Twin sisters, monozygotic with the fragile X mutation, but with a different phenotype

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Twin sisters, monozygotic with the fragile X mutation, but with a different phenotype

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Abstract

The absence of the fragile X mental retardation protein (FMRP) results in fragile X syndrome. All males with a full mutation in the *FMR1* gene and an inactive *FMR1* gene are mentally retarded while 60% of the females with a full mutation are affected. Here we describe monozygotic twin sisters who both have a full mutation in their *FMR1* gene, one of whom is normal while the other is affected. Using molecular and protein studies it was shown that owing to preferential X inactivation in the affected female a minority of the cells expressed the normal *FMR1* gene, while in her sister most cells expressed the normal *FMR1* gene. This shows that X inactivation took place in the female twins after separation of the embryos and that for a normal phenotype *FMR1* expression is necessary in the majority of cells.

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Keywords: fragile X syndrome; mental retardation; monozygotic twins; Lyonisation

Fragile X syndrome is the most common form of X linked inherited mental retardation in humans with a prevalence of 1:4000 males and 1:6000 females.[1] The basic genetic defect is an increase in length of a stretch of CGG triplet repeats (>200 repeats, full mutation) within the *FMR1* gene.[2] The large number of CGG repeats leads to silencing of the *FMR1* gene and consequently the absence of the encoded *FMR1* protein (FMRP).[3 4] The absence of FMRP in the brain is responsible for the observed mental retardation. Males carrying the full mutation are always affected, whereas females carrying a full mutation show mental impairment in only approximately 60% of cases. Females carrying a full mutation are characterised by cells with and without FMRP expression, which can be explained by inactivation of the X chromosome (Lyonisation). Thus, in cells without FMRP expression the normal *FMR1* allele is inactivated, whereas in cells with FMRP expression the mutant *FMR1* allele is inactivated. It has been suggested that an insufficient number of FMRP expressing neurones in the brain in affected females causes the mental retardation as a result of the proportion of mutant *FMR1* alleles on the active X chromosome.

Monozygous twins are genetically identical and concordance for cognitive function between monozygous twins carrying a full mutation would be expected. At least three cases of live born male monozygotic twins with the fragile X syndrome have been published and three pairs of brothers were concordant for the mental retardation.[5–7] One of the male twins had a different size of the full mutation.[6] No information about the time of twinning was available. Of the four sister pairs with retardation,[5 6 8 9] one pair was concordant for mental retardation.[5] Of the other three, only one sister was mentally retarded. Two of the three pairs had significant differences in X inactivation pattern showing that in the normal girl the X chromosome with the full mutation was preferentially inactivated. For an overview of twin studies in fragile X syndrome see Helderman *et al*.[10]

Here we describe the unexpected observation of different phenotypes in monozygotic twin sisters with the full mutation. One sister is affected and is living in an institute for the mentally handicapped without having typical features of fragile X syndrome, but this is often seen in affected females. The other sister is intellectually normal and has finished secondary school. Unfortunately their IQ values are not available.

Molecular studies on DNA isolated from blood showed that both had a full mutation (fig 1), but in the normal sister in almost all blood cells the normal X chromosome was active ("N active"), while in the affected sister the normal X chromosome is inactive in approximately 50% of her blood cells ("N active + N inactive"). However, it should be noted that in DNA studies performed so far, only a weak correlation has been found between the X inactivation pattern and cognitive function.[11–15] However, this weak correlation is not strong enough to be a prognostic indicator.

Recently, we described a non-invasive antibody test for fragile X syndrome using hair root analysis.[16] This test offers an affordable and alternative tool for identifying fragile X patients. We have hypothesised that this new test might be of value for predicting the mental capacities of females carrying a full mutation because hair roots originate, like brain tissue, from the ectoderm during embryonic development.

The affected sister shows FMRP expression in 35% of 20 hair roots, whereas her mentally normal sister shows it in 79% of 18 hair roots (fig 1). In controls (>130 persons), FMRP expression in hair roots was found to be between 77 and 100%, whereas affected females carrying a full mutation showed FMRP expression in only some of their hair roots (<55%).[16]

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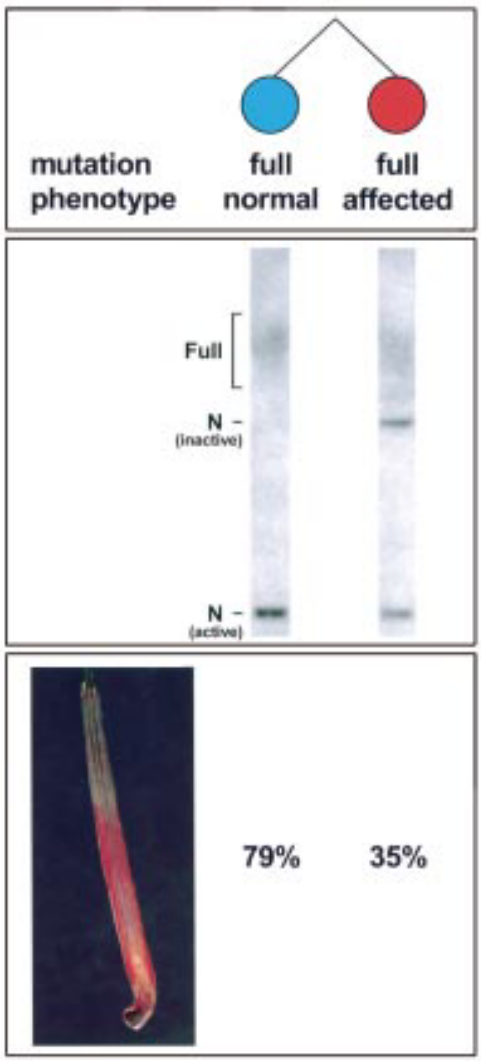

Figure 1 (Upper panel) Family pedigree showing monozygotic twin sisters with fragile X mutation and their phenotype. (Middle panel) Analysis of the FMR1 gene with probe pP2 of HindIII and EagI digested DNA isolated from blood; N active: normal allele on active X chromosome; N inactive: normal allele on inactive X chromosome; Full: inactive allele with full mutation. (Lower panel) Percentage of hair roots expressing FMRP using an indirect alkaline phosphatase immunolabelling technique.[16]

In conclusion, we were able to show in this case a correlation between FMRP expression in hair roots and cognitive function in these monozygotic twin sisters, who are discordant for the fragile X syndrome. We can explain the discordance of mental capacity between the twin sisters by the fact that the number of FMRP expressing neurones in the brain of the affected sister is insufficient to prevent mental retardation. In contrast, her normal sister has a sufficient number of FMRP expressing neurones in the brain to develop normal cognitive function in adult life. The results look promising for the development of a diagnostic test to predict the mental status of females carrying the full mutation. In addition, these results also show that the Lyonisation and inactivation patterning took place in the female twins after separation of the embryos.

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