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Decreased fragile X mental retardation protein (FMRP) is associated with lower IQ and earlier illness onset in patients with schizophrenia

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ABSTRACT

The purpose of this study was to investigate Fragile X Syndrome (FXS)-related mechanisms in schizophrenia, including CGG triplet expansion, *FMR1* mRNA, and fragile X mental retardation protein (FMRP) levels in lymphocytes. We investigated 36 patients with schizophrenia and 30 healthy controls using Southern blot analysis, mRNA assay, and enzyme-linked immunosorbent assay (ELISA). General intellectual functions were assessed with the Wechsler Adult Intelligence Scale-III, and the clinical symptoms were evaluated with the Positive and Negative Syndrome Scale. Results revealed that, relative to healthy controls, CGG triplet size and *FMR1* mRNA were unaltered in patients with schizophrenia. However, the FMRP level was significantly reduced in patients compared with controls. We found an association between lower FMRP levels, reduced IQ, and earlier illness onset in schizophrenia. Chlorpromazine-equivalent antipsychotic dose did not correlate with FMRP levels. These results raise the possibility of impaired translation of *FMR1* mRNA, altered epigenetic regulation, or increased degradation of FMRP in schizophrenia, which may play a role in dysfunctional neurodevelopmental processes and impaired neuroplasticity.

1. Introduction

Fragile X mental retardation protein (FMRP) is an RNA binding protein, which is abundant in the soma and dendritic spines of neurons. FMRP is a translational suppressor for several genes encoding proteins critical for synaptic transmission and plasticity, such as subunits of glutamate receptors and postsynaptic proteins (O'Donnell and Warren, 2002; Bear et al., 2008; Rousseau et al., 2011). The absence of FMRP characterizes fragile X syndrome (FXS), which is a prevalent form of inherited mental disabilities (Reiss and Hall, 2007). In FXS, the absence of FMRP is due to the silencing of the *FMR1* gene, which is caused by the expansion of a CGG trinucleotide repeat (Xq27.3, >200 repeats in the full syndrome; 55–200 repeats in premutation carriers) and increased methylation of the promoter region (O'Donnell and Warren, 2002; Bear et al., 2008; Rousseau et al., 2011).

There is increasing evidence that *FMR1*/FMRP is also involved in the pathogenesis of other disorders, such as premature ovarian insufficiency, fragile X-associated tremor/ataxia syndrome, and autism (Hagerman et al., 2010). Moreover, the spectrum of fragile X-related disorders might include some forms of schizophrenia, anxiety, and mood disorders (Bourgeois et al., 2009; Fatemi and Folsom, 2011). This hypothesis was recently supported by the observation of Fatemi et al. (2010), who described reduced FMRP expression in the lateral cerebellum of patients with schizophrenia, bipolar disorder, and major depressive disorder. FMRP is implicated in glutamatergic and GABA-ergic processes, which are key pathophysiological mechanisms of schizophrenia (Fatemi and Folsom, 2011).

The purpose of the present study was to investigate whether FMRP is decreased in the peripheral blood of patients with schizophrenia and to assess its relationship with *FMR1* mRNA level. In addition, we explored the relationship between FMRP expression and clinical characteristics of the patients. We hypothesized that FMRP is associated with IQ, given that the absence of this protein is accompanied by a severe cognitive disability in patients with FXS.

2. Methods

2.1. Participants

Participants comprised 36 outpatients with schizophrenia and 30 healthy volunteers. The control participants were university/hospital employees and their non-biological relatives and acquaintances. All participants were assessed with

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the Structured Clinical Interview for DSM-IV axis I disorders (SCID-CV; First et al., 1996) and the Wechsler Adult Intelligence Scale-III (Wechsler, 1997). Full medical records were available, and all patients had a previous diagnosis of schizophrenia. We used the SCID-CV to confirm this diagnosis. The control participants had no Axis I psychiatric disorders and had a negative family history for psychotic disorders. Exclusion criteria included neurological disorders, head injury, and history of psychoactive substance dependence. For the assessment of clinical symptoms, the Positive and Negative Syndrome Scale (PANSS) was used (Kay et al., 1987). In the PANSS, positive and negative symptoms are evaluated by 7–7 items (minimum score=7, maximum score=49 for positive and negative symptoms separately), and general psychopathology symptoms are evaluated by 16 items (minimum score=16, maximum score=112). All patients received antipsychotic medications at the time of testing (olanzapine: $n=14$, risperidone: $n=11$, quetiapine: $n=4$, risperidone plus quetiapine: $n=7$). Antipsychotic doses were converted into chlorpromazine equivalents (Woods, 2003). The patients did not receive any other psychotropic medications at the time of testing. Table 1 summarizes the clinical and demographic characteristics of the participants. The study was carried out in accordance with the Declaration of Helsinki and was approved by the local ethics board. All participants gave written informed consent.

2.2. Molecular biological measurements

All molecular measurements were based on published standard protocols. Peripheral blood was drawn from the cubital vein of the participants. CGG repeat size was determined using Southern blot analysis (Steyaert et al., 2003). We measured *FMR1* mRNA using Affymetrix Quantigene (Vala Sciences Inc., CA) by adopting the protocol of Tassone et al. (2000). For the quantitative measurement of FMRP levels, we used a new enzyme-linked immunosorbent assay (ELISA) method (Iwahashi et al., 2009). This method uses a combination of avian and murine antibodies, and it is highly sensitive and specific for FMRP in peripheral blood lymphocytes. The correlation of this ELISA approach with Western blot analysis of lymphocyte extracts is $r=0.99$ (Iwahashi et al., 2009). Further advantages of this method are that it is suitable for the quantification of FMRP levels in biologically relevant ranges, specific for the intact FMRP protein, applicable in non-transformed cells, and scalable for large sample numbers. Concentrations of FMRP were determined using SoftMax Pro 4-parameter fit logistics curve (pM; Iwahashi et al., 2009).

3. Results

Table 2 summarizes the results. Given that the variables were not normally distributed, as revealed by Kolmogorov–Smirnov tests, we used non-parametric Mann–Whitney *U*-tests. Patients with schizophrenia displayed normal CGG triplet size and *FMR1* mRNA level. In contrast, the level of FMRP was significantly reduced in patients relative to controls (Table 2). Earlier age at illness onset and lower IQ were associated with decreased FMRP levels in patients with schizophrenia (illness onset: Spearman's $R=0.59$, $p<0.001$; IQ: Spearman's $R=0.66$, $p<0.001$). Regarding the IQ, a similar trend was observed in healthy controls, but it did not reach the level of statistical significance (Spearman's $R=0.24$, $p=0.17$) (Fig. 1). There was no significant difference between male and female participants ($p>0.5$).

Table 1
Demographic and clinical characteristics.

	Schizophrenia ($n=36$)	Control ($n=30$)
Male/female	22/14	20/10
Age (years)	35.5 (6.9)	36.4 (8.3)
IQ*	97.5 (9.6)	103.3 (8.9)
Age of illness onset (years)	24.0 (4.6)	–
PANSS positive	19.7 (5.4)	–
PANSS negative	17.8 (6.3)	–
PANSS general	46.7 (9.1)	–
Antipsychotics (chlorpromazine-equivalent, mg/day)	460.5 (120.9)	–

Data are mean (standard deviation). PANSS—Positive and Negative Syndrome Scale.

* $t(64)=-2.50$, $p<0.05$.

Table 2
CGG triplet size, *FMR1* mRNA, and FMRP levels.

	Schizophrenia ($n=36$)			Control ($n=30$)		
	Mean	S.D.	Range	Mean	S.D.	Range
CGG triplet size	28.4	3.7	17–32	28.7	3.1	19–33
FMR1 mRNA level	1.6	0.4	1–1.9	1.7	0.3	1–1.8
FMRP level*	78.4	75.3	18–340	124.8	91.0	40–420

FMR1—Fragile X Mental Retardation 1; FMRP—Fragile X Mental Retardation Protein.

* $Z=-3.25$, $p<0.005$ (Mann–Whitney *U* test).

We conducted a multiple regression analysis to control for age and gender in patients with schizophrenia (data were log-transformed); this model also included age at illness onset and IQ. The analysis indicated that illness onset and IQ were significant predictors of the FMRP level (illness onset: $b^*=0.43$, $t(31)=2.67$, $p<0.05$; IQ: $b^*=0.46$, $t(31)=3.30$, $p<0.05$).

Finally, PANSS scores and the daily chlorpromazine-equivalent dose of antipsychotics did not correlate with FMRP levels (Spearman's R: $-0.2<R<0.2$, $p>0.1$).

4. Discussion

The results of the present study demonstrated that the mechanism behind low peripheral FMRP in patients with schizophrenia is different from that observed in FXS. Patients with FXS display CGG triplet expansion, and decreased *FMR1* mRNA and FMRP, whereas patients with schizophrenia are characterized by reduced FMRP together with normal CGG triplet size and *FMR1* mRNA. This suggests that the dysfunction may be related to abnormal translation of *FMR1* mRNA, although the CGG triplet is a key factor in determining translational efficacy, and it was not altered in patients. An alternative explanation is that the degradation of FMRP is accelerated in schizophrenia. Future studies should also take into consideration the potential role of epigenetic regulations of *FMR1* (Stöger et al., 2011).

The most important finding of the study was that decreased FMRP was associated with earlier illness onset and lower IQ. This suggests that reduced FMRP may be related to neurodevelopmental changes, neuroplasticity, and cognitive functions. In an extensive meta-analysis of thousands of cases and controls, it was confirmed that greater premorbid IQ decrement is associated with earlier illness onset (Khandaker et al., 2011). In the case of cross-sectional studies, however, neuropsychological functions are linked to current IQ instead of to IQ trajectory during the disease course (Kremen et al., 2008).

Similarly to our previous study (Kéri and Benedek, 2011, 2012), we found no significant correlation between IQ and FMRP in healthy controls, although the tendency was similar to that revealed in patients with schizophrenia. This similar tendency may suggest that FMRP may be implicated in neurodevelopment and neuroplasticity underlying the emergence of general cognitive abilities, and it is not disease specific for schizophrenia. Previously, we observed similar correlations between visual functions and FMRP expression in healthy controls (Kéri and Benedek, 2011, 2012), with a special reference to contrast sensitivity functions disrupted in FXS and schizophrenia (Slaghuis, 1998; Kogan et al., 2004). In this case, similarly to IQ, FMRP may be important, even under non-pathological circumstances, in the physiological range of expression.

Loat et al. (2006) studied the relationship between *FMR1* allele length and cognitive functions in low ability, control, and high-IQ children. In males, there was a significant negative correlation

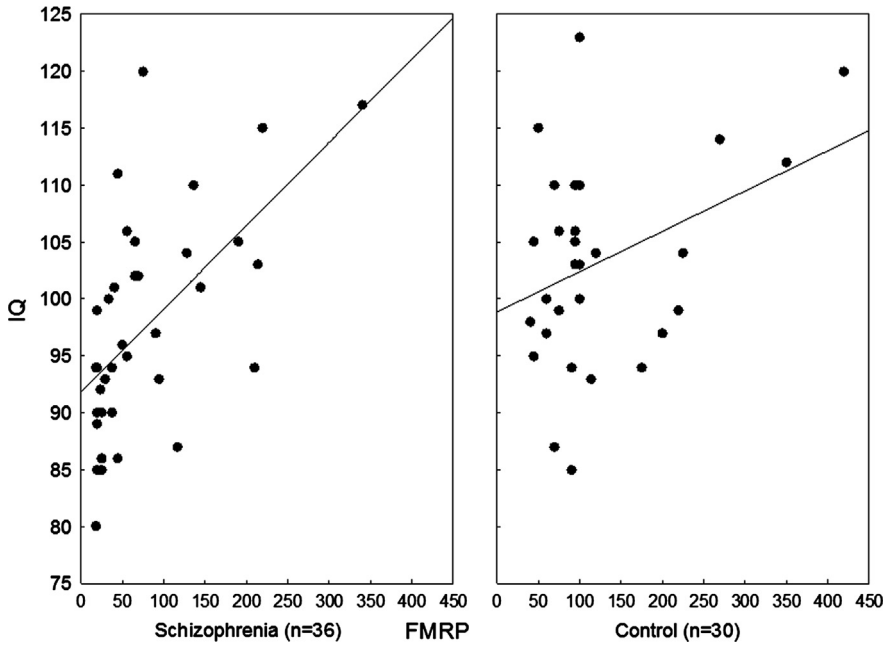

Fig. 1. Correlations between IQ and FMRP (fragile X mental retardation protein) in patients with schizophrenia and controls.

between allele length, non-verbal ability, and IQ. Mínguez et al. (2009) demonstrated that in premutation carriers, IQ scores tend to decrease when the number of CGG repeats is higher and the FMRP values are lower (but see also Franke et al., 1998, 1999). In patients with schizophrenia, we found a similar relationship between FMRP and IQ, but in this case, CGG triplet size was normal.

The most important limitation of the present study is that we used a correlational approach with a cross-sectional design, and we cannot establish a firm causal relationship between the dependent measures. Therefore, it remains to be confirmed whether reduced FMRP plays a causal role in schizophrenia and the associated cognitive deficit. Although we did not find a correlation between FMRP and antipsychotic dose, the results must be replicated in unmedicated patients. Furthermore, that illness-onset was correlated with FMRP levels, but current antipsychotic dosage was not, could be indicative that long-term antipsychotic medication may have a greater effect on FMRP levels. Long-term antipsychotic administration may lead to decreased IQ, although it can be seen only when unusually high doses and multiple medications are administered (Hori et al., 2006). Based on the medical records of our patients, however, there was no evidence for unusually high antipsychotic doses.

Although FXS exhibits many similarities with autism, its relationship with psychotic disorders is less convincing. In female premutation carriers, early studies revealed pronounced schizotypal features and lower IQ (Reiss et al., 1988; Freund et al., 1992). Nicolson et al. (1999) suggested that cytogenetic abnormalities are increased in cases of childhood-onset schizophrenia, consistent with a model that earlier onset is due to a marked impairment of neurodevelopmental processes. However, the authors did not confirm the role of fragile-X associated mechanisms (see also DeLisi et al., 1988; Jönsson et al., 1995; Franke et al., 1996). Others concluded that mutations in the *FMR1* gene do not have a role in schizophrenia, but it is possible that *FMR1* mutations can modify the clinical appearance of the illness, with a special relevance to developmental delay (Ashworth et al., 1996). Khin et al. (1998) described a male psychotic patient who had an *FMR1* gene methylation mosaicism. More recently, Tam et al. (2010) found a copy number variant in cytoplasmic FMR1-interacting protein 1 (CYFIP1) gene in patients with schizophrenia.

The results of our study are consistent with the data indicating that fragile-X-related mechanisms are not altered at gene and mRNA levels in schizophrenia. However, a reduced level of FMRP was associated with lower IQ and earlier illness onset, which may represent the neurodevelopmental aspects of schizophrenia.

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References

Ashworth, A., Abusaad, I., Walsh, C., Nanko, S., Murray, R.M., Asherson, P., McGuffin, P., Gill, M., Owen, M.J., Collier, D.A., 1996. Linkage analysis of the fragile X gene FMR-1 and schizophrenia: no evidence for linkage but report of a family with schizophrenia and an unstable triplet repeat. Psychiatric Genetics 6, 81–86.

Bear, M.F., Dölen, G., Osterweil, E., Nagaraja, N., 2008. Fragile X: translation in action. Neuropsychopharmacology 33, 84–87.

Bourgeois, J.A., Coffey, S.M., Rivera, S.M., Hessl, D., Gane, L.W., Tassone, F., Greco, C., Finucane, B., Nelson, L., Berry-Kravis, E., Grigsby, J., Hagerman, P.J., Hagerman, R.J., 2009. A review of fragile X premutation disorders: expanding the psychiatric perspective. Journal of Clinical Psychiatry 70, 852–862.

DeLisi, L.E., Reiss, A.L., White, B.J., Gershon, E.S., 1988. Cytogenetic studies of males with schizophrenia. Screening for the fragile X chromosome and other chromosomal abnormalities. Schizophrenia Research 1, 277–281.

Fatemi, S.H., Folsom, T.D., 2011. The role of fragile X mental retardation protein in major mental disorders. Neuropharmacology 60, 1221–1226.

Fatemi, S.H., Kneeland, R.E., Liesch, S.B., Folsom, T.D., 2010. Fragile X mental retardation protein levels are decreased in major psychiatric disorders. Schizophrenia Research 124, 246–247.

First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B.W., 1996. Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV). American Psychiatric Press, Washington, DC.

Franke, P., Leboyer, M., Gänsicke, M., Weiffenbach, O., Biancalana, V., Cornillet-Lefebre, P., Croquette, M.F., Froster, U., Schwab, S.G., Poustka, F., Hautzinger, M., Maier, W., 1998. Genotype-phenotype relationship in female carriers of the premutation and full mutation of FMR-1. Psychiatry Research 80, 113–127.

Franke, P., Leboyer, M., Hardt, J., Sohne, E., Weiffenbach, O., Biancalana, V.V., Cornillet-Lefebre, P., Delobel, B., Froster, U., Schwab, S.G., Poustka, F., Hautzinger, M., Maier, W., 1999. Neuropsychological profiles of FMR-1 premutation and full-mutation carrier females. Psychiatry Research 87, 223–231.

Franke, P., Maier, W., Hautzinger, M., Weiffenbach, O., Gänsicke, M., Iwers, B., Poustka, F., Schwab, S.G., Froster, U., 1996. Fragile-X carrier females: evidence for a distinct psychopathological phenotype? American Journal of Medical Genetics 64, 334–339.

Freund, L.S., Reiss, A.L., Hagerman, R., Vinogradov, S., 1992. Chromosome fragility and psychopathology in obligate female carriers of the fragile X chromosome. Archives of General Psychiatry 49, 54–60.

Hagerman, R., Hoem, G., Hagerman, P., 2010. Fragile X and autism: intertwined at the molecular level leading to targeted treatments. Molecular Autism 1, 12.

Hori, H., Noguchi, H., Hashimoto, R., Nakabayashi, T., Omori, M., Takahashi, S., Tsukue, R., Anami, K., Hirabayashi, N., Harada, S., Saitoh, O., Iwase, M., Kajimoto, O., Takeda, M., Okabe, S., Kunugi, H., 2006. Antipsychotic medication and cognitive function in schizophrenia. Schizophrenia Research 86, 138–146.

Iwahashi, C., Tassone, F., Hagerman, R.J., Yasui, D., Parrott, G., Nguyen, D., Mayeur, G., Hagerman, P.J., 2009. A quantitative ELISA assay for the fragile x mental retardation 1 protein. Journal of Molecular Diagnostics 11, 281–289.

Jönsson, E., Björck, E., Wahlström, J., Gustavsson, P., Sedvall, G., 1995. Screening for CGG trinucleotide repeat expansion in the fragile X mental retardation 1 gene in schizophrenic patients. Psychiatric Genetics 5, 157–160.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. Schizophrenia Bulletin 13, 261–276.

Kéri, S., Benedek, G., 2011. Fragile X protein expression is linked to visual functions in healthy male volunteers. Neuroscience 192, 345–350.

Kéri, S., Benedek, G., 2012. Why is vision impaired in fragile X premutation carriers? The role of fragile X mental retardation protein and potential FMR1 mRNA toxicity. Neuroscience 206, 183–189.

Khandaker, G.M., Barnett, J.H., White, I.R., Jones, P.B., 2011. A quantitative meta-analysis of population-based studies of premorbid intelligence and schizophrenia. Schizophrenia Research 132, 220–227.

Khin, N.A., Tarleton, J., Raghu, B., Park, S.K., 1998. Clinical description of an adult male with psychosis who showed FMR1 gene methylation mosaicism. American Journal of Medical Genetics 81, 222–224.

Kogan, C.S., Boutet, I., Cornish, K., Zangenehpour, S., Mullen, K.T., Holden, J.J., Der Kaloustian, V.M., Andermann, E., Chaudhuri, A., 2004. Differential impact of the FMR1 gene on visual processing in fragile X syndrome. Brain 127, 591–601.

Kremen, W.S., Seidman, L.J., Faraone, S.V., Tsuang, M.T., 2008. IQ decline in cross-sectional studies of schizophrenia: methodology and interpretation. Psychiatry Research 158, 181–194.

Loat, C.S., Craig, G., Plomin, R., Craig, I.W., 2006. Investigating the relationship between FMR1 allele length and cognitive ability in children: a subtle effect of the normal allele range on the normal ability range? Annals of Human Genetics 70, 555–565.

Mínguez, M., Ibáñez, B., Ribate, M.P., Ramos, F., García-Alegría, E., Fernández-Rivas, A., Ruiz-Parra, E., Poch, M., Alonso, A., Martinez-Bouzas, C., Beristain, E., Tejada, M.I., 2009. Risk of cognitive impairment in female premutation carriers of fragile X premutation: analysis by means of robust segmented linear regression models. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 150B, 262–270.

Nicolson, R., Giedd, J.N., Lenane, M., Hamburger, S., Singaracharlu, S., Bedwell, J., Fernandez, T., Thaker, G.K., Malaspina, D., Rapoport, J.L., 1999. Clinical and neurobiological correlates of cytogenetic abnormalities in childhood-onset schizophrenia. American Journal of Psychiatry 156, 1575–1579.

O'Donnell, W.T., Warren, S.T., 2002. A decade of molecular studies of fragile X syndrome. Annual Review of Neuroscience 25, 315–338.

Reiss, A.L., Hagerman, R.J., Vinogradov, S., Abrams, M., King, R.J., 1988. Psychiatric disability in female carriers of the fragile X chromosome. Archives of General Psychiatry 45, 25–30.

Reiss, A.L., Hall, S.S., 2007. Fragile X syndrome: assessment and treatment implications. Child and Adolescent Psychiatric Clinics of North America 16, 663–675.

Rousseau, F., Labelle, Y., Bussières, J., Lindsay, C., 2011. The fragile x mental retardation syndrome 20 years after the FMR1 gene discovery: an expanding universe of knowledge. Clinical Biochemist Reviews 32, 135–162.

Slaghuis, W.L., 1998. Contrast sensitivity for stationary and drifting spatial frequency gratings in positive- and negative-symptom schizophrenia. Journal of Abnormal Psychology 107, 49–62.

Steyaert, J., Legius, E., Borghgraef, M., Fryns, J.P., 2003. A distinct neurocognitive phenotype in female fragile-X premutation carriers assessed with visual attention tasks. American Journal of Medical Genetics Part A 116, 44–51.

Stöger, R., Genereux, D.P., Hagerman, R.J., Hagerman, P.J., Tassone, F., Laird, C.D., 2011. Testing the FMR1 promoter for mosaicism in DNA methylation among CpG sites, strands, and cells in FMR1-expressing males with fragile X syndrome. PLoS One 6, e23648.

Tam, G.W., van de Lagemaat, L.N., Redon, R., Strathdee, K.E., Croning, M.D., Malloy, M.P., Muir, W.J., Pickard, B.S., Deary, I.J., Blackwood, D.H., Carter, N.P., Grant, S.G., 2010. Confirmed rare copy number variants implicate novel genes in schizophrenia. Biochemical Society Transactions 38, 445–451.

Tassone, F., Hagerman, R.J., Taylor, A.K., Gane, L.W., Godfrey, T.E., Hagerman, P.J., 2000. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. American Journal of Human Genetics 66, 6–15.

Wechsler, D., 1997. The Wechsler Adult Intelligence Scale-III: Administration and Scoring Manual. The Psychological Corporation, San Antonio, TX.

Woods, S.W., 2003. Chlorpromazine equivalent doses for the newer atypical antipsychotics. Journal of Clinical Psychiatry 64, 663–667.