Frequently Asked Questions: **Fragile X syndrome (FXS) Genetic Testing**

Frequently asked questions addressed in this document:

Summary of key points:

- FXS genetic testing is indicated when there is a strong clinical suspicion of this disorder. Clinical indications include global developmental delay (GDD)*/intellectual disability (ID) with or without dysmorphic features.
- Isolated ASD in the absence of GDD/ID is *not* an indication for FXS testing.
- Delay in a single developmental domain, such as isolated speech delay, is *not* an indication for FXS testing.
- FXS genetic testing is not required prior to assessment at Sunny Hill Health Centre if the individual does not meet clinical criteria for testing.
- Clear and appropriate clinical indications must be provided on the requisition for the test to be initiated.

*GDD: significant delay (at least 2 SDs below the mean using standardized tests) in at least two of the following developmental domains:

- Gross or fine motor delay
- Speech/language
- Cognition
- Social/personal
- Activities of daily living

GDD, as defined by the Canadian Pediatric Society[1], is reserved for children <5 years. *Suspected* GDD or ID based on the results of developmental screening tools and/or clinical judgement is acceptable if formal testing is pending or cannot be completed.

Diagnostic rate

The diagnostic rate of FXS is very low and does not support testing without appropriate clinical indications. In individuals with ID with or without ASD, the diagnostic rate has been reported as **0-2.5%** in recent literature[2-6]. In the Division of Genome Diagnostics, the diagnostic rate was **0.63%** (0.23-1.34%) from 2016-2020. Positive diagnoses are typically supported by clinical features and/or family history[2-6]. By comparison, chromosomal

microarray and exome sequencing have diagnostic rates up to **30%** in individuals with neurodevelopmental disorders[2,7].

1. Who should be tested?

- Individuals of either sex with confirmed or suspected GDD/ID *see CPS definition above[1].
- Individuals who are too young to undergo formal psychoeducational testing but for whom GDD/ID is strongly suspected.
- Any of the above with clinical features of fragile X syndrome and/or a positive family history.
    - Clinical features of FXS include GDD/ID, as well as other features which may emerge at the time of puberty, including elongated face, prominent ears, connective tissue features and macroorchidism[8] (see Appendix for a recent clinical checklist).

2. What is NOT considered an indication for FXS testing?

- Isolated neurological conditions without co-morbid GDD/ID, including:
    - ASD (including high-functioning autism)
    - Attention Deficit Hyperactivity Disorder (ADHD) and/or behavioural challenges
    - Epilepsy
    - Developmental Coordination Disorder
    - Anxiety
    - Males with learning difficulties
    - Delay in a single developmental domain, e.g., speech/language delay
- Individuals with 'developmental delay'. Clarification is required to ensure delay spans multiple developmental domains and GDD/ID is suspected.

3. Are patients with ASD/suspected ASD eligible for FXS genetic testing?

- Individuals with isolated and/or high-functioning ASD, i.e., without co-morbid GDD/ID, are NOT eligible for FXS genetic testing.
    - The Canadian Pediatric Society (CPS) guideline addressing diagnostic assessment ASD does NOT include FXS genetic testing as a required investigation in the work-up of ASD[9]. Rather, laboratory testing or further investigations can be performed "**only if clinically indicated"**. See step 4 in the CPS statement for more details**.**
    - The diagnostic rate of FXS in individuals with *isolated* ASD has been reported as **0%** in recent scientific literature[3].
    - The utility of testing in individuals with *high-functioning* ASD has yet to be clearly established but is expected to be very low in the absence of co-morbid ID.
    - CMA has a higher diagnostic rate in this population (5-10%) and should be pursued as a first-tier genetic test[3,9].

4. I have referred my patient to Sunny Hill Health Centre for an assessment. Is FXS testing necessary prior to the appointment?

- FXS genetic testing is **not** required prior to assessment at Sunny Hill Health Centre.

- If clinical suspicion of FXS is high in your patient, then Sunny Hill requests that this genetic testing be performed prior to the assessment. However, if the patient does not have a presentation consistent with FXS then genetic testing is not required for the assessment to proceed.

5. When should patients with a family history of an *FMR1*-related disorder be tested?

- Patients presenting with ID or significant GDD, regardless of sex, are eligible for testing and should be tested during childhood.
- In the absence of clinical suspicion of fragile X syndrome, genetic testing of individuals with a family history of an *FMR1*-related disorder should be deferred until the patient is old enough to provide informed consent.
    - Testing for family history alone, i.e. in the absence of clinical suspicion for FXS, may be performed in consenting adults to assess reproductive risk *OR* risk for *FMR1*-related adult onset disorders.
    - See www.genebc.ca for more information about carrier testing and *FMR1*-related adult onset disorders.

6. What information is required on the requisition when ordering FXS genetic testing?

- The **clinical indication(s)** for genetic testing should be clearly provided on the requisition. See who should be tested above for a review of the indications for testing.
    - Testing will not proceed without the provision of clinical indications.
- Please indicate if GDD or ID is *confirmed* or *suspected*.
    - For individuals >5 years with *suspected* ID where no formal diagnosis has been made, please indicate on the requisition the reasons why a formal diagnosis has not been pursued (e.g., pending assessment, child is non-verbal and not testable, etc.)

References

1 Belanger, S. A. & Caron, J. Evaluation of the child with global developmental delay and intellectual disability. *Paediatr Child Health* **23**, 403-419, doi:10.1093/pch/pxy093 (2018).

2 Borch, L. A., Parboosingh, J., Thomas, M. A. & Veale, P. Re-evaluating the first-tier status of fragile X testing in neurodevelopmental disorders. *Genet Med* **22**, 1036-1039, doi:10.1038/s41436-020-0773-x (2020).

3 Weinstein, V., Tanpaiboon, P., Chapman, K. A., Ah Mew, N. & Hofherr, S. Do the data really support ordering fragile X testing as a first-tier test without clinical features? *Genet Med* **19**, 1317-1322, doi:10.1038/gim.2017.64 (2017).

4 Hartley, T. *et al.* Fragile X testing as a second-tier test. *Genet Med* **19**, doi:10.1038/gim.2017.147 (2017).

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6 Mullegama, S. V. *et al.* Is it time to retire fragile X testing as a first-tier test for developmental delay, intellectual disability, and autism spectrum disorder? *Genet Med* **19**, doi:10.1038/gim.2017.146 (2017).

7 Srivastava, S. *et al.* Meta-analysis and multidisciplinary consensus statement: exome sequencing is a first-tier clinical diagnostic test for individuals with neurodevelopmental disorders. *Genet Med* **21**, 2413-2421, doi:10.1038/s41436-019-0554-6 (2019).

8 Lubala, T. K. *et al.* Fragile X checklists: A meta-analysis and development of a simplified universal clinical checklist. *Mol Genet Genomic Med*, doi:10.1002/mgg3.398 (2018).

9 Brian, J. A., Zwaigenbaum, L. & Ip, A. Standards of diagnostic assessment for autism spectrum disorder. *Paediatr Child Health* **24**, 444-460, doi:10.1093/pch/pxz117 (2019).

Appendix

Table 1: Common clinical findings in males with Fragile X syndrome. Intellectual disability was a prerequisite for inclusion in the meta-analysis from which these data are derived, and is therefore not captured in the table below. Clinical findings are ordered by decreasing odds ratio and key discriminating features are highlighted in red. Table adapted from Lubala *et. al.,* 2018[8]

Clinical feature	Frequency in Fragile X syndrome	Frequency in ID (without FRX)	Odds ratio (95% CI)
Large and prominent ears	84% (173/206)	22% (756/3458)	18.62 (14.38-24.1)
Soft, velvety skin	88% (38/43)	52% (95/181)	16.85 (10.4-27.3)
Flat feet	70% (26/37)	37% (43/115)	11.53 (6.79-19.56)
Large testicles (post-puberty)	71% (129/181)	10% (291/2915)	7.14 (5.53-9.22)
Plantar crease	86% (84/98)	23% (162/707)	3.74 (2.67-5.24)
Elongated face	72% (109/151)	20% (533/2728)	3.69 (2.84-4.81)
Family history of ID	81% (166/205)	24% (807/3418)	3.43 (2.76-4.27)
Tactilely defensive	65% (108/166)	19% (626/3274)	3.40 (2.63-4.40)
Autistic-like behaviour*	76% (162/213)	25% (854/3457)	3.08 (2.48-3.83)
Hand flapping	59% (75/128)	29% (404/1391)	2.91 (2.20-3.84)
Transverse palmar crease	26% (30/115)	10% (104/1064)	2.68 (1.70-4.18)
Hyper extensible joints	68% (150/220)	25% (849/3336)	2.68 (2.15-3.34)
Poor eye contact	86% (139/161)	34% (517/1506)	2.51 (1.96-3.22)
Hand biting	39% (45/115)	21% (218/1062)	1.91 (1.31-2.77)
Short attention	79% (91/115)	48% (511/1063)	1.65 (1.23-2.21)
Perseverative speech	66% (107/161)	46% (675/1466)	1.44 (1.11-1.87)
Hyperactivity	74% (120/162)	53% (829/1576)	1.41 (1.10-1.81)
ADHD	75% (122/162)	55% (870/1576)	1.36 (1.06-1.75)

***** Autistic-like behaviour encompasses tactile defensiveness, perseverative speech, hand flapping and poor eye contact. This item was scored positive when *one* feature was observed.

Table 2: Clinical scoring for the seven most discriminant fragile X features, as recommended by from Lubala *et. al.,* 2018[2]. Scores >5 have a significant yield of fragile X syndrome.

Clinical feature	Score
Skin soft and velvety on the palms with redundancy of skin on the dorsum of the hand	2
Flat feet	2
Large and prominent ears	2
Plantar crease	1
Large testicles	1
Family history of ID	1
Autistic-like behaviour	1