N-glycosylation profiling of plasma provides evidence for accelerated physiological aging in post-traumatic stress disorder

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INTRODUCTION

In the aftermath of traumatic events, the probability of developing post-traumatic stress disorder (PTSD), which is characterized by a cluster of intrusive symptoms, avoidance behavior and hyper-arousal,1 increases with the number of different traumatic event types experienced.2,3 This cumulative effect of traumatic load is presumably based on the development of a neuronal fear network: the connectivity between nodes is strengthened and the network is enlarged by every additional traumatic event experienced.4

In addition to psychological suffering, traumatic stress also increases the risk of developing somatic illnesses, such as cardiovascular, inflammatory and autoimmune diseases.5 As a result, trauma exposure is associated with premature mortality and an increased risk for carcinogenesis.6–8 One underlying biological mechanism might be that chronic stress is associated with an accelerating process of cellular aging.9 Individuals with PTSD not only show typical age-related diseases10,11 and report an older subjective age than controls,12,13 but they even show characteristics indicative of accelerated aging. For instance, naïve T lymphocytes decrease with age and are the lowest in centenarians,14 and individuals with PTSD also show a reduction in naïve T lymphocytes.15 Next, the proportion of memory T lymphocytes increases with normal aging.16,17 and individuals with PTSD also display a higher proportion of memory T lymphocytes compared with age-matched controls.15 Gene expression of the pro-inflammatory cytokine interleukin-6 is increased after menopause and andropause in elderly adults18–21 and the concentration19 and spontaneous production20 of interleukin-6 is enhanced in individuals with PTSD. C-reactive protein, which is a marker for low-grade inflammation, is elevated in women over the age of 60 years22 and also in individuals with PTSD.23 On a bio-molecular level, DNA damage increases with age,24 and DNA damage, detected as DNA single-strand breaks, also accumulates in individuals with PTSD.24 Contrary to expectations, elevated levels of DNA damage are not related to impaired DNA repair, but rather seem to accelerate DNA repair capacity, as neither old people23 nor individuals with PTSD show deficits in DNA repair capacity24 yet, DNA repair processes might still be altered in individuals with PTSD and also with age,25 and this should be investigated in more detail in future studies. Finally, telomeres, which stabilize the end of chromosomes, gradually shortened by advancing age in many cell types,26 and telomere shortening is again also associated with childhood maltreatment27 and PTSD.28

N-glycosylation is an enzymatic process that produces sugar chains covalently linked to macromolecules like proteins and lipids. N-glycans present on proteins are oligosaccharides covalently attached to protein at asparagine (Asn) residues by an N-glycosidic bond mediating numerous biological functions. In contrast to proteins, N-glycans are not controlled by the genome, but secondary gene products and can be altered by environmental influences.29 Previous studies identified nine prominent N-glycan structures in human plasma, named peak 1 through peak 9,30 the concentration of N-glycans in human plasma varies with age,31 and whereas N-glycan peak 1 (agalactosylated core-α1,6-fucosylated biantennary; NGA2F) increases with age, N-glycan peak 6 (bighalactosylated core-α1,6-fucosylated biantennary; NAc2F) decreases.32 The log ratio of these two N-glycans [log10 (peak1/peak6)], called GlycoAge Test, is a biomarker for physiological aging and gives information beyond chronological age.33 Interestingly, juvenile patients with Cockayne syndrome, a
progeroid disease that is due to a genetic deficiency in transcription-coupled nucleotide excision repair,34 show high values in the GlycoAge Test.33

We hypothesize that trauma exposure might lead to accelerated aging. We examined the N-glycosylation profile in the plasma of individuals with PTSD, of trauma-exposed individuals who were not diagnosed with PTSD and of a low-stress control group. We hypothesized that individuals with PTSD would present higher values in the GlycoAge Test, compared with the low-stress control group. Further, we hypothesized that trauma-exposed individuals should be positioned between individuals with PTSD and low-stress controls.

MATERIALS AND METHODS

Participants

We analyzed the N-glycosylation profile of 13 individuals (2 women, 11 men) with PTSD (DSM IV-TR),35 in comparison with a high-stress (trauma-exposed individuals: n = 9; 3 women, 6 men) and a low-stress (n = 10; 8 women, 2 men) control group. Individuals with PTSD were refugees living in Germany (4 from Africa, 1 Balkan and Eastern Europe, 8 Middle East and Afghanistan) with a history of severe war and torture experiences. Survivors of multiple traumata commonly also exhibit depressive symptoms. In individuals with PTSD, the average scores on the Hamilton rating scale (HAM-D)35 ranged from 0 to 39, with 7 of the 13 patients reaching a threshold of > 26.36

As non-PTSD subjects differed substantially in the number of traumatic experiences, we divided this group by median split into a high-stress (Trauma-exposed: 4 from Africa, 1 Balkan and Eastern Europe, 4 Middle East and Afghanistan) group with substantial trauma exposure (traumatic load: M = 1.18, s.d. = 0.63) and a low-stress group (3 from Africa, 2 Balkan and Eastern Europe, 5 Middle East and Afghanistan) without substantial trauma exposure (traumatic load: M = 0.23, s.d. = 0.09). The traumatic load in individuals with PTSD was significantly higher (traumatic load: M = 1.61, s.d. = 0.49; F(2,29) = 25.88; P < 0.0001). Traumatic load was specified by the proportion of specific war and torture experiences, assessed using the Vivo checklist of war, detention and torture events37 and general traumatic event types, assessed by the Clinical Administration PTSD Scale (CAPS).38

Twelve subjects reported taking psychotropic medication: eight individuals with PTSD (2 benzodiazepines, 5 antidepressants, 1 neuroleptics, 1 lithium), three trauma-exposed individuals (1 benzodiazepines, 2 antidepressants) and one control subject (benzodiazepines). To control for possible effects of medication, we compared the N-glycosylation profile of individuals with PTSD with (n = 8) and without medication (n = 6). Individuals with PTSD were recruited by the Center of Excellence for Psychotraumatology. Control subjects were recruited through advertisements, which were posted in town and university.

Exclusion criteria were comorbid psychological disorders (other than major depression episode (MDE), acute infections and chronic inflammatory diseases. MDE was not used as an exclusion criterion because the comorbidity of MDE in individuals with PTSD is high,39 and MDE symptomatology overlaps with PTSD pathology in a wide range (for example, diminished interest, restricted range of affect, difficulty with falling or staying asleep, lack of concentration).

Psycho-diagnostic interviews took place at the Center of Excellence for Psychotraumatology, University of Konstanz, Germany. Blood analyses were performed in the Laboratory for Molecular Toxicology, University of Konstanz, Germany, and the Department for Molecular Biomedical Research, Ghent University, Belgium. The Ethics Committee of the University of Konstanz approved the study. All study subjects signed an informed consent and received a remuneration of 30 €.

Psycho-diagnostic interview

Psycho-diagnostic interviews were conducted by trained clinical psychologists specialized in the field of trauma. Whenever participants were not fluent in German or English, interviews were conducted with the help of trained interpreters. Traumatic events, PTSD diagnosis and PTSD symptom severity were assessed with the CAPS.38 Traumatic experiences were assessed using the Vivo checklist of war, detention and torture experiences.37 The Hamilton depression rating scale (HAM-D)35 was used for quantification of depressive symptoms. To exclude potential comorbid psychiatric disorders, the Mini International Neuropsychiatric Interview (MINI)40 was applied.

Clinical characteristics

Groups did not differ with respect to age. Individuals with PTSD experienced significantly more different traumatic event types and showed higher traumatic load compared with low-stress controls. PTSD symptom severity (CAPS score) and depressive symptoms (HAM-D score) were highest in individuals with PTSD. Trauma-exposed individuals were between the two groups (Table 1).

Analysis of N-glycosylation

To standardize the time of blood collection, blood was always collected at 10:00 a.m. using Coagulation 9 NC Monovettes (Sarstedt, Germany). Blood samples were coded to guarantee blinding of the laboratory staff. In a first step, blood plasma was isolated and quick-frozen at ~ 80 °C at the Laboratory for Molecular Toxicology, University of Konstanz. In a second step, frozen plasma samples were shipped on dry ice to the Department for Molecular Biomedical Research, Ghent University, Belgium for N-glycosylation analysis. N-glycosylation was analyzed in 2 μl of plasma.

Nine N-glycan structures (peak 1—peak 9), which were identified to be most prominent in previous studies,10 and the GlycoAge Test [log10 (peak/peak0)],31 were measured using a DNA sequencing equipment (3130 Genetic Analyzer; Applied Biosystems, Grand Island, NY, USA), fluorophore-assisted carbohydrate electrophoresis (DSA-FACE).41

Statistical analysis

Statistical analyses were performed using R 2.11.0.42 using an alpha level of 0.05. Group differences in clinical characteristics and in the N-glycosylation profile were analyzed using ANOVAs. Age, use of tobacco, psychotropic medication and gender were included separately as covariates into the model. The Akaike Information Criterion (AIC) was used for evaluating the appropriateness of models,43 with the model without covariates having the lowest AIC. In the case of significant effects, post hoc comparison. Residuals in all models were tested for deviations from the normal distribution. Residuals in the models of the GlycoAge Test, the N-glycan peak 2, the N-glycan peak 8 and the N-glycan peak 9 were not normally distributed; therefore, these variables were re-analyzed using

| Variables | Low-stress Controls | Trauma-exposed | PTSD | Statistics | P-value |
|-----------|---------------------|----------------|------|------------|---------|
| Age       | M (s.d.)            | M (s.d.)       | M (s.d.) | F(2,29) = 1.73 | 0.19 |
| CAPS      | 3.2 (1.69)          | 1.58 (0.5)     | 0.5   | F(2,29) = 84.11 | <0.0001 |
| Number different event types (CAPS) | 3.2 (1.69) | 7.56 (3.36) | 7.56 (3.36) | <0.0001 |
| Traumatic load* | 0.23 (0.09) | 1.18 (0.63) | 1.18 (0.63) | <0.0001 |
| HAM-D score | 2.60 (6.13) | 9.00 (12.16) | 9.00 (12.16) | <0.0001 |

Abbreviations: CAPS, clinical administered PTSD scale; M, mean; s.d., standard deviation. *Traumatic load was specified by the proportion of specific war and torture experiences, assessed by the Vivo checklist of war, detention and torture events37 and general traumatic event types, assessed by the Clinical Administered PTSD Scale (CAPS).38

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RESULTS

Individuals with PTSD and trauma-exposed individuals presented higher values in the GlycoAge Test compared with low-stress controls \( (W = 25; P = 0.01) \). Trauma-exposed individuals differed neither significantly from individuals with PTSD \( (W = 70; P = 0.47) \), nor from controls \( (W = 22; P = 0.06) \), but were positioned in-between these two groups. Interestingly, traumatic load was positively correlated with the GlycoAge Test \( (r = 0.41; P = 0.02, \text{Figure 1}) \).

With respect to a possible effect of gender, we included gender as an additional factor into the model and results did not change. Further, there was no significant main effect of gender \( F_{(1,28)} = 0.24; P = 0.63 \) and no significant interaction between group \( \times \) gender \( F_{(2,28)} = 2.05; P = 0.15 \) in the GlycoAge Test.

Individuals with PTSD with psychotropic medication \( (n = 7) \); median \(-0.33 \); range \(-0.53 \) to \(-0.23 \) did not differ significantly in the GlycoAge Test \( (W = 22; P = 0.41) \) from individuals with PTSD without medication \( (n = 6) \); median \(-0.51 \); range \(-0.62 \) to \(-0.34 \).

In addition to the effect on GlycoAge Test, also in the N-glycan peak 2 individuals with PTSD presented significantly higher values \( (r^2 = 6.04; P = 0.05) \). In post hoc tests, individuals with PTSD \( (W = 30; P = 0.03) \) and trauma-exposed individuals \( (W = 20; P = 0.04) \) differed significantly from low-stress controls. There were no significant differences between PTSD and trauma-exposed individuals \( (W = 65.5; P = 0.66, \text{Table 2 and Figure 2}) \).

Further, individuals with PTSD, surprisingly, presented reduced values in N-glycan peak 7 \( (F_{(2,29)} = 4.35; P = 0.02) \). Individuals with PTSD differed significantly from the low-stress control group \( (t_{(11.9)} = 2.75; P = 0.02) \), and trauma-exposed individuals were positioned in-between, but did not differ significantly from the PTSD or the low-stress control group \( (\text{Table 2 and Figure 3}) \).

However, as we did not have a priori hypotheses for the N-glycans peak 2 and peak 7, but rather made exploratory analyses, we corrected these results for multiple comparisons. After a stepwise Holm correction,\(^{44} \) group differences in the N-glycan peak 2 and in the N-glycan peak 7 did not remain significant.

The N-glycosylation of peaks 1, 3, 4, 5, 6, 8 and 9 did not differ significantly between individuals with PTSD and low- or high-stress controls \( (\text{Table 2}) \).

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Translational Psychiatry (2013), 1–5

**Table 2.** N-Glycans in PTSD, trauma-exposed and low-stress control subjects

| Variables              | Low-stress controls (n = 10) | Trauma-exposed (n = 9) | PTSD (n = 13) | Statistics | P-value |
|------------------------|-----------------------------|------------------------|--------------|------------|---------|
| M (s.d.)               | M (s.d.)                    | M (s.d.)               |              |            |         |
| Peak 1                 | 6.46 (2.72)                 | 8.28 (2.35)            | 8.48 (2.03)  | \( F_{(2,29)} = 2.36 \) | 0.11    |
| Peak 2                 | 1.04 (0.51)                 | 1.35 (0.34)            | 1.41 (0.44)  | \( \chi^2 = 6.04 \) | 0.05    |
| Peak 3                 | 6.99 (1.78)                 | 7.17 (1.88)            | 7.69 (2.20)  | \( F_{(2,29)} = 0.28 \) | 0.69    |
| Peak 4                 | 6.19 (1.43)                 | 6.41 (1.31)            | 6.13 (1.42)  | \( F_{(2,29)} = 0.11 \) | 0.89    |
| Peak 5                 | 40.31 (5.24)                | 40.80 (5.37)           | 40.35 (4.72) | \( F_{(2,29)} = 0.03 \) | 0.97    |
| Peak 6                 | 22.77 (1.78)                | 20.30 (2.87)           | 20.49 (3.35) | \( F_{(2,29)} = 2.44 \) | 0.11    |
| Peak 7                 | 6.39 (1.58)                 | 5.63 (1.26)            | 4.91 (0.73)  | \( F_{(2,29)} = 4.35 \) | 0.02    |
| Peak 8                 | 6.91 (1.75)                 | 6.34 (1.96)            | 6.91 (1.75)  | \( \chi^2 = 0.52 \) | 0.77    |
| Peak 9                 | 1.77 (0.70)                 | 2.48 (1.11)            | 1.97 (1.25)  | \( \chi^2 = 2.47 \) | 0.29    |
| GlycoAge Test          | \(-0.58 (0.20)\)            | \(-0.40 (0.12)\)       | \(-0.39 (0.10)\) | \( \chi^2 = 7.00 \) | \(0.03\) |

Abbreviations: M, mean; s.d., standard deviation; \( \chi^2 \), Kruskal–Wallis test. Bold P-values indicate significance on an alpha level of 0.05 (two-sided).
therefore not only be a marker of physiological aging but might also be associated with individuals with PTSD, although this effect did not withstand statistical corrections. The N-glycan peak 7 in individuals with PTSD, although this effect did not withstand statistical corrections. However, we report this effect because this finding might be interesting for future studies. To the best of our knowledge, there has been only one study on the N-glycan peak 7 so far, which was found to be decreased in patients with hepatocellular carcinoma compared with liver cirrhosis patients, with a negative correlation between the N-glycan peak 7 and tumor development. At the present state of knowledge, it is difficult to pinpoint a mechanistic link that could explain the concordant N-glycan peak 7 effect in these two quite diverse disease conditions.

Limitation of the study: there was no possibility to control for unfavorable environments (unhealthy food, poor medication, etc.), which might influence the effect of accelerated aging. However, interestingly, traumatic load was positively correlated with the GlycoAge Test (r = 0.41; P = 0.02, Figure 1). This argues against the hypothesis that exposure to unfavorable living conditions is a main driver of this effect; on the other hand, trauma exposure in itself is an unfavorable environment. Thus, the influence cannot be controlled for.

In conclusion, we present evidence that traumatic stress leads to an accelerated process of physiological aging, as revealed by a shift in the N-glycosylation profile that is typical of persons with higher age, which is possibly mediated by a state of low-grade inflammation.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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