 45–54       | No                 | No known clinical risk                         | Possible expansion to a premutation in future generations                 |
|  <b>Premutation</b>              | 55–200      | No                 | Increased risk of FXPOI, FXTAS and other FXPAC | Possible expansion to a full mutation during maternal transmission        |
|  <b>Full mutation</b>            | >200        | Yes                | Fragile X syndrome                             | Transmission according to the inheritance pattern of the <b>FMR1</b> gene |

### Available Tests

Eurofins Genoma offers different **FMR1** testing options, which differ according to the analytical workflow and the possible need for additional testing.

### A472 – X Fragile FRAXA

**A472 – X Fragile FRAXA** is the **first-line assay** for determining the number of **CGG repeats** within the 5' untranslated region (5' UTR) of the **FMR1** gene.

The analysis is performed by **fluorescent PCR followed by capillary electrophoresis**.

This method allows accurate sizing of detected alleles up to approximately **80 CGG repeats**.

In most cases, the first-line assay provides a conclusive result.

In some situations, however, the following findings may occur:

- only one allele is detected in an individual with two X chromosomes;
- no allele is detected in an individual with a single X chromosome;
- the electrophoretic profile does not allow exclusion of an expansion exceeding the sizing capability of the assay.

In these cases, **reflex testing** using a dedicated second-level assay may be recommended.

The laboratory report will include the test result, the interpretation of the identified allele category and, whenever appropriate, a recommendation to proceed with additional testing.

**Estimated turnaround time (TAT): 10 working days**

#### A1639 – FMR1 (FRAXA) Long-Range PCR - Second-line (Reflex) Assay

**A1639 – FMR1 (FRAXA) Long-Range PCR** is a **reflex assay** intended for cases in which the first-line analysis does not allow complete characterization of the **FMR1** genotype.

The analysis is performed using a **three-primer CGG Repeat-Primed PCR**, which allows:

- detection of large CGG repeat expansions;
- differentiation, in individuals with two X chromosomes, between true homozygosity and the presence of a second expanded allele not detected by the first-line assay;
- investigation of cases in which no allele has been identified in an individual with a single X chromosome;
- characterization of alleles within the **premutation** or **full mutation** ranges.

Whenever technically feasible, the assay can be performed on the **same biological sample already available in the laboratory**, avoiding the need for a new sample collection.

When the patient has initially undergone **A472** only, **A1639 must be requested separately**.

**Estimated turnaround time (TAT):  
15 working days from the request for reflex testing**

#### A2098 – X Fragile FRAXA Complete

**A2098 – X Fragile FRAXA Complete** provides a comprehensive analytical workflow.

The test includes:

1. **First-line analysis** by fluorescent PCR and capillary electrophoresis.
2. **Automatic reflex testing** by CGG Repeat-Primed PCR whenever the first-line result requires further characterization.

The second-level assay is therefore performed **only when necessary** to achieve a more complete characterization of the result, without requiring an additional request or authorization.

This solution offers several advantages:

- a complete diagnostic pathway from the outset;
- no need for subsequent requests for additional testing;
- reduced administrative and organisational delays;
- use of the same biological sample whenever technically feasible;
- simplified workflow for **preconception carrier screening, Assisted Reproductive Technology (ART) programmes and gamete donor evaluation.**

**Estimated turnaround time (TAT): 15 working days. The turnaround time may be longer in cases requiring reflex testing.**

| Test                                    | Purpose                                              | When is it indicated?                                                                                                                                         | Estimated TAT* |
|-----------------------------------------|------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| <b>A472 – X Fragile FRAXA</b>           | First-line analysis                                  | All patients                                                                                                                                                  | 10             |
| <b>A1639 – FMR1 Long-Range PCR</b>      | Reflex assay                                         | Only when further characterization is required                                                                                                                | 15§            |
| <b>A2098 – X Fragile FRAXA Complete</b> | Comprehensive workflow with automatic reflex testing | Preconception carrier screening, Assisted Reproductive Technology (ART), gamete donor evaluation and all situations in which a complete workflow is preferred | 15             |

\* Turnaround times are expressed in **working days**.

§ Turnaround time is calculated **from the request for reflex testing**, not from the date the original sample was received

## Biological Sample Requirements

The analysis is generally performed on DNA extracted from a **peripheral blood sample**.

In specific clinical settings, the test may also be performed on DNA obtained from other biological specimens, including:

- chorionic villi;
- amniotic fluid;
- fetal tissue;
- previously extracted DNA, provided that it meets the laboratory's quality and quantity requirements.

The type of specimen required may vary according to the clinical indication and the requested analysis.

The suitability of the sample is assessed by the laboratory before testing. If the sample quantity or quality is insufficient, or if the result is not interpretable, a new specimen may be requested.

## Test Limitations

This test is intended exclusively to determine the number of **CGG repeats** within the **5' untranslated region (5' UTR)** of the **FMR1** gene.

The analysis does **not** detect:

- single nucleotide variants (SNVs) or small insertions/deletions (indels) within the **FMR1** gene;
- partial or complete deletions or duplications of the **FMR1** gene;
- chromosomal rearrangements involving the **FMR1** locus;
- methylation status of the **FMR1** promoter;
- X-chromosome inactivation pattern;
- the number and position of **AGG interruptions** within the CGG repeat sequence;
- pathogenic variants in other genes associated with intellectual disability, autism spectrum disorder, primary ovarian insufficiency or neurological disorders;
- sex chromosome abnormalities.

Genetic alterations of **FMR1** other than CGG repeat expansions account for only a small proportion of Fragile X syndrome cases; however, they cannot be excluded by this analysis.

Rare sequence variants within the primer binding regions may interfere with PCR amplification and result in failure to detect one allele, a phenomenon known as **allelic dropout**.

The first-line assay accurately sizes alleles up to approximately **80 CGG repeats**. Larger expansions may require reflex testing.

Even the reflex assay has some limitations:

- it may detect the presence of a CGG repeat expansion without always allowing precise sizing;
- it does not exclude deletion of the **FMR1** gene or of a second allele;
- it does not assess promoter methylation;
- it does not allow accurate determination of tissue-specific mosaicism.

When the clinical presentation strongly suggests an **FMR1-associated condition**, a negative result may require additional genetic investigations.

In individuals with a known family history, a negative result should be interpreted, whenever possible, in comparison with the molecular finding previously identified in the affected family member.

If the molecular result of the affected relative is unavailable, a negative result may not be sufficient to completely exclude the familial risk.

## Implications for Family Members

Identification of an **intermediate allele**, a **premutation** or a **full mutation** may have implications for other family members.

Depending on the result, testing of additional relatives may be recommended following appropriate **genetic counselling**.

## Reproductive Options

When an **FMR1 expansion** is identified, the individual or couple may discuss the available reproductive options with a clinical geneticist and reproductive medicine specialists.

The main options include:

- **prenatal diagnosis**, through molecular analysis of fetal DNA obtained by **chorionic villus sampling (CVS)** or **amniocentesis**;
- **Preimplantation Genetic Testing for Monogenic Disorders (PGT-M)** within an **Assisted Reproductive Technology (ART)** programme.

Prenatal diagnosis can determine whether the fetus has inherited the at-risk allele and allows characterization of the identified expansion.

PGT-M makes it possible to identify embryos that have inherited the familial at-risk allele before embryo transfer.

The feasibility of PGT-M must be assessed in advance and may require genetic testing of the couple and, in some cases, additional family members.

The most appropriate reproductive strategy depends on several factors, and the advantages, limitations and possible outcomes of each option should be discussed with the appropriate healthcare professionals.

## Genetic Counselling

The interpretation of the test result should always take into account the individual's **clinical, family and reproductive history**.

**Genetic counselling is strongly recommended** whenever an intermediate allele, a premutation or a full mutation is identified, as well as when reproductive risk assessment or cascade testing of family members is being considered.

In families with a known history of **FMR1-associated conditions**, genetic counselling is strongly recommended **before** undergoing testing.