A Metabolomic and Systems Biology Perspective on the Brain of the Fragile X Syndrome Mouse Model

Laetitia Davidovic, Vincent Navratil, Carmela M. Bonaccorso, Maria Vincenza Catania, Barbara Bardoni, Marc-Emmanuel Dumas

SUPPLEMENTARY INFORMATION

Generation and Characterization of the polyclonal anti-FMRP antibody

The C-terminus domain spanning amino acids 516-632 of the longest isoform of FMRP Iso1 (Adinolfi et al. 1999) was used to raise a polyclonal antibodies against FMRP in rabbit. This domain is highly evolutionarily conserved among human, mouse and rat sequences as shown by the convergent identity matrix (*SI Fig. 1A*). Human C-terminus diverges mostly from rat and mouse sequences by a 17 aa insertion (*SI Fig. 1A*). Conversely, analysis of human sequences reveals that this domain is highly divergent from the corresponding C-terminal domains of the homologues of FMRP, FXR1P and FXR2P as exemplified by the low identity matrix (*SI Fig. 1B*). These data support the choice of this epitope to generate a polyclonal antibody specifically recognizing FMRP in human and murine tissues with minimal crossreaction with the homologues of FMRP, FXR1P and FXR2P. The His-tagged FMRP C-terminus was purified as described previously (Bechara et al. 2009) and used to produce polyclonal antibodies in rabbit using a standard protocol (Eurogentec). The anti-FMRP IgG were then affinity purified from rabbit R60 serum with the same fusion protein used for immunization as described previously (Davidovic et al. 2007). To assess the specifity of the R60 antibody, 20 μg of lysates from *Fmr1*-knockout fibroblasts (STEK) stably transfected with empty vector pTL1 or expressing pTL1-*FMR1* Isoform 1 (ISO1 described in (Castets et al. 2005)) as well as 30 μg of brain extracts from *Fmr1*-KO and WT mouse brain were loaded on a 7.5% SDS-PAGE. Proteins transferred onto a 0.45 μm nitro-cellulose membrane were revealed using the affinity-purified R60 antibody diluted 1/1000 (*SI Fig. 1B*). No bands were detected in the KO samples. FMRP was detected as a single band in the STEK expressing FMRP ISO1 and as several bands corresponding to its different isoforms in WT brain lysates, as expected and previously shown (Devys et al. 1993). These data confirm that the R60 antibody specifically recognizes FMRP in western blotting.

SUPPLEMENTARY INFORMATION REFERENCES

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SUPPLEMENTARY TABLES LEGENDS

SI Table 1. FXS metabolic pathways for each brain region. The metabolic signatures from Table 1 were mapped onto the KEGG pathway, and a metabolite-set enrichment analysis (MSEA) was performed to test whether each pathway was significantly affected. The most pertinent KEGG pathways are highlighted in **bold**.

SI Table 2. Compilation of known and putative FMRP mRNA or protein interactors. Interaction types are annotated as pri for protein/RNA interaction and ppi for protein-protein interaction. Original references are indicated by their Pubmed Identifier (PMID), molecular interactions replicated by different studies (pri and ppi) are framed.

SI Table 3. Pivotal betweenness of the proteins present in the *FMR1* iMIM network. We have determined how many shortest paths travel through each protein present in the iMIM network (Fig. 5), as summarized by the pivotal betweenness, to identify the key nodes in the network.

SI Table 4a. Gene Ontology (GO) analysis of significantly enriched biological processes considering all the proteins in the *FMR1* iMIM network. The list of all the proteins present in the iMIM network (*i.e.* present on a pivotal shortest path or an alternative shortest path) was used for enrichment in GO terms to identify the pathways involved in the network.

SI Table 4b. Gene Ontology (GO) analysis of significantly enriched biological processes considering FMRP mRNA targets included in the *FMR1* iMIM network. Biological processes highlighted in **bold** are highly relevant to pathways perturbed in FXS.

SI Table 5a. KEGG analysis of significantly enriched biological functions considering all the proteins of the *FMR1* iMIM network ($p<0.05$).

SI Table 5b. KEGG analysis of significantly enriched biological functions considering FMRP mRNA targets present in the *FMR1* iMIM network ($p <0.05$).

SUPPLEMENTARY FIGURE LEGEND

SI Fig. 1. Characterization and validation of the rabbit polyclonal antibodies raised against the C-terminus of FMRP. (*a*) Sequence alignment and identity matrix generated with the ClustalX webtool of the C-termini of *Mus musculus* (mm), *Rattus norvegicus* (r n) and *Homo sapiens* (hs) FMRP. The arrow depicts the 17 aa insertion in human FMRP C-terminus. **(*b*)** Sequence alignment and identity matrix generated with the ClustalX webtool of the C-terminus of human FXR1P, FXR2P and FMRP C-termini. ***(c)*** Western-blotting analysis of lysates from *Fmr1*-knockout fibroblasts (STEK) stably transfected with empty vector pTL1 or expressing pTL1-*FMR1* Iso1 and from *Fmr1*-KO and WT mouse brain using the R60 antibody.