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Research paper

# Alternative splicing of *SMPD1* coding for acid sphingomyelinase in major depression

Cosima Rhein[a,b,*], Martin Reichel[a,1], Marcel Kramer[c,d], Andrea Rotter[a], Bernd Lenz[a], Christiane Mühle[a], Erich Gulbins[e], Johannes Kornhuber[a]

[a] *Department of Psychiatry and Psychotherapy, University Hospital, Friedrich-Alexander University Erlangen-Nürnberg (FAU), Schwabachanlage 6, D-91054 Erlangen, Germany*
[b] *Department of Medicine and Stony Brook Cancer Center, Stony Brook University, Stony Brook, New York, USA*
[c] *Department of Anesthesiology and Intensive Care Medicine, Jena University Hospital, Jena, Germany*
[d] *Genome Analysis, Leibniz Institute on Aging – Fritz Lipmann Institute, Jena, Germany*
[e] *Department of Molecular Biology, University of Duisburg-Essen, Essen, Germany*

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ABSTRACT

*Background:* Major depressive disorder (MDD) is a psychiatric disorder characterized by key symptoms that include depressed mood and a loss of interest and pleasure. A recently developed pathogenic model of MDD involves disturbed neurogenesis in the hippocampus, where the acid sphingomyelinase (ASM)/ceramide system plays an important role and is proposed as a molecular target for antidepressant action. Because alternative splicing of *SMPD1* mRNA, coding for ASM, is relevant for the regulation of ASM enzymatic activity, we investigated the frequency of alternatively spliced ASM isoforms in peripheral blood cells of MDD patients versus healthy controls.

*Methods:* Because the full-length transcript variant 1 of *SMPD1* (termed ASM-1) is the only known form within the splicing pattern that encodes an enzymatically fully active ASM, we determined a fraction of splice isoforms deviating from ASM-1 using PCR amplification and capillary electrophoresis with laser-induced fluorescence analysis.

*Results:* ASM alternative splicing events occurred significantly less frequently in MDD patients compared to healthy subjects. After 5 days of antidepressant treatment, the frequency of alternatively spliced ASM isoforms decreased in those patients who were treated with a functional inhibitor of ASM activity (FIASMA) but remained constant in MDD patients treated with other antidepressant drugs. This effect was more pronounced when healthy male volunteers were treated with the FIASMAs fluoxetine or paroxetine, in contrast to a placebo group.

*Limitations:* Patients were treated with different antidepressant drugs, depending on individual parameters and disease courses.

*Conclusions:* This study shows that the ASM alternative splicing pattern could be a biological target with diagnostic relevance and could serve as a novel biomarker for MDD.

## 1. Introduction

Major depressive disorder (MDD) is a severe and often chronic psychiatric disorder that is characterized by depressed mood, the decline of motivation and the loss of feelings of pleasure and interest. According to the International Statistical Classification of Diseases and Related Health Problems, ICD-10, additional symptoms include episodes of sadness, pessimism, negative beliefs about the self, behavioural passivity, changes in sleep, appetite and sexual interest, and suicidal thoughts and impulses. The lifetime prevalence of MDD is more than 10% (Belmaker and Agam, 2008; DeRubeis et al., 2008). The pathogenesis of MDD is not clearly understood. Environmental factors, e.g., psycho-social stress, and genetic aspects in combination with a dysregulation of the cytokine system, the neurotransmitter systems, the hormonal systems or the circadian rhythm have been discussed for triggering the pathologic symptoms (Dowlati et al., 2010; Howren et al., 2009; Krishnan and Nestler, 2008; Zhang et al., 2004). Recently, the monoamine hypothesis, which claims that antidepressants act via

* Corresponding author.
*E-mail address:* Cosima.Rhein@uk-erlangen.de (C. Rhein).
[1] Present address: Department of Nephrology and Hypertension, Friedrich-Alexander University Erlangen-Nürnberg (FAU), Erlangen, Germany.

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an influence on the monoaminergic system, has been expanded by theories involving an imbalance between hippocampal atrophy and neurogenesis as an aetiologic factor for MDD (Kempton et al., 2011; Pittenger and Duman, 2008). Hippocampal atrophy is observed in MDD patients, and antidepressant medication seems to rebalance cellular plasticity in the direction towards neurogenesis (David et al., 2009; Santarelli et al., 2003; Warner-Schmidt and Duman, 2006).

Currently, the enzyme acid sphingomyelinase (ASM, EC 3.1.4.12) is being investigated as a potential target for antidepressant action. ASM cleaves the sphingolipid sphingomyelin, an abundant component of neuronal membranes, into ceramide. Ceramide is a bioactive lipid that affects downstream signalling in the cell and mediates increased apoptotic potential (Grassmé et al., 2001; Gulbins and Grassmé, 2002; Gulbins and Kolesnick, 2002, 2003). Accordingly, it was shown that depressed patients displayed significantly higher levels of ASM activity in their cultivated blood cells compared to a control group. Additionally, the severity of depression, indicated by the Hamilton Scale of Depression (HAMD), and ASM activity levels in these cells were positively correlated (Kornhuber et al., 2005). In a genetic mouse model overexpressing ASM, the ASM/ceramide system mediated the effects of antidepressant drugs (Gulbins et al., 2013). The relationship between MDD and ASM activity is further strengthened by studies of the mode of action of antidepressant drugs. A variety of antidepressant drugs such as desipramine and imipramine indirectly reduce ASM activity (Albouz et al., 1986) and are therefore called functional inhibitors of ASM (FIASMAs) (Kornhuber et al., 2011, 2010, 2008). Thus, ASM is hypothesized to play a major role in the pathophysiology of MDD.

The exact mechanism of ASM activity regulation is not well understood. ASM activity is regulated at different processing steps during maturation, ranging from transcriptional to post-translational modifications (Jenkins et al., 2009; Kornhuber et al., 2015). Importantly, alternative splicing also regulates ASM activity. The full-length transcript variant 1 of *SMPD1* (NM_000543.4; termed ASM-1) is the only currently known splice form that encodes an enzymatically fully active protein. In different cell culture models, alternatively spliced ASM variants exerted a dominant-negative effect on ASM-1 and reduced cellular ASM activity levels (Rhein et al., 2012).

To search for a potential biomarker, and to gain a deeper understanding of the relationship between MDD, antidepressants and ASM regulation, we investigated the patterns of alternatively spliced ASM transcripts in peripheral blood cells of patients suffering from MDD, and the influence exerted by antidepressant drugs in MDD patients and in healthy subjects. The results indicate that alternative splicing processes of ASM are relevant for the aetiology and diagnosis of MDD.

## 2. Results

### 2.1. The frequency of ASM alternative splicing events is decreased in untreated MDD patients compared to healthy controls

The region between exons 4 and 6 shows the highest variability when comparing the genomic structures of all alternatively spliced ASM transcripts (Kramer et al., 2015; Rhein et al., 2012). We therefore determined the fraction of the splice isoforms deviating from the full-length transcript variant 1 of ASM (NM_000543.4; termed ASM-1) in the region between exons 4 and 6 as a functional measure ("percentage of ASM isoform fraction") and calculated a reciprocal value for ASM-1 ("percentage of ASM-1 fraction"). Due to methodical reasons, the ASM-1 fraction contains splice isoforms that share the region between exons 4 and 6 with ASM-1 but differ regarding the region between exons 2 and 4. With respect to the region between exons 2 and 4, we focused on the most frequent splicing event, an insertion of 20 nucleotides from intron 2 (Exon 2(in2)-3-4), that results among others in ASM-7, the isoform with the highest physiological impact so far (Rhein et al., 2012). Upon RNA isolation from peripheral blood cells and cDNA synthesis, we were able to quantify ASM transcripts using PCR amplification with 5′-6-carboxyfluorescein (FAM)-labelled primers and subsequent capillary electrophoresis with laser-induced fluorescence analysis.

The frequency of ASM alternative splicing events was compared between patients suffering from MDD and healthy controls. MDD patients were initially untreated, i.e., they did not receive any antidepressant medication. Compared to the healthy controls (n=50) that had 29.0% ± 2.3% alternatively spliced ASM isoforms in their blood cells, alternative splicing in patients (n=22) was significantly reduced to 25.0% ± 3.3% (ANOVA, $F(1,69)=34.8$, $p < 0.001$), which suggests more ASM-1 transcripts coding for enzymatically active ASM protein in MDD patients. Similarly, patients had significantly lower levels of the exon2-4 splice event Exon 2(in2)-3-4 compared to healthy controls (4.8% ± 1.4% versus 5.5% ± 0.9%; ANOVA, $F(1,69)=7.1$, $p < 0.01$). Due to the significant influence of age on ASM isoform fractions, age was included as a covariate in all analyses. Thus, the frequency of alternative splicing events of ASM seems to discriminate untreated MDD patients from healthy control subjects. To estimate the percentage of patients with decreased percentage of ASM isoforms, a receiver operating characteristic (ROC) curve for the combination 'percent ASM isoforms' and the state variable 'diagnosis' was calculated. The determined area under the curve was 0.861, and considering the defined cutoff value of 27.6% of ASM isoforms, sensitivity and specificity was 80% and 86%, respectively. Accordingly, all but three patients exhibited lowered levels of ASM isoforms, while 40 of 50 controls displayed normal values. A logistic regression analysis including the covariates 'percent isoforms' ($p < 0.001$; odds ratio=2.0) and 'age' ($p < 0.01$; odds ratio=1.1) resulted in an 86% correct classification of cases. This result indicates the diagnostic value of ASM splicing patterns for discriminating healthy from depressed subjects.

### 2.2. Treating MDD patients with FIASMAs transiently decreases the frequency of ASM alternative splicing events

Patients suffering from MDD were investigated to detect changes in ASM isoform levels after treatment with antidepressants for 3 or 7 days, respectively, and 28 days. To control for effective treatment, patients underwent clinical psychometric testing for measuring depressive symptoms. The Beck Depression Inventory (BDI) as a self-rating scale (Beck et al., 1996) and the HAMD as an observer-rating scale (Hamilton, 1969) were administered on day 1 (untreated state) and day 28 (treated state). The general linear model for repeated measures revealed a significant amendment of depressive symptoms after 28 days of medical treatment (BDI: $F(1,21)=29.91$, $p < 0.001$; HAMD: $F(1,21)=34.17$, $p < 0.001$).

After averaged 5 days of treatment, those patients who were treated with FIASMAs (Kornhuber et al., 2011) (n=8) showed a statistical tendency for a decrease of approximately 2% in ASM alternative splicing events from 25.2% to 23.4% (general linear model for repeated measures, adjusted for age, $F(1,6)=5.4$, $p=0.06$, Table 1A). This result suggests an increase in ASM-1 transcripts coding for enzymatically active ASM protein upon treatment at day 5. In contrast, no trend was observed for the patients treated with other drugs than FIASMAs (n=14, $F(1,12)=0.40$; Table 1B). After 28 days of treatment, no changes in ASM alternative splicing were detected with regard to the untreated state at day 1 in both groups. When analyzing the splicing patterns upon treatment more closely, it is obvious that this effect was most pronounced for splice isoforms with retained intron 5 in the region exon 4-6. Similarly, the frequency of the splicing event in region exon 2-4 was significantly decreased selectively upon a 5-D-treatment with a FIASMA (Table 1A). Thus, FIASMAs seem to decrease the frequency of ASM alternative splicing events in a time-restricted manner, especially of those with intron 5 retention and exon 2 elongation.