Testing Criteria for Fragile X Syndrome: Report of the outcome from the UKGTN Fragile X Workshop

December 2nd, 2008 at Chandos House, 2 Queen Anne Street, London W1G 9LQ

Summary

The UKGTN organised an Expert Workshop on December 2nd 2008 to develop testing criteria for Fragile X genetic testing. NHS clinicians, molecular scientists and commissioners have raised concerns about the lack of standardised testing criteria to assure appropriateness of genetic testing for Fragile X. Twenty four invited experts participated in the workshop. Participants included clinical geneticists who have a special interest in the diagnosis and management of Fragile X, paediatricians, clinical endocrinologists, consultant psychiatrists, genetic counsellors, patient representatives and molecular and cytogenetic scientists (Annex A). The workshop programme consisted of a series of presentations and structured discussions which allowed a consensus to be reached on the testing criteria for each clinical group and purpose. The workshop programme is presented in Annex B for information.

The workshop focused on the development and agreement of testing criteria for the following patient groups and clinical purposes:

1. Male child with developmental delay (DD) / learning difficulty (LD)
2. Female child with DD / LD
3. Adults with LD
4. Female adult with premature ovarian failure (POF)
5. Male adults with fragile-X tremor ataxia (FXTAS)

The agreed consensus criteria will be reviewed by the UKGTN Gene Dossier working group and then presented for endorsement to the UKGTN Steering Group. The proposed criteria are presented in Annexes C-E.

Key points from meeting

The molecular labs represented had all experienced a reducing positive detection rate for FraX molecular test requests over the 15 years since introduction of the test. Data from GOS, Salisbury and W.Midlands suggested a 3% positive rate in 1993 (with only 50% of tests being for children < 6yrs), compared with 0.6% positive in 2008.

1. **Fragile X testing in the case of Developmental Delay/Learning Disability**

It was agreed that the reduced positive detection rate was due to the following reasons:

- Concentration of assessment and community paediatric input to detect delay earlier in children has led to a shift to younger age in the target
population for Fragile X testing, for which there is no specific younger age limit.

- The range of problems in children presenting for assessment at this age is much wider, and hence a focus on the level of delay appropriate for Fragile X is less likely
- The characteristic physical features reported for adults are less applicable at this younger age
- The parents of younger children are less likely to have completed their families, so there is more urgency for diagnosing or excluding Fragile X
- The expertise of clinicians for recognising behavioural/personality pointers to Fragile X in young children may be less widespread.

The over-riding consideration is that Fragile X is a diagnosis which a clinician cannot afford to miss, primarily due to the serious genetic implications, but also to avoid otherwise unnecessary multiple subsequent investigations. Therefore, as the overwhelming majority of Fragile X test requests are for young children with developmental delay, where Fragile X as a cause needs to be excluded, it is clear that a low positive detection rate is to be expected when a clinical test is used in such a manner to exclude a diagnosis.

The Group concluded that chromosomal testing for Fragile X is no substitute for DNA tests; although most children with learning problems should also be having karyotyping (probably in the coming few years to be replaced by micro-array CGH technology on DNA) since the detection of different cytogenetic anomalies in the target population is higher than identifying positive cases of Fragile X.

Previous attempts to use physical and behavioural/personality characteristics to define Fragile X syndrome and thereby develop testing criteria have involved a scoring system from a check list (see below for background notes on FraX). This may have a place in testing the older male child or adult male population with learning difficulty; however it has not been applicable to the younger child with developmental delay. Most clinical centres who have piloted such scoring criteria have subsequently abandoned these due to coincidentally finding cases of Fragile X which would otherwise not have been tested. The practical availability of expertise required for accurate differentiation of a psychological/personality profile is also very limited.

The finding of a premutation or intermediate-sized allele in the context of testing for FraX as a potential cause of developmental delay/learning difficulty will in most cases be coincidental to the clinical presentation, but individual interpretation, according to clinical presentation, particularly for premutation alleles, may be required. In particular, for large premutations, the possibility of mosaicism for a full mutation should be investigated.

2. Girls with Fragile X syndrome (manifesting carriers)

The number of requests in girls for Fragile X testing is uniformly lower than in boys. This is due to:

(i) More boys than girls in the total population being referred to paediatric services for having learning/behaviour problems. Equal numbers of males and females can be expected to be born with a full Fragile X expansion, but only a proportion (approximately a third) of girls will manifest this sufficiently to be referred to paediatric services (IQ in affected girls is typically 80-85).
(ii) The personality/behaviour of affected girls is also noted to be more passive than the disruptive effect in boys, and may unfortunately often be blamed inappropriately on their parental environment. Girls with Fragile X do not typically show the same physical features, although can have a characteristic personality including inattention, autistic-like communication problems, shyness & social anxiety, gaze avoidance, and difficulty with maths or visuo-spatial skills.

It should be noted that for approximately 10% of tests in females it is necessary to add a Southern Blot to the DNA analysis on account of homozygosity for a single repeat size in conjunction with an associated common Xq27 haplotype.

3. **Fragile X testing for Premature Ovarian Failure/Insufficiency (POF/POI) and Tremor-ataxia syndrome (FXTAS)**

The clinical utility of Fragile X testing for Premature Ovarian Failure and Tremor-Ataxia syndrome were also discussed. UKGTN felt that it could not develop Testing Criteria for these tests in the absence of a Gene Dossier submission from a member laboratory, as neither of these tests are currently included in the UKGTN Directory of Molecular Genetic Tests.

**Summary Testing Criteria**

The agreed Testing Criteria for Fragile X testing in males and females in the case of Developmental Delay/Learning Disability are presented in Annexes C and D. The Testing Criteria for Fragile X carrier or premutation testing in relatives in a family with a known mutation is presented in Annexe E. Annex E should not be applied to families where there is an intermediate allele which is stable across generations, as there is currently no consistent evidence that cascade testing in that situation has clinical utility. However, where consistent repeat stability cannot be demonstrated, cascade testing and prenatal diagnosis may be appropriately offered to women carrying as few as 56 repeats, thereby allowing also for standard errors in sizing accuracy.

**Workshop Recommendations**

1. The majority of tests performed are to exclude Fragile X Syndrome for developmental delay/learning delay in children.

2. Testing criteria have been proposed for male (full mutation) and female (index) children. These criteria also apply to older children and adults.

3. Applications for member laboratories to offer FraX gene testing with POF or for FXTAS should be made through the UKGTN Gene Dossier process.

**Conclusion**

The Workshop Recommendations including the proposed consensus Testing Criteria will be considered by the UKGTN Gene Dossier Working Group and following agreement by UKGTN Steering Group.

The Testing Criteria will then be disseminated to NHS molecular genetic laboratories and will be made freely available to NHS clinicians on the UKGTN website.
Background

Fragile X syndrome

Fragile X syndrome occurs in individuals with an FMR1 full mutation and is nearly always characterized by moderate mental retardation (IQ 35-70) in affected males and milder learning difficulty in affected females, although clinically affected individuals occasionally have an atypical presentation with an IQ above 70. Adult males with an FMR1 full mutation accompanied by aberrant methylation may have a characteristic appearance (head size >50th centile, large protruding ears, long face, joint laxity, and large testes after puberty). Behavioural abnormalities, sometimes including autism spectrum disorder, are common and particularly manifest as gaze avoidance, delayed speech and echolalia, tactile defensiveness, hand-flapping and hand-biting, poor impulse control and distractability. In the past these features have formed the basis of a check list as an attempt at a scoring system for Fragile X, but sensitivity has remained too low, especially in children.

Female carriers of a Fragile X full mutation exhibit recognised learning difficulties in around one third of cases, although many female carriers will show traits from inattention, autistic-like communication problems, shyness & social anxiety, gaze avoidance, and difficulty with maths or visuo-spatial skills.

More than 99% of individuals with fragile X syndrome have a loss-of-function mutation in the FMR1 gene caused by an increased number of CGG trinucleotide repeats (typically >200) accompanied by aberrant methylation of the FMR1 gene. Rarely (<1% cases) other mutations within FMR1 cause fragile X syndrome (eg. deletions, missense or splice site mutations).

From a normal copy number of <50 CGG repeats, trinucleotide expansions of between 59*-200 repeats (*NB. see definitions, below) are termed premutations (due to their potential for expansion in the next generation), and usually remaining unmethylated, are not usually considered to associate with learning difficulty, although some recent evidence suggests there may possibly be an increased risk of autism spectrum disorder and/or attention deficit disorder in some premutation males, and of social avoidance traits in some female carriers. However it is apparent that premutations can be associated in females with a substantially increased risk for premature (<40yrs) ovarian failure (POF), and in males (and occasionally females) with a late onset neurological presentation as fragile-X tremor-ataxia syndrome (FXTAS).

Intermediate alleles (50-58* repeats) (*NB.see definitions, below) are asymptomatic, do not expand into the affected full mutation range, and may be stable across generations, although expansion into the premutation range has been recorded.

**Fragile X-associated tremor/ataxia syndrome (FXTAS)** is characterized by late-onset (>50 yrs) progressive cerebellar ataxia and intention tremor, associated with an FMR1 premutation. Other neurologic findings include short-term memory loss, executive function deficits, cognitive decline, dementia, parkinsonism, peripheral neuropathy, lower-limb proximal muscle weakness, and autonomic dysfunction. The penetrance of FXTAS I males is age-related; prevalence is estimated at 40% overall for males >50 yrs with premutations. FXTAS may account for 2-4% of non-familial late-onset (>40yrs) ataxia in men.
Premature Ovarian Failure (POF).
This is defined as ovarian failure/insufficiency under age 40 yrs., and is estimated to affect 1% of women, but 16-25% of female FraX permutation carriers, particularly with expansion sizes between 80-140 CGG repeats. It is estimated that FraX premutation accounts for 5% of all females with POF, and 13% where POF is familial.

Definitions:
Full mutation = > 200 CGG repeats
Pre-mutation = 59*-200 CGG repeats
Intermediate allele = 50-58* CGG repeats
*NB. A single family has been published subsequent to the meeting, reporting expansion to full mutation (> 500 repeats) in one generation of an ‘intermediate’ allele of 56 repeats (Fernandez-Carvajal et al J.Mol.Diag. 2009; 11: 306-310)

Epidemiology

Full mutation occurs in approximately 1/ 4000-1/6000 males
Symptomatic full mutation may occur in approximately: 1/ 8000 females
Moderate to severe LD (IQ<70) in total population: ~ 1 /100 males
Full mutation in severe LD adult males: ~ 2 %

Premutation female carriers: 1 /150- 1/300 females
Premutation males, assume: 1 /300 – 1/600 males

Premature ovarian failure /insufficiency (<40yrs): 1/100 women
Premutation in all POF females: 5 %
Premutation in familial POF: 13.2 %
Premutation in sporadic POF: 3.5 %

FXTAS Premutation in non-familial late-onset (>40yrs) ataxia in men: 2-4 %

Distribution of types of Fragile X test

For a typical general genetic service (Bristol 2005-7), the Fragile X tests requests divided as:

Males with LD: 73 %
Females with LD: 19 % mostly young children
POF: 2 %
FXTAS: < 0.5 %
Carrier testing in family: 5 %
Prenatal testing: 1 %

SCOBEC data (2003): Fragile X testing represents 17 % of test requests into member molecular labs. Equivalent to 180 tests per million population and 12,600 tests across UK.

Birmingham data (2008): 23 % of molecular genetic test workload
Positive pick up: 1993: 3 % (50% of these were children < 6 yrs)
(GOS data) – definitely lower by 2008
2008: 0.6 % (Salisbury, Birmingham)
ANNEX A

Fragile X workshop invitees

Chair: Peter Lunt (UKGTN Clinical Advisor)

Attendees
Clinical Endocrinology: Gerard Conway (London)
Clinical Genetics: Angela Barnicoat (London), Jenny Morton (Birmingham), Mary Porteous (Edinburgh), Helen Stewart (Oxford and member of Gene Dossier and Directory Working group)
Cytogenetics: Jonathan Waters (London)
Genetic Counselling/PGD: Susan Fairgrieve (Newcastle), Alison Lashwood (London), Margaretha van Mourik (Glasgow)
Molecular Genetics: James McPherson (Salisbury), Anna Murray (Exeter), Michael Sweeney (Dublin), Jon Warner (Edinburgh)
Neurology: None available
Paediatrics: Gillian Baird (London), Alison Salt (London), Patricia Jackson (Edinburgh)
Psychiatry: Jeremy Turk (London), Manga Sabaratnam (London)
Patient Representation: Lynne Zwink (Fragile X Society)
UKGTN: Jacqui Hoyle (Communications Specialist), Mark Kroese (Public Health Adviser), Fiona Stewart (Chair of Gene Dossier and Directory Working Group), Jacquie Westwood (Project Director and Commissioner)

Apologies
Clinical Genetics: Angus Clarke (Cardiff), Shehla Mohammed (London), Miranda Splitt (Oxford)
Molecular Genetics: Valerie Davison (Birmingham), Anneke Seller (Oxford), Su Stenhouse (UKGTN Scientific Advisor)
Neurology: Patrick Chinnery (Newcastle), Simon Hammans (Southampton), Huw Morris (Cardiff)
Paediatrics: Geoffrey Debelle (Birmingham), Paul Gringras (London), Anne O’Hare (Edinburgh), Jane Williams (Nottingham)
Psychiatry: Michael Kerr (Cardiff), Chris Oliver (Birmingham)
ANNEX B

UK Genetic Testing Network Workshop:
Fragile X Syndrome Testing Criteria

CONTENT OF PROGRAMME

1. Welcome and Introductions PL

2. Fragile X testing & workshop in context PL
   - Fragile X testing in context: (number of tests, cost, pick-up rate) PL
   - Gene dossier path – might it be appropriate? PL
   - Clinical scenarios for testing:
     - male child developmental delay (DD)
     - male child other presentations (e.g., hypotonia, epilepsy, autistic disorder)
     - female child DD
     - male adult learning difficulties (LD)
     - female adult learning difficulties
     - female adult premature ovarian failure (POF)
     - pregnancy testing/preimplantation genetic diagnosis (PGD)
     - male adult tremor-ataxia
   - Proportion of test requests in each scenario All
   - Pick-up rate in each scenario All
   - Technical testing considerations in each scenario All

3. Definition of Fragile X at molecular/cytogenetic level JMcP/JWat

4. Male child developmental delay AB/ASa/JT
   - Is Fragile X primarily a test to make the diagnosis, or to exclude it? GB/All
   - Does cytogenetic testing for Fragile X have any role? JWat
   - Studies of sensitivity of clinical features as ‘checklist criteria’ for Fragile X AB/JT/ASa
   - Experience of one lab (Bristol) from request form information transferred to database
   - Any studies of exclusion criteria, by which a test is not done?
   - Experience of one lab (Cardiff) in ceasing Fragile X test as a lab-initiated add-on

5. Male child other presentation JT/MP/All
   - Comments on experience of pick-up rate
   - Possible behavioural phenotype/cognitive profile of intermediate/ permutation allele

6. Female child developmental delay All
   - Typical clinical/behavioural/educational profile
   - Is Fragile X primarily a test to make the diagnosis, or to exclude it? JWat
   - Studies of sensitivity of clinical features as
‘checklist criteria’ for Fragile X
- Experience of one lab (Bristol) from request form info
  transferred to database
- Any studies of exclusion criteria, with which a test is not done?
- Technical aspect of ‘single normal band’ situation
  (ie. how frequent; does one then do Southern analysis ?)  JMcP/ SS/ JonW etc

7. Male adult LD  MSa/All
- Are there many UK cases remaining untested?
- Typical clinical / behavioural / educational profile
- Is Fragile X primarily a test to make the diagnosis, or to exclude it?
- Does cytogenetic testing for Fragile X have any role?  JWat
- Studies of sensitivity of clinical features as ‘checklist criteria’
  for Fragile X

8. Female adult LD  MSa/All
- Typical clinical / behavioural / educational profile
- Is Fragile X primarily a test to make the diagnosis, or to exclude it?
- Does cytogenetic testing for Fragile X have any role?  JWat
- Studies of sensitivity of clinical features as ‘checklist criteria’
  for Fragile X

9. Female adult POF  AM/GC
- Definition of POF
- Age limits
- Proportion of POF due to Fragile X, proportion of Fragile X who
  have POF.
- Any female FXTAS risk prediction?
- Premutation size requirement?

10. Pregnancy testing/PGD  AL/All
- reproductive age/pregnant women for carrier status
- PGD

11. Male adult with tremor-ataxia (in absence of SH/PCh)  PL
- Incidence /prevalence
- Presentation & clinical features
- Age distribution
- Premutation size requirement?
- Any late-onset neurology in premutation females?
- Suggested gene dossier for FXTAS

12. Other considerations  All
- eg. mosaicism, deleted allele, TN repeat reduction, Fragile XE,
  intermediate phenotypes, any ethnicity influence.

13. Summary and Conclusions  PL
- can there be possible testing criteria in each scenario?
- if so, would they be for inclusion or exclusion of access
- if criteria can be recommended, how would they be implemented?

14. Planned Construction of a report  PL
## ANNEX C

### 1. Fragile X syndrome (full mutation) in Males

**Referrers:**

- Paediatrician, Community Paediatrician or Paediatric neurologist
- Child Psychiatrist
- Clinical Geneticist / Genetic counsellor
- Learning disability consultant / Psychiatrist
- Neurologist

**Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:**

<table>
<thead>
<tr>
<th>Criteria – for index case, (or for testing via their mother in lieu)</th>
<th>Tick if patient meets criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>AND Moderate to severe Developmental delay / learning difficulty (IQ if measured would be 35-70 range)</td>
<td></td>
</tr>
<tr>
<td>AND Does not have profound psychomotor handicap necessitating total care</td>
<td></td>
</tr>
<tr>
<td>AND FraX test specifically requested by referrer (ie. not laboratory-initiated)</td>
<td></td>
</tr>
</tbody>
</table>

**Guidance notes:**

- Typically will have:
  - Gaze avoidance
  - Resists physical contact (tactile defensiveness)
  - Repetitive speech /perseveration (delayed echolalia) & behaviours
  - on autistic spectrum (hand-flapping, hand biting).
  - Inattentive & distractable; motor hyperactivity /restlessness
  - Shy & socially anxious, but also sociably friendly
  - Particular difficulty with numeracy and visuo-spatial skills
  - Head circumference > 50th centile
  - Joint laxity

- Also may have:
  - Family history of learning difficulty
  - Long ears – post-7yrs
  - Large testes – post-pubertally
  - Epilepsy

- Very unlikely to have:
  - High functioning (normal intelligence) autism / Asperger syndrome
  - Classic 'aloof' severe autistic phenotype
  - Microcephaly
### ANNEX D

#### 2. Fragile X syndrome in Females (as index case)

**Referrers:**

<table>
<thead>
<tr>
<th>Paediatrician, Community Paediatrician or Paediatric neurologist</th>
<th>Child Psychiatrist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Geneticist / Genetic counsellor</td>
<td>Learning disability consultant / Psychiatrist</td>
</tr>
<tr>
<td>Neurologist</td>
<td></td>
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</tbody>
</table>

**Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:**

<table>
<thead>
<tr>
<th>Criteria – for index case: (parents are not a substitute)</th>
<th>Tick if patient meets criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
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<tr>
<td>AND learning difficulty (usually mild, IQ often 80-85; but can be moderate or severe LD)</td>
<td></td>
</tr>
<tr>
<td>AND Does not have profound psychomotor handicap necessitating total care</td>
<td></td>
</tr>
<tr>
<td>AND FraX test specifically requested by referrer (ie. not laboratory-initiated)</td>
<td></td>
</tr>
</tbody>
</table>

**Guidance notes:**

- Often will have:
  - Attention difficulty
  - Gaze avoidance
  - Resists physical contact (tactile defensiveness)
  - Communication problems, impulsive social faux pas in speech
  - Shy, self-conscious & socially anxious behaviour
  - Obsessionality
  - Passivity
  - Particular difficulty with maths and visuo-spatial skills
  - Poor adaptive skills / vulnerable
- Also may have:
  - Family history of learning difficulty

- Very unlikely to be presenting primarily with:
  - High functioning autism / Asperger syndrome
  - Classic ‘aloof’ severe autistic phenotype
  - Microcephaly
### ANNEX E

#### 3. Fragile X carrier, or premutation-test in relatives with known family history

**Referrers:**

<table>
<thead>
<tr>
<th>Clinical Geneticist / Genetic counsellor</th>
<th>Obstetrician / fetal medicine specialist</th>
</tr>
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**Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Tick if patient meets criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known family history of FraX, premutation FraX, or intermediate allele of uncertain stability and ≥ 56 repeats (but excluding generationally stable intermediate allele), AND potentially sharing same X chromosome.</td>
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<tr>
<td>OR Female with close family history of male relative with severe learning difficulty without specific diagnosis, especially if he fits guidance notes for affected male (see specific dossier).</td>
<td></td>
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</table>