Parasuicidal behavior and neuroinflammation: a genetics & gene expression approach

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Objective

Parasuicidal and self-harm behavior are debilitating symptoms affecting both mood disorder and personality disorder patients. Previous publications suggest that a complex interaction between genetic predisposition and acquired environmental factors are causing parasuicidal behavior [1,2]. Pro-inflammatory cytokines have made a recent comeback in neuroscience [3]. Post-mortem brain data has shown increased expression patterns of these pro-inflammatory cytokines but also of a protein-family that precedes and mediates their production: the toll-like receptor family (TLR). TLR-3 in particular is an interesting candidate in the context of psychiatric research since it is expressed in neurons and since its increased expression in the prefrontal cortex has been associated with suicide [4]. The neuroinflammation hypothesis revolves around the debate whether these findings reflect the cause of a phenotype (trait) or whether it is a reaction to a concomitant disease process (state). When studying parasuicidal behavior one is confronted with a similar problem, is parasuicidal behavior a trait and depends on the individual or is it a state independent of the genetic predisposition of the individual? The hypothesis of the present study is that genetic polymorphisms of the TLR-3 correlate with the phenotype self-harm within a sample of affective disorder patients.

Patients and Methods

The present study was realized within the context of an FWF-funded project (VieSAD) aiming to link well-defined phenotypes of suicidal behavior with genetic polymorphisms within a sample of affective disorder patients. Eight hundred forty eight Caucasian affective disorder patients were recruited at the Department of Psychiatry and Psychotherapy of the Medical University Vienna, the Karl Landsteiner University for Health and Science and the Zentren für seelische Gesundheit, BBRZ-Med. Suicidality and history of suicidal behavior were assessed using SCAN (Schedules for Clinical Assessment in Neuropsychiatry), SBQ-R (Suicidal Behaviors Questionnaire-Revised) and LPC – (Lifetime Parasuicide Count). Self-harm behavior was assessed using the LPC (Item 1). DNA samples of peripheral blood cells (PBMCs) were genotyped with iPLEX® Reagents and the MassARRAY® System at the Sequencing & Genotyping Core Facility of the Medical University of Innsbruck (rs5743305, rs7657186, rs7668666, rs3775292 and rs76713360). Data was analyzed by calculating Chi-squared values and Fisher's exact test for genotype and allele associations as well as Haploview for Linkage-Disequilibrium and Haplotype association analysis. Peripheral expression of TLR-3 and GAPDH as endogenous control was quantified using EvaGreen-based quantitative real-time PCR.

Results

After Bonferroni correction ($p < 0.01$) for multiple testing no significant associations between genotypes, alleles, haplotypes and self-harm behavior were found. Lifetime history of self-harm (yes/no) was analyzed as a dichotomous trait applying the standard chi-square statistics (and Fisher's exact test for the SNPs with allele frequency <5) finding neither genotypic nor allelic association with any of the tested SNPs (Table 1). The selection criteria for haplotypes used in the haplotype analyses were adjacent SNPs with pairwise $r^2 > 0.80$.

In the analysis, haplotypes with frequencies above 0.01 were tested. According to the selection criteria, two SNPs (rs5743305 and rs7657186) with strong $r^2 > 0.80$ were in one block (Figure 1). Further association analysis for the adjacent block did not reveal any significant associations between the most frequent haplotypes G-C/G-A/A-C and lifetime history of self-harm (Table 2). Preliminary gene expression results indicate no significant differences in TLR3 expression levels in peripheral blood cells of 18 individuals of the same patients sample (Table 3, Figure 2,3).

TLR3			Genotypes		Alleles		HWE	
SNP ID	N° of patients with intentional self-harm	N° of patients without intentional self-harm	χ^2	p	χ^2	p	Patients with intentional self-harm (p-value)	Patients without intentional self-harm (p-value)
rs76713360	281 (CC: 200, CT: 77, TT: 4)	396 (CC: 303, CT: 86, TT: 7)	2.96*	0.22*	1.78	0.18	0.26	0.75
rs5743305	125 (AA 10, AT: 71, TT: 44)	216 (AA 35, AT: 99 TT: 82)	6.11	**0.047**	0.53	0.47	**0.01**	0.58
rs7657186	276 (AA: 9, AG: 85, GG: 182)	388 (AA: 21, AG: 135, GG: 232)	3.41	0.18	3.32	0.07	0.81	0.81
rs7668666	274 (AA: 21, AC: 109, CC: 141)	274 (AA: 30, AC: 150, CC: 206)	0.34	0.85	0.33	0.57	0.66	0.71
rs3775292	282 (CC: 12, CG: 85, GG: 185)	400 (CC: 22 CG: 145, GG: 233)	3.80	0.15	3.54	0.06	0.57	0.93

Table 1: Personal history of parasuicidal behavior, single marker analyses were established with standard chi-squared testing.

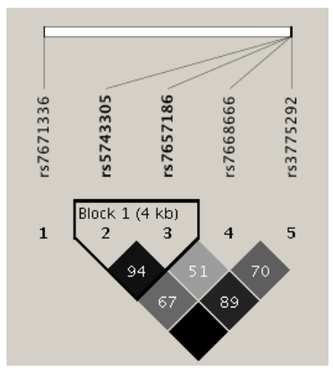

Figure 1: Linkage disequilibrium (LD) analysis was performed using Haploview software. The selection criteria for haplotypes used in the analyses were adjacent SNPs with pairwise $r^2 > 0.80$; also only haplotypes with frequencies above 0.01 were tested. According to the selection criteria, two of five SNPs of the TLR3 gene (rs5743305 and rs7657186) with strong $r^2 > 0.80$ were in one block.

No. of Markers	Haplotype	Frequency	Alleles increasing in cases	Estimated haplotype frequency %		χ^2	p
				Patients with intentional self-harm	Patients without intentional self-harm		
2	rs5743305-rs7657186	0.51	G-C	0.53	0.50	0.99	0.32
2	rs5743305-rs7657186	0.28	G.A	0.29	0.27	0.38	0.54
2	rs5743305-rs7657186	0.21	A-C	0.19	0.23	3.42	0.06

Table 2. Estimated frequency of haplotypes and association significance: Chi-Square values and Pearson's p-values

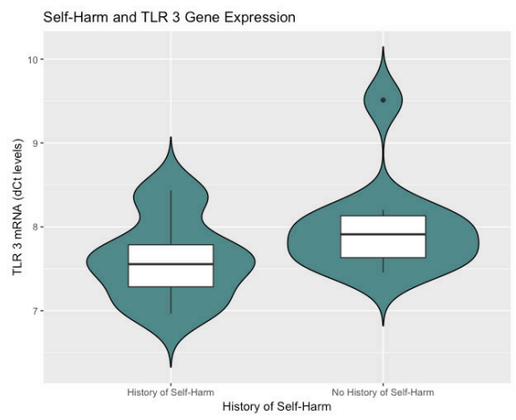

Figure 2: dCT values of Toll-Like-Receptor 3 mRNA levels, comparing patients with a history of self-harm to patients without any history of self-harm

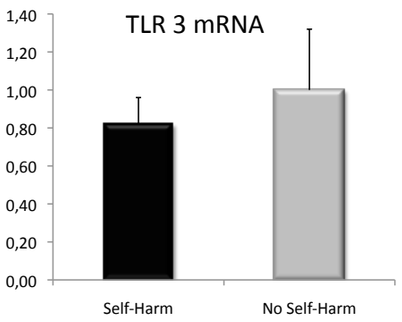

Figure 3: Fold change in TLR 3 mRNA in peripheral blood cells of affective disorder patients

Group	FC	SEM
Self-Harm	0,82	0,14
No Self-Harm	1,00	0,32

Table 3: Fold change in TLR 3 mRNA in peripheral blood cells of affective disorder patients. FC = fold change, SEM = Standard error of the mean

Conclusion

Our data suggests that there is no association between self-harm behavior and certain polymorphisms of TLR-3, but further replication is needed to strengthen the evidence.

Preliminary gene expression findings of TLR-3 in PBMCs also suggest no association, but gene expression analyses within this study are on-going and we hypothesize that previous gene expression findings of increased neuroinflammation in post-mortem brain tissue might be influenced not by genetic but epigenetic and post-translational mechanisms and that further analyses of an increased patient sample might allow to replicate the post mortem brain findings on a peripheral level.

Larger sample sizes might also be required to ultimatively elucidate the link between self harm behavior and the neuroinflammation system.

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Conflict of Interest

SK has received grant/research support from Bristol Myers-Squibb, Eli Lilly, GlaxoSmithKline, Lundbeck, Pfizer, and Servier; he has served as a consultant or on advisory boards for AstraZeneca, Bristol-Myers Squibb, Eli Lilly, GlaxoSmithKline, Janssen, Lundbeck, Novartis, Pfizer, Schwabe and Servier; and he has served on speakers' bureaus for Angelini, AOP Orphan Pharmaceuticals AG, AstraZeneca, Bristol Myers-Squibb, Eli Lilly, Janssen, Lundbeck, Neuraxpharm, Pfizer, Pierre Fabre, Schwabe, Servier.

The other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.