Mutations in protein N-arginine methyltransferases are not the cause of FTLD-FUS


a Department of Neuroscience, Mayo Clinic, Jacksonville, FL, USA
b Department of Neuropathology, University of Tübingen, Tübingen, Germany
c German Center for Neurodegenerative Diseases, Tübingen, Germany
d Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada
e Department of Neurology, Mayo Clinic, Rochester, MN, USA
f Neuroscience Research Australia, Sydney, NSW, and UNSW Medicine, University of New South Wales, Sydney, NSW, Australia
g Disciplines of Medicine and Pathology, Sydney Medical School, The University of Sydney, NSW, Australia
h Department of Neurology, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands, and Vumc Alzheimercenter, Amsterdam
i Department of Neurology, University of California, San Francisco, CA, USA

Abstract

The nuclear protein fused in sarcoma (FUS) is found in cytoplasmic inclusions in a subset of patients with the neurodegenerative disorder frontotemporal lobar degeneration (FTLD-FUS). FUS contains a methylated arginine-glycine-glycine domain which is required for transport into the nucleus. Recent findings have shown that this domain is hypomethylated in patients with FTLD-FUS. To determine if the cause of hypomethylation is the result of mutations in protein N-arginine methyltransferases (PRMTs), we selected 3 candidate genes (PRMT1, PRMT3 and PRMT8) and performed complete sequencing analysis and real-time PCR mRNA expression analysis in 20 FTLD-FUS cases. No mutations or statistically significant changes in expression were observed in our patient samples, suggesting that defects in PRMTs are not the cause of FTLD-FUS.
1. Introduction

Frontotemporal Dementia (FTD) refers to a group of clinical syndromes characterized by progressive changes in behaviour and personality or language. A subgroup of these patients are pathologically characterized by fused in sarcoma (FUS)-positive and TAR DNA binding protein 43 (TDP-43)-negative cytoplasmic inclusions in neuronal and glial cells and referred to as FTLD-FUS (Josephs, et al., 2008, Mackenzie, et al., 2010, Neumann, et al., 2009, Rohrer, et al., 2009, Rohrer, et al., 2011, Seelaar, et al., 2010). Although mutations in FUS are known to cause around 4% of familial amyotrophic lateral sclerosis (ALS) (Kwiatkowski, et al., 2009, Vance, et al., 2009); any genetic causes of FTLD-FUS remain unknown (Snowden, et al., 2011, Urwin, et al., 2010).

FUS belongs to the FET family of proteins along with Ewing’s sarcoma protein (EWS), and the TATA-binding protein-associated factor 15 (TAF15) both of which coaggregate in inclusions with FUS in FTLD-FUS cases (Mackenzie and Neumann, 2012, Neumann, et al., 2011). In addition to FET proteins the cytoplasmic inclusions in FTLD-FUS also contain the nuclear import protein Transportin (TRN) (Neumann, et al., 2012). TRN binds to the shared proline-tyrosine rich C-terminal nuclear localisation signal (PY-NLS) of the FET proteins and transports them between the nucleus and cytoplasm (Chook and Suel, 2011). FET proteins contain an extensively methylated RGG domain (Arginine-Glycine-Glycine) and there is strong evidence to show that dimethylation of the arginine residues in this domain can disrupt the nuclear localisation of the FET proteins (Jobert, et al., 2009, Tradewell, et al., 2012). Moreover, recent findings show a novel TRN binding motif in the RGG domain of FET proteins and the hypomethylation of arginine in this region increases the binding capacity of TRN to FET proteins, the consequence of which is mislocalisation (Dormann, et al., 2012). Since type I Protein N-arginine methyltransferases (PRMTs) dimethylate arginine residues in vivo, we hypothesised that mutations in the genes encoding PRMT1, PRMT3 and PRMT8, all previously shown to methylate FET proteins in vitro (Kim, et al., 2008, Pahlich, et al., 2005), could contribute to the hypomethylation observed in FTLD-FUS.

2. Subjects, Materials and Methods

Through an international collaboration, we collected samples from 20 FTLD-FUS patients from the United States, Canada, Germany, the Netherlands and Australia (Sup. Table 1). All patients had the atypical FTLD-U subtype of FTLD-FUS. DNA of all 20 patients was sequenced for all coding exons and both 3′and 5′untranslated regions of PRMT1, PRMT3 and PRMT8. DNA fragments were amplified using Apex products, purified using the Agencourt Ampure system and sequenced using Big Dye Terminator V3.1 products. Sequencing purification was performed using the Agencourt CleanSEQ method, run on an ABI3730 DNA-analyser with Sequencher used for analysis.

For quantitative real-time PCR mRNA analysis a subset of 8 patients with FTLD-FUS and 7 control brains was used to quantify RNA levels in frontal cortex. RNA was prepared using a Qiagen RNeasyplus mini kit, and cDNA was made using Invitrogen Superscript III first-strand kit. Samples used for the study are indicated in Sup. Table 1. Expression levels of PRMT1 (Hs01587651), PRMT3 (Hs00411605) and PRMT8 (Hs00998598) as well as the housekeeping gene RPLP0 (Hs00420895) and the neuronal marker Synaptophysin (Hs00300531) were measured on an Applied Biosystems 7900HT fast real-time PCR system and analysed using relative quantification (ΔΔCt) in SDS 2.2.2.

3. Results

Sequencing analysis of PRMT1, PRMT3 and PRMT8 in FTLD-FUS patients did not reveal any novel sequence variants. In PRMT3 and PRMT8 we did identify a number of known
sequence variants (Sup. Table 2). Quantitative mRNA expression analysis of \textit{PRMT1} and 
\textit{PRMT3} in frontal cortex brain samples did not show a significant difference in expression 
between the FTLD-FUS cases and controls (P values of 0.523 and 0.2602 respectively). In 
contrast, we found that expression levels of \textit{PRMT8} were significantly lower in the frontal 
cortex of subjects with FTLD-FUS in comparison to controls (P=0.0231) (Sup. Fig. 1). 
However, when the neuronal marker synaptophysin was used for normalization no statistical 
difference was observed between FTLD-FUS patients and control brains (P=0.3842), 
suggesting that the decrease in \textit{PRMT8} expression was due to the neuronal specificity of 
\textit{PRMT8} (Sup. Fig. 2).

4. Discussion

While mutations in \textit{FUS} explain the disease in all ALS patients with FUS pathology, the 
cause of FUS pathology in patients with FTLD remains unknown (Snowden, et al., 2011, 
Urwin, et al., 2010). Importantly, recent studies highlight important differences between 
ALS-FUS and FTLD-FUS suggesting distinct pathomechanisms. First, in contrast to ALS-
FUS, pathological inclusions in FTLD-FUS cases contain EWS and TAF15, as well as TRN, 
suggesting a more general defect in TRN-mediated nuclear import in FTLD-FUS 
the inclusions in ALS-FUS patients contain methylated FUS, while inclusions in FTLD-FUS 
patients are not methylated (Dormann, et al., 2012, Tradewell, et al., 2012).

Based on these findings, we hypothesized that the hypomethylation of FUS seen in FTLD-
FUS patients was a consequence of mutations in or the altered expression of the N-arginine 
methylation proteins \textit{PRMT1}, \textit{PRMT3} or \textit{PRMT8}. However, in-depth sequencing of all 
coding, as well as 5′ and 3′ untranslated regions, and quantitative real-time mRNA 
expression analysis did not identify any mutations or differences in expression between 
FTLD-FUS patients and controls. These results indicate that the mislocalisation of FET 
proteins in FTLD-FUS is not a consequence of any genetic variants in \textit{PRMT1}, \textit{PRMT3} or 
\textit{PRMT8}.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Chook YM, Suel KE. Nuclear import by karyopherin-beta s: Recognition and inhibition. Biochimica 
2010.10.014 
Dormann D, Madl T, Valori CF, Bentmann E, Tahirovic S, Abou-Ajram C, Kremmer E, Ansorge O, 
Mackenzie IRA, Neumann M, Haass C. Arginine methylation next to the PY-NLS modulates 
Transportin binding and nuclear import of FUS. Embo Journal. 2012; 31(22):4258–75.10.1038/ 


