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Diagnosis and Prevention of Fragile-X Syndrome. From the Family Study to the Population Screening Programme: Eighteen Years of Activity

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INTRODUCTION

Fragile-X syndrome, which derives its name from the expression of a fragile site (FRAXA) at Xq27.3 associated with the phenotype, has achieved distinction as the most common inherited cause of mental retardation. It is the first disorder shown to be due to dynamic mutation in heritable instable DNA.

In 1991 the mutation responsible for Fragile-X syndrome was delineated as an expansion of the trinucleotide (CGG) sequence within an evolutionarily conserved gene, at the position of the fragile-X site.

The DNA of the promoter in the 5' UTR region of FMR-1 gene becomes abnormally methylated when the CGG sequence exceeds approximately 230 repeats, resulting in the transcriptional suppression of FMR-1. Based on the length of CGG repeat in the FMR-1 gene, the alleles are usually classified as normal, premutation or full mutation. CGG instability correlates with the length of repeats and number of AGGs within the FMR-1 CGG tract. In a minority of cases the Fragile-X syndrome may be due to deletion, or to point mutation in the FMR-1 gene.

Eighteen years of activity

Our adventure with Fragile-X syndrome starts in April 1974, with the identification of a fragile site on Xq27 in two mentally retarded brothers with very large ears.

Few years later, the papers by Giraud (1976), Harvey (1977) and Sutherland (1977) led us to reconsider the available data concerning our patients, and to ascertain that we were dealing with Fragile-X syndrome. Lubs (1969) first had described a marker X chromosome in mentally retarded males but his observation went unconfirmed for years.

We hence started a programme of cytogenetic analysis in a large survey of boys referred to our Center of Human Genetics because mentally retarded and/or for unexplained special educational needs. We have examined, using cytogenetic analysis, 268 boys: 15 fra-X probands belonging to 12 families were diagnosed by fragile site identification. The efficiency, was 5.6% due to a significant, clinical, preselection.

In 1991 we have introduced in the diagnostic protocol a first molecular technique, using linkage analysis with highly informative microsatellite markers to identify carriers of the Fragile-X syndrome, in Fragile-X families previously cytogenetically assessed. This method proved to be especially useful for establishing the carrier status in 6 pregnant women. The indirect tracking was soon replaced by the direct DNA analysis for the detection of amplification and methylation in the FMR-l gene, using the double digest

Table 1 - General data

Number of unrelated, multigenerational fra-x families		**50**
Number of Full Mutated Subjects		**78**
– Males	59	
Mosaic Full Mutation/Premutation 2		
Mosaic full Mutation/Normal 1		
– Females	19	
Number of premutated subjects		**66**
– Carrier Females	59	
– Normal Transmitting Males	4	
– Males with unstable alleles (50-55-56)	3	

Number of Full Mutated Subjects per Sibship

N° of Families	N° of Full Mutated Subjects per Sibship	Total N° of Full Mutated Subjects
2	4	8
1	3	3
13	2	26
41	1	41
		total 78

Index Subiects		**49**
– Males	MR and/or unexplained educational needs	46
– Females	MR	3

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EcorI+EagI and a probe telomeric to the CpG island (StB12.3), proposed by Rousseau and Mandel (1991). Using this technique we once again examined patients previously diagnosed on cytogenetic basis, other mentally retarded subjects, both males and females and their relatives at risk.

In 1992, we oriented our attention to the new molecular technique, the PCR, that was recognized useful in measuring the number of CGG repats (Fu, 1991). In 1992 we prepared a protocol of analysis that associates a method of nonradioactive PCR, Southern blotting and cytogenetic testing, for the screening of referrals for Fragile-X mutations. In particular, for the non-radioactive PCR technique, we used the method proposed by Wang and Mathew and published in 1993, partly modified, for the detection of both normal and mutant alleles.

This method permits rapid identification of full mutation and allows accurate resolution of normal alleles as well as simultaneous detection of carrier alleles. The limits of this techinque are represented by the "negative" result of amplification of very large expanded alleles in affected males and in a part of carrier females and normal transmitting males. The largest FRAXA allele amplified with this protocol, in our experience, contained about 135 repeats: however it is important to underline that all premutated males and 80% premutated females were accurately identified with the non-radioactive PCR analysis. Confirmation of the presence of expanded alleles must be carried out by Southern blot which is also essential to clarify the females shown to be homozygous for a normal allele by PCR analysis in Metaphor minigel or in non-denaturing 10% polyacrylamide gel.

This protocol enables to easily perform the molecular analysis in all subjects at risk in each Fragile-X family, with the main goal of prevention. In fact the increasingly better knowledge of transmission mechanisms and of genotype/phenotype correlation in the Fragile-X syndrome made it of primary importance to extend the FMR-1 mutation analysis from the propositus (i) to all, male and female relatives, possibly affected, carrier females or normal transmitting males. The results obtained by using the combined method of molecular analysis are reported in Table 2.

Table 2 - Results from Fragile-X screening with combined method of molecular analysis

Number of Fragile-X Families studied		**44**
– Families previously identified by cytogenetic analysis	6	
– New Families	38	
Number of MR subjects analyzed		**475**
– Number of new Index Patients identified	42	
– Efficiency	8.8%	
Number of relatives at risk analyzed		**361**
– Number of Fra-X subjects identified	73	
– Full mutations	21	
– Premutations	52	

From the prevention at the family level we are now looking towards an efficient screening programme at the population level.

The epidemiological data on the frequency of individuals having clinical effects from the FMR-1 gene mutations and of premutated females and males are more and more significant and comparable:

- The estimated prevalence rate of the Fragile-X syndrome is 1 in 1250 males and 1 in 2500 females.
- The estimated prevalence rate of the premutation is 1 in 1538 males and 1 in 769 females; the most liberal estimate of premutation prevalence would include the subjects with a stable 52 repeat allele increasing the estimated prevalence to approximately 1 in 384 males and 1 in 192 females.

The availability of reliable DNA testing systems and also FMRP expression analysis using Western blotting, or the more recent antibody test, strengthens the case for the introduction of population screening programmes which could be targeted in different ways. Screening the population at large or screening population subgroups? And which subgroups?

The choice of the screening policy and approaches is especially critical and difficult, and is a priority subject for discussion and collaborative planning at national and international levels.

A number of papers report the preliminary data on screenings for Fragile X in different countries. On the basis of such experiences and also guided by the results obtained in the last four years of research and diagnostic activity on Fragile-X syndrome, we decided to start performing, in our Center, experimental screenings in four different subgroups of population. A proposal is now being evaluated, for a multiregional screening for all newborn males to determine the frequency of full mutation in Italy.

On January 1995 we started to screen the following four subgroups:

1) *Institutionalized Retarded Males* (previous studies have demonstrated a prevalence of the Fra-X syndrome as high as 6%)
2) *Women with Precocious Ovarian Failure* (it has been suggested that Fra-X premutated women have an increased incidence of Premature Ovarian Failure - POF)
3) *DNA Registry Subjects with Negative Family History for Mental Retardation*: testing was offered at our Human Genetics Center during genetic counseling, and widely accepted on a self-pay basis
4) *Students of the Medical School of Medicine*, both males and females, aged 18-20, with informed consensus

The preliminary data are shown in Table 3. The four experimental screenings are expected to be completed within two years: the results should provide data instrumental in taking the final decisions about the screening options for Fragile-X syndrome in our country.

The combined protocol of molecular analysis has shown to be highly effective; its potential applications and the positive acceptance of the testing by the general population (as may be inferred from the favourable response among the subjects included in all four subgroups), contribute in forming a valid basis towards the aimed goal of Fragile-X syndrome prevention.

Table 3 - Experimental screening for Fragile-X syndrome (preliminary data, not included in Table 1)

1. Institutionalized Mentally retarded Males	Adults	subjects analyzed	56
		subjects affected	2
	Children	screening just started	–
2. Women showing POF		subjects analyzed	20
		subjects affected	1
3. DNA registry		subjects analyzed	158
		premutations	1
		unstable alleles – maes (40-45) – 1 female (45)	3
4. Students from the Medical School		subjects analyzed	53
		Fra-X subjects	0

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