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

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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 methytransferases (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 (ΔΔ Ct) 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 *PRMT1* and *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 *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 *PRMT8* expression was due to the neuronal specificity of *PRMT8* (**Sup. Fig. 2**).

4. Discussion. While mutations in *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 (Mackenzie and Neumann, 2012,Neumann, et al., 2011,Neumann, et al., 2012). Second, 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 PRMT1, PRMT3 or 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 *PRMT1*, *PRMT3* or *PRMT8.*

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Disclosure Statement

The authors disclose no conflicts of interest.

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