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## $\gamma'$ fibrinogen levels are associated with blood clot strength in traumatic brain injury patients

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## ABSTRACT

**Background:**  $\gamma'$  fibrinogen is an alternatively-spliced fibrinogen variant that displays different coagulation parameters *in vitro* than the major form of fibrinogen. Purified  $\gamma'$  fibrinogen has slower clotting kinetics than unfractionated fibrinogen, but forms clots that are stronger and resistant to fibrinolysis. However, these properties have only been investigated in human populations in a limited number of studies. We therefore performed a retrospective analysis to test the hypothesis that  $\gamma'$  fibrinogen levels influence coagulation *in vivo*.

**Methods:** In the present study, we utilized blood samples that were collected from traumatic brain injury patients to probe the relationship between  $\gamma'$  fibrinogen levels and traditional coagulation parameters.

**Results:** The results show that the levels of  $\gamma'$  fibrinogen were inversely associated with clotting kinetics, indicated by a shortened INR. In addition, the levels of  $\gamma'$  fibrinogen were associated with stronger clots by thrombelastography. However, these changes were not associated with significant changes in hemorrhage progression.

**Conclusions:** These findings verify that  $\gamma'$  fibrinogen properties observed in purified systems result in similar properties in a clinical setting, and may affect coagulation.

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## Introduction

$\gamma'$  (pronounced “gamma prime”) fibrinogen is an alternatively-spliced variant of fibrinogen that displays different coagulation parameters *in vitro* than the major form of fibrinogen. In particular, purified  $\gamma'$  fibrinogen has slower clotting kinetics than unfractionated fibrinogen, but forms clots that are mechanically stronger and resistant to fibrinolysis. However, these properties have only been investigated in human populations in a limited number of studies.

Fibrinogen consists of three types of polypeptide chains,  $\alpha$ ,  $\beta$ , and  $\gamma$ , that are assembled as a six-chain dimeric protein. In  $\gamma'$  fibrinogen, one or both of the  $\gamma$  chains are substituted with a splice variant that results in the truncation of the carboxyl four amino acids and their replacement with twenty amino acids. This

substitution introduces a high affinity thrombin binding site but removes a platelet binding site for  $\alpha$ IIb $\beta$ 3.  $\gamma'$  fibrinogen constitutes about 7% of total fibrinogen, although it has a wide reference interval from approximately 88–551  $\mu$ g/ml.<sup>1</sup>  $\gamma'$  fibrinogen is a known inflammatory marker and is associated with C-reactive protein levels.<sup>2–4</sup> In addition,  $\gamma'$  chain mRNA levels can be induced 8.3-fold in the HepG2 liver cell line *in vitro* by the inflammatory cytokine interleukin-6 (IL-6).<sup>5</sup> Elevation of  $\gamma'$  fibrinogen is a risk factor for cardiovascular disease death, peripheral arterial disease, and heart failure, as shown in a prospective study of 10,601 individuals in the Atherosclerosis Risk in Communities (ARIC) study.<sup>2</sup> However, mechanistic evidence for its association with thrombosis remains controversial.<sup>6,7</sup>

$\gamma'$  fibrinogen displays opposing biologic activities that could either contribute to hemostasis or inhibit hemostasis. On one hand, binding of the  $\gamma'$  chain to thrombin allosterically modulates thrombin's active site.<sup>8</sup> Consequently,  $\gamma'$  fibrinogen forms clots more slowly than unfractionated fibrinogen,<sup>9–12</sup> and the binding of thrombin to the  $\gamma'$  chain inhibits thrombin cleavage of fibrinogen itself,<sup>11,12</sup> factor V,<sup>13</sup> factor VIII,<sup>14</sup> and platelet PAR-1.<sup>15,16</sup> This

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inhibition of thrombin activity would theoretically produce an anticoagulant effect rather than thrombosis. In fact, the historical activity of “antithrombin I”<sup>17</sup> has been ascribed to  $\gamma'$  fibrinogen.<sup>18</sup> In addition,  $\gamma'$  fibrinogen increases plasma activated protein C sensitivity.<sup>19</sup> Furthermore, transgenic mice expressing the human  $\gamma'$  chain showed decreased thrombosis,<sup>20</sup> and exogenous human  $\gamma'$  fibrinogen slowed arterial thrombosis in a mouse model compared to unfractionated fibrinogen.<sup>21</sup> These characteristics of  $\gamma'$  fibrinogen could potentially explain the association between decreased  $\gamma'$  fibrinogen levels and venous thrombosis observed in some studies,<sup>22–24</sup> although it should be noted that a more recent, larger prospective study failed to show any association between  $\gamma'$  fibrinogen and venous thrombosis.<sup>25</sup>

On the other hand,  $\gamma'$  fibrinogen produces clots *in vitro* that are about three-times stronger than unfractionated fibrinogen and about eight-fold more resistant to fibrinolysis,<sup>4,9–11</sup> which could contribute to thrombosis, although the increased mechanical strength of  $\gamma'$  fibrinogen clots has been called into question.<sup>26</sup> However, binding of thrombin to the  $\gamma'$  chain protects thrombin from inactivation by antithrombin III<sup>27</sup> and  $\alpha_2$ -macroglobulin<sup>28</sup> and increases thrombin generation in plasma.<sup>28</sup> Thus, there is mechanistic evidence both for and against a direct role for  $\gamma'$  fibrinogen in thrombosis. Our hypothesis, based strictly on previous work *in vitro*, was that  $\gamma'$  fibrinogen levels would be associated with slower clot formation, but ultimately form stronger clots.

We therefore investigated the association between  $\gamma'$  fibrinogen and clot characteristics in a secondary analysis of a cohort of traumatic brain injury (TBI) patients who underwent a detailed coagulation workup using both routine coagulation assays and thrombelastography. We recently completed a study showing that the TBI patients in this cohort with hemorrhage progression had fibrinolytic activation that lead to hyperfibrinolysis, resulting in increased levels of D-dimers.<sup>29</sup> Since  $\gamma'$  fibrin displays resistance to fibrinolysis *in vitro*<sup>4,9–11</sup> we investigated the role of  $\gamma'$  fibrinogen levels in these TBI patients. We reasoned that a cohort with such a detailed coagulation assay workup would reveal associations between  $\gamma'$  fibrinogen and traditional coagulation parameters that may affect hemostasis. In addition, we sought to determine whether  $\gamma'$  fibrinogen is associated with clinical outcomes, particularly intracranial hemorrhage progression. Our results show that although  $\gamma'$  fibrinogen levels are not significantly associated with hemorrhage progression, they are significantly associated with slower coagulation parameters and increased clot strength.

## Materials and methods

### Patients

We conducted a retrospective analysis of stored blood samples taken from a single center prospective observational study of TBI patients presenting to an urban Level I trauma center from October 2011 to December 2014. The primary aim of this study was to examine the association between progression of intracranial hemorrhage and thrombelastography (TEG) values. The association between  $\gamma'$  fibrinogen and coagulation parameters was a secondary analysis. Adult trauma patients with isolated blunt TBI (head Abbreviated Injury Scale (AIS)  $\geq 3$  and  $\leq 2$  in other regions) were enrolled. Exclusion criteria included age less than 15 years, pregnancy, anticoagulant or anti-platelet drug use within 30 days of the injury, red cell, plasma, or platelet transfusion in the first 6 h after admission, use of recombinant factor VIIa, or the presence of a known coagulation disorder. Consent was obtained under an IRB-approved waiver of informed consent. Attempts were made to consent the patient, or a legally authorized representative if the patient was not able to provide consent, as soon as possible. In the

situation where no legally authorized representative was available or the patient did not become consentable, the patient was excluded from the study. Patient characteristics including demographics, admission physiologic and laboratory values, AIS scores and Injury Severity Score (ISS) were recorded. Blood used in this study was obtained at admission and 6 h to allow comparison with computed tomography (CT) analyses described below.

### Laboratory investigations

CT of the head was performed at admission and 6 h later. The ABC/2 method<sup>30</sup> was used to calculate the volume of epidural, intraparenchymal, and subdural hemorrhages. Briefly, the CT image showing the largest area of hemorrhage was identified, and the largest diameter (A) was measured. The diameter orthogonal to this was then measured (B), and the total number of 10 mm slices in which hemorrhage was detectable was counted (C). Hemorrhage progression was defined as an increase  $\geq 30\%$  in total hemorrhage volume measured at 6 h after admission, or by the attending radiologist's interpretation for subarachnoid hemorrhage. Previous literature has established that hemorrhage growth of approximately 30% on serial head CT is a conservative definition for progression of intracranial hemorrhage.

Hemoglobin, hematocrit, platelet count, and sodium levels were measured on admission using standard clinical laboratory instrumentation. TEG (Hemoscope, Niles, IL) was performed on fresh whole blood at admission and 6 h later using a TEG 5000 instrument with kaolin-activated cups. TEG values either obtained or calculated were R-value, K-value,  $\alpha$ -angle, maximum amplitude (MA), G-value, fibrinolysis at 30 min (LY30), and coagulation index (CI). CI was calculated according to the manufacturer's formula of  $CI = -0.6516R - 0.3772K + 0.1224MA + 0.0759\alpha - 7.7922$ . Citrated blood samples were processed for platelet-poor plasma and stored at  $-80^\circ\text{C}$  for subsequent assays. Coagulation assays were performed using a STA Compact instrument (Diagnostica Stago, Parsippany, NJ) and included prothrombin time/international normalized ratio (PT/INR), activated partial thromboplastin time (aPTT), D-dimers, and total fibrinogen. Coagulation factor immunoassays included prothrombin fragments 1 + 2 (f1.2) (MyBioSource, Santa Clara, CA), thrombin-antithrombin (TAT) complexes (MyBioSource), plasminogen activator inhibitor-1 (PAI-1) (ThermoFisher, Waltham, MA), tissue plasminogen activator (tPA) (ThermoFisher), plasmin-antiplasmin complexes (PAP) (MyBioSource), and  $\gamma'$  fibrinogen (Gamma Therapeutics, Inc., Portland, OR). Cytokines were measured using multiplexed fluorescent microbead immunoassays (BioRad, Richmond, CA) on a Bio-Plex 200 instrument (BioRad). This is a broad panel of inflammatory markers that includes mostly interleukins.

### Statistical analysis

Data are presented as mean  $\pm$  standard deviation or median (interquartile range [IQR]), or percentage as appropriate. Univariate comparisons were made using Student's t-test for normally distributed data, Mann-Whitney U tests for non-normally distributed data, and Chi square or Fisher's exact test for proportions. Pearson's Product Moment correlation was calculated using pairwise-complete data. Analyses were performed using JMP 13 and SAS 9.4 software (SAS Institute, Cary, NC).

## Results

The patients enrolled in this study had a median age of 47 years and were predominately (57%) female (Table 1). Both mean and median values are presented to show the distribution of values.

**Table 1**  
Patient characteristics.

Variable	n	Mean ( $\pm$ SD)	Median (range)
Age (years)	73	47.4 ( $\pm$ 19.5)	48 (16–89)
Female (%)	73	57 ( $\pm$ 0.8)	n/a
Time from injury to admission (min)	73	52.1 ( $\pm$ 33.9)	43 (13–190)
ISS	73	23.2 ( $\pm$ 11.3)	21 (3.4–50)
Pre-Hospital Total High GCS	71	12 ( $\pm$ 3.9)	14 (3–15)
Systolic Blood Pressure (mm Hg)	73	151.5 ( $\pm$ 27.1)	148 (91–238)
Heart Rate (beats/minute)	72	90.1 ( $\pm$ 19)	87.5 (54–161)
Hemoglobin (g/l)	73	14.1 ( $\pm$ 1.7)	14.3 (9.9–17.7)
Hematocrit (%)	73	41.5 ( $\pm$ 4.8)	42 (28.8–52.1)
Platelets ( $\times 10^9/l$ )	73	230.1 ( $\pm$ 68)	227 (120–422)
Sodium (mEq/l)	66	140 ( $\pm$ 3.4)	140 (123–146)
PAI-1 (ng/ml)	72	29,894 ( $\pm$ 33,480)	18,143 (2833–167,807)
PAP (ng/ml)	33	8.6 ( $\pm$ 10.9)	4.5 (0.8–50.3)
$\gamma'$ Fibrinogen ( $\mu$ g/ml)	73	99 ( $\pm$ 40.3)	89.9 (35.8–204.6)

Patients had a median GCS of 14 and a median injury severity score of 21.  $\gamma'$  fibrinogen levels on admission showed no significant differences with age or gender, although the levels 6 h after admission showed a non-significant trend towards higher levels with age (Table 2).

We first investigated the association between  $\gamma'$  fibrinogen and intracranial hemorrhage progression in the TBI patients. Patients who had an intracranial hemorrhage that progressed by  $\geq 30\%$  from admission to 6 h later had no significant difference in  $\gamma'$  fibrinogen levels. Similarly, their  $\gamma'$  fibrinogen levels at 6 h were not significantly different either ( $113 \pm 71 \mu\text{g/ml}$  vs.  $112 \pm 78 \mu\text{g/ml}$ ,  $p = 0.535$ ). These results suggest that  $\gamma'$  fibrinogen levels did not influence hemorrhage progression in the TBI patients.

We then investigated the association between  $\gamma'$  fibrinogen levels and cytokines. Previous studies had demonstrated an association between  $\gamma'$  fibrinogen levels and C-reactive protein<sup>2–4</sup>, a well-established inflammatory marker.<sup>31</sup> In addition, previous *in vitro* studies demonstrated an 8.3-fold increase in  $\gamma'$  chain mRNA levels in the HepG2 human liver cell line after exposure to IL-6.<sup>5</sup> We therefore measured a panel of different cytokines in the patient plasma samples using a fluorescent microbead assay. In contrast to the previous *in vitro* findings, the results showed no significant association between IL-6 and  $\gamma'$  fibrinogen levels (Table 3). However, there was a significant association between IL-8 and  $\gamma'$  fibrinogen levels both at admission and 6 h later. IL-8 is a chemokine produced by endothelial cells and macrophages, and is stored in endothelial Weibel-Palade bodies.<sup>32</sup> It is a known neutrophil

**Table 2**  
Association between  $\gamma'$  Fibrinogen and Patient Characteristics.

Variable	n	Mean ( $\pm$ SD)	P-value
<b>Admission</b>			
Age, years			0.2182
<55	41	93.55 $\pm$ 37.09	
55–64	16	98.17 $\pm$ 43.53	
65+	16	113.96 $\pm$ 43.87	
Gender			0.1566
female	16	107.08 $\pm$ 33.6	
male	57	96.78 $\pm$ 42.02	
<b>Six Hours</b>			
Age, years			0.0516
<55	36	89.6 $\pm$ 39.64	
55–64	13	102.57 $\pm$ 52.87	
65+	17	116.98 $\pm$ 42.47	
Gender			0.7583
female	14	94.75 $\pm$ 44.74	
male	52	100.41 $\pm$ 44.25	

**Table 3**  
Association between  $\gamma'$  Fibrinogen and Cytokines.

Variable	Pairwise n	R (95% CI)	P-value
<b>Admission</b>			
IL-1 $\beta$	28	0.065 (–0.32 – 0.43)	NS
IL-4	5	0.13 (–0.85 – 0.91)	NS
IL-6	67	–0.047 (–0.28 – 0.20)	NS
IL-8	63	–0.030 (–0.51 – 0.061)	0.0155
IL-10	34	–0.20 (–0.51 – 0.15)	NS
IFN- $\gamma$	14	–0.15 (–0.63 – 0.41)	NS
TNF $\alpha$	64	0.070 (–0.18 – 0.31)	NS
<b>Six Hours</b>			
IL-1 $\beta$	26	–0.22 (–0.056 – 0.19)	NS
IL-4	4	0.34 (–0.92 – 0.98)	NS
IL-6	63	–0.17 (–0.40 – 0.077)	NS
IL-8	60	–0.26 (–0.48 – 0.007)	0.0444
IL-10	29	–0.099 (–0.45 – 0.28)	NS
IFN- $\gamma$	11	0.023 (–0.58 – 0.62)	NS
TNF $\alpha$	59	0.064 (–0.20 – 0.31)	NS

All values were log-transformed prior to analysis. R = Pearson's Correlation Coefficient; NS=Not Significant.

chemotactic factor, but the reason for its association with  $\gamma'$  fibrinogen levels in this study is unknown.

The association between  $\gamma'$  fibrinogen levels and other coagulation parameters was investigated using both standard clinical assays performed on samples obtained at admission and at 6 h, and assays were performed on plasma samples that were stored at  $-80^\circ\text{C}$  until assay (see Materials and methods). The INR showed a significant inverse association with  $\gamma'$  fibrinogen levels both at admission and at 6 h (Table 4). This is the opposite of results seen *in vitro*, in which  $\gamma'$  fibrinogen formed fibrin clots more slowly than unfractionated fibrinogen.<sup>9–12</sup> None of the other coagulation parameters that were measured showed a significant association with  $\gamma'$  fibrinogen except total fibrinogen, including the aPPT, prothrombin f1.2 fragments, thrombin-antithrombin complexes, PAI-1, tPA, plasminogen-antiplasmin complexes, or D-dimers.

TEG was next used to investigate the kinetic and mechanical characteristics of whole blood clots and their association with  $\gamma'$  fibrinogen. The R-value is the time until the first evidence of clot formation, as seen by a deflection in the TEG tracing. The R-value showed a non-significant trend to increase with higher  $\gamma'$  fibrinogen

**Table 4**  
Association between  $\gamma'$  Fibrinogen and Coagulation Parameters.

Variable	Pairwise n	R (95% CI)	P-value
<b>Admission</b>			
INR	69	–0.32 (–0.52 – 0.088)	0.0078
aPTT	68	–0.13 (–0.036 – 0.11)	NS
f1.2	65	0.024 (–0.22 – 0.27)	NS
TAT	64	–0.040 (–0.28 – 0.21)	NS
PAI-1	72	–0.064 (–0.29 – 0.17)	NS
tPA	73	–0.041 (–0.27 – 0.19)	NS
PAP	33	–0.16 (–0.47 – 0.20)	NS
D-dimer	71	–0.22 (–0.43 – 0.015)	0.067
Total fibrinogen	69	0.64 (0.48–0.76)	0.0001
<b>Six Hours</b>			
INR	60	–0.33 (–0.54 – 0.085)	0.0095
aPTT	60	0.096 (–0.16 – 0.34)	NS
f1.2	59	0.013 (–0.24 – 0.27)	NS
TAT	57	0.051 (–0.21 – 0.31)	NS
PAI-1	65	–0.097 (–0.33 – 0.15)	NS
tPA	66	–0.24 (–0.45 – 0.007)	0.057
PAP	28	–0.13 (–0.48 – 0.26)	NS
D-dimer	65	–0.24 (–0.46 – 0.0015)	0.052
Total fibrinogen	60	0.58 (0.38–0.73)	0.0001

Values for  $\gamma'$  fibrinogen were log-transformed prior to analysis. R = Pearson's Correlation Coefficient.

levels (Table 5), which may reflect the known delay in fibrinopeptide release from  $\gamma'$  fibrinogen and was seen previously in TEG experiments using purified  $\gamma'$  fibrinogen.<sup>11</sup> The MA is the maximum amplitude, and represents the greatest deflection of the TEG curve during clotting. An increase in the MA is indicative of increased clot strength. A significant increase in MA with increasing  $\gamma'$  fibrinogen levels was observed both at admission and 6 h. This increase in clot strength is consistent with the 3-fold increase in G' (storage modulus) observed previously in torsion pendulum experiments<sup>10</sup> and the increased MA in TEG experiments performed with purified  $\gamma'$  fibrinogen.<sup>11</sup> The calculated G-value, a log derivation of the MA, was also significantly increased at admission and at 6 h. The K-value is the time it takes the TEG tracing to reach a 20 mm deflection from the end of the R-value, and represents the velocity of clot assembly. The K-value significantly decreased with increasing  $\gamma'$  fibrinogen levels, both at admission and at 6 h, indicating more rapid clot formation once the fibrinopeptides were removed (Table 5). The  $\alpha$ -angle was significantly greater at admission, although this became non-significant at 6 h. As a result of these changes, the calculated coagulation index (CI), which reflects the overall coagulability, was also significantly increased at admission, although this became non-significant at 6 h.

One TEG parameter that was not consistent with previous *in vitro* studies was the LY30, the percentage of clot lysis after 30 min. Previous studies of fibrin clots made with purified  $\gamma'$  fibrinogen demonstrated significant resistance to fibrinolysis by several different analytical methods, including TEG,<sup>11</sup> turbidity,<sup>4,9</sup> and laser scanning confocal microscopy.<sup>10</sup> However, we found no significant association between the LY30 and  $\gamma'$  fibrinogen levels in our study (Table 5). It is possible that this may reflect differences in fibrinolysis in purified systems compared to whole blood, which contains platelets that are the main contributor to clot strength, but the true reasons for the difference are presently unclear.

## Discussion

The results from this study provide clinical verification of previous *in vitro* studies regarding  $\gamma'$  fibrinogen's properties, in this case using blood samples obtained from TBI patients. The shortening of the INR observed in the present study with increasing  $\gamma'$  fibrinogen levels is opposite to *in vitro* findings with purified  $\gamma'$  fibrinogen, which demonstrated that  $\gamma'$  fibrinogen forms fibrin clots more slowly than unfractionated fibrinogen.<sup>9–12</sup> The increased MA, G-value, and CI TEG values mirror *in vitro* findings

with purified  $\gamma'$  fibrinogen, which demonstrated a 3-fold increase in G' (storage modulus) in torsion pendulum experiments<sup>10</sup> and an increased MA in TEG experiments.<sup>11</sup> These results extend the results found by Pieters et al.,<sup>4</sup> who observed a delay in clot lysis in human blood samples with increasing  $\gamma'$  fibrinogen levels.

The results do not, however, resolve the biological paradox of  $\gamma'$  fibrinogen's opposing activities. On the one hand, the inverse association found between INR values with  $\gamma'$  fibrinogen does not support the "antithrombin I" concept of  $\gamma'$  fibrinogen's anticoagulant activity. On the other hand, the association found between the other TEG parameters and  $\gamma'$  fibrinogen, particularly the association between  $\gamma'$  fibrinogen levels and MA, support the prothrombotic concept of  $\gamma'$  fibrinogen activity. However,  $\gamma'$  fibrinogen levels were not significantly associated with clinical intracranial hemorrhage progression in the present study. If  $\gamma'$  fibrinogen's "antithrombin I" activity was dominant, then one might predict that hemorrhage progression would be associated with increased levels of  $\gamma'$  fibrinogen. If, however,  $\gamma'$  fibrinogen's stronger fibrin clot strength and fibrinolytic resistance predominated, then hemorrhage progression would be associated with decreased levels of  $\gamma'$  fibrinogen. Since neither a positive nor negative association was observed between  $\gamma'$  fibrinogen levels and hemorrhage progression, the biological effects of  $\gamma'$  fibrinogen *in vivo* are still unclear. It is possible that this is due to the small sample size of this study, although there was very little difference in  $\gamma'$  fibrinogen levels between patients whose hemorrhage progressed and those that did not. However, it may simply be that the properties of  $\gamma'$  fibrinogen do not have a major biologic consequence on intracranial hemorrhage progression in TBI patients.

This study has several limitations, mainly that this is a secondary analysis of a prospective study that was not powered to investigate the relationship between  $\gamma'$  fibrinogen and either clinical outcomes or coagulation parameters. However, despite these shortcomings, it is notable that significant associations were discovered between  $\gamma'$  fibrinogen and INR, as well between  $\gamma'$  fibrinogen and TEG values.

This leaves open the question of why  $\gamma'$  fibrinogen shows a significant association with arterial thrombosis, particularly heart attack<sup>1,2,33,34</sup> and stroke.<sup>35–37</sup> It may be, as some have postulated, that  $\gamma'$  fibrinogen merely serves as a surrogate marker of inflammation, much like C-reactive protein.<sup>2</sup> Previous studies have, in fact, demonstrated a significant association between  $\gamma'$  fibrinogen levels and C-reactive protein<sup>2–4</sup>. However, somewhat unexpectedly,  $\gamma'$  fibrinogen levels did not show a significant association with the inflammatory cytokine IL-6 in the present study, even though IL-6 has been shown to increase  $\gamma'$  fibrinogen expression *in vitro* in the HepG2 human liver cell line.<sup>5</sup> This may be due to the fact that  $\gamma'$  fibrinogen has a much longer half-life than IL-6, 88 h,<sup>38</sup> and may therefore change concentration in response to IL-6 *in vivo* much slower than the 6-h time point measured. Further studies are therefore necessary to determine the role of inflammation in the regulation of  $\gamma'$  fibrinogen levels.

## Conclusions

The results show that the levels of  $\gamma'$  fibrinogen were associated with faster clotting kinetics, indicated by a shortened INR. In addition, the levels of  $\gamma'$  fibrinogen were associated with stronger clots as demonstrated by increased TEG MA, G-value, and CI. However, these changes were not associated with hemorrhage progression in traumatic brain injury patients. The findings show that the  $\gamma'$  fibrinogen properties observed *in vitro* do not always result in similar properties in a clinical setting, which may affect coagulation.

**Table 5**  
Association between  $\gamma'$  Fibrinogen and Thrombelastography Parameters.

Variable	Pairwise n	R (95% CI)	P-value
<i>Admission</i>			
R-value	71	0.21 (−0.021 – 0.43)	0.0742
K-value	71	−0.28 (−0.48 – 0.053)	0.0170
$\alpha$ -angle	71	0.27 (0.044–0.48)	0.0204
MA	71	0.49 (0.28–0.65)	0.0001
G-value	70	0.53 (0.34–0.68)	<0.0001
LY30	49	−0.18 (−0.44 – 0.11)	NS
CI	70	0.26 (0.029–0.47)	0.0282
<i>Six Hours</i>			
R-value	63	0.17 (−0.079 – 0.40)	NS
K-value	63	−0.33 (−0.54 – 0.094)	0.0075
$\alpha$ -angle	63	0.22 (−0.029 – 0.44)	0.0833
MA	63	0.47 (0.25–0.64)	0.0001
G-value	63	0.45 (0.23–0.63)	0.0002
LY30	46	−0.11 (−0.39 – 0.18)	NS
CI	61	0.14 (−0.12 – 0.38)	NS

Values for R-value, K-value, LY30, and  $\gamma'$  fibrinogen were log-transformed prior to analysis. R = Pearson's Correlation Coefficient.

## Authors' contributions

David H. Farrell: drafting of manuscript and analysis and interpretation of data; Elizabeth A. Rick: acquisition of data; Elizabeth N. Dewey: analysis and interpretation of data; Martin A. Schreiber: study conception and design and revision of manuscript; Susan E. Rowell: study conception and design and revision of manuscript.

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## Ethical approval

This study was approved by the Institutional Review Board at Oregon Health & Science University and followed the ethical principles of the Declaration of Helsinki and its later amendments.

## Informed consent

Consent was obtained under an IRB-approved waiver of informed consent. Attempts were made to consent the patient, or a legally authorized representative if the patient was not able to provide consent, as soon as possible. In the situation where no legally authorized representative was available or the patient did not become consentable, the patient was excluded from the study.

## Declaration of competing interest

OHSU and David H. Farrell have a significant interest in Gamma Therapeutics, a company that may have a commercial interest in the results of this research and technology. This potential individual and institutional conflict of interest has been reviewed and managed by OHSU. Dr. Farrell was blinded to the results until the analysis by Ms. Dewey.

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