Effects of Sequence Variations in Innate Immune Response Genes on Infectious Outcome in Trauma Patients: A Comprehensive Review

Sequence variations in trauma patients

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ABSTRACT

Objective: Infectious complications, sepsis and multiple organ dysfunction syndrome (MODS) remain important causes for morbidity and mortality in patients who survive the initial trauma. Increasing evidence suggests that genetic variants, particularly Single Nucleotide Polymorphisms (SNPs), are critical determinants for interindividual differences in both inflammatory responses and clinical outcome in sepsis patients. Although the effect of SNPs on sepsis and MODS has been studied in many populations and diseases this review aimed to summarize the current knowledge on the effect of SNPs on infectious complication specifically in trauma patients.

Methods: review of available literature in PubMed database.

Results: The following genes have been studied in populations of trauma patients: *CD14, HMGB1, IFNG, IL1A, IL1B, IL1RN, IL4, IL6, IL8, IL10, IL17F, IL18, MBL2, MASP2, FCN2, TLR1, TLR2, TLR4, TLR9, TNF, LTA, GR, MYLK, NLRP3, PRDX6, RAGE, HSPA1B, HSPA1L, HSP90, SERPINE1, IRAK1, IRAK3, VEGFA, LY96, ANGPT2, LBP, MicroRNA and mtDNA.* In this review we discuss the genes of the Pattern Recognition Receptors (PRR), Signal Transducing Adaptor Proteins (STAP) and Inflammatory Cytokines of the innate immune system.

Conclusions: A number of genetic variations have so far been studied in cohorts of trauma patients. Studies are often unique and numbers sometimes small. No definitive conclusions can be reached at this time about the influence of specific sequence variations on outcome in trauma patients.

KEYWORDS

Injury

Sepsis

Multiple Organ Dysfunction Score

Inflammatory response

Single Nucleotide Polymorphism

INTRODUCTION

Trauma is a major public health problem worldwide, ranking as the fourth leading cause of death. In 2010, there were 5.1 million deaths from injuries and the total number of deaths from injuries was greater than the number of deaths from HIV/AIDS, tuberculosis and malaria combined (3.8 million) (1, 2). Infectious complications, sepsis and multiple organ dysfunction syndrome (MODS) remain important causes for morbidity and mortality in patients who survive the initial trauma (3). Although the rate of MODS in trauma patients has diminished over the last decade, MODS-related mortality, intensive care unit stay, and mechanical ventilation duration have not changed significantly (4, 5). These complications increase the burden of cost to society.

The primary inflammatory insult determines the magnitude of systemic inflammation and subsequent immune exhaustion, which makes patients prone for septic complications. Both a proinflammatory and anti-inflammatory response appear to coexist in trauma patients, possibly leading to both additional tissue damage by the immune system as well as increased susceptibility for subsequent infections (6). The development of the systemic inflammatory response (SIRS) with liberation of proinflammatory cytokines is recognized as a part of the physiologic response to trauma. Tissue injury following trauma results in depressed cell-mediated immunity (especially T-cell) leading to an increased risk of infectious complications (7). Cytokine production varies between individuals, due to genetic background and certain allelic variants of cytokine genes; in particular, single nucleotide polymorphisms (SNPs) in coding regions of cytokine genes are associated with higher or lower cytokine production. Polymorphism may be considered as an important genetic risk factor for susceptibility to posttraumatic sepsis and a potential target for immunotherapy. Increasing evidence suggests that genetic variants, particularly SNPs, are critical determinants for interindividual differences in both inflammatory responses and clinical outcome in sepsis patients (8). Although the effect

of SNPs on sepsis and MODS has been studied in many populations and diseases this review aims to summarize the current knowledge on SNPs in genes of the innate immune system in trauma patients only.

A literature search was performed in PubMed by using "genetic variation", "trauma", and "innate immunity" and synonyms as search string. The search was finalized by cross-checking references. Studies describing the effect of SNPs in innate immune response genes on infectious complications in trauma patients were included. An overview of the SNPs included is shown in Supplemental Table S1.

1. Pattern Recognition Receptors and Complexes

1.1 Toll-Like Receptors and associated genes

Toll-Like Receptor 1 (*TLR1*)

Three SNPs in TLR1 were studied in trauma patients (Table 1) (9). The *TLR1* -7202G allele (rs5743551) and the *TLR1* 742AG(p.Asn248Ser) (rs4833095) were associated with increased risk of mortality in sepsis and Gram-positive sepsis, respectively.

Toll-Like Receptor 2 (*TLR2*)

Five SNP in *TLR2* have been studied in a trauma population (Table 1).

The *TLR2* 19216T>C (rs3804099) CC genotype conferred a significantly higher risk of developing sepsis and higher MOD scores than those with a TT or TC genotype (10).

The *TLR2* p.R753Q SNP was studied by two authors (11, 12). McDaniel *et al*. found the AG genotype significantly more often in septic patients (62,5%) than in aseptic patients (25%) in African-American patients (not so in whites) (12). Bronkhorst *et al*. found no association with sepsis or mortality in a mixed ethnic cohort of 219 trauma patients (11).

For the *TLR2* -16934T>A the TA genotype increased the risk of a Gram-positive infection and SIRS in a trauma population by (11) .

Toll-Like Receptor 4 (*TLR4*)

SNPs in *TLR4* have been studied in trauma patients (11-15) and in burns patients (16-19) (Table 1). In trauma patients multiple SNPs in *TLR4* have been studied making comparison difficult (11-15).

The *TLR4* 896A>G (rs 4986790) was studied in four cohorts of burns patients. Three studies that used the same growing cohort used sepsis as endpoint (16, 17, 19) and two studies used mortality as endpoint (18, 19)The *TLR4* 896A>G was significantly associated with an increased risk for severe sepsis (16, 17). Shalhub could not confirm this (19). Moreover, no association with mortality was found (18, 19). Carriage of the TLR4 896G allele was associated with a decreased risk of complicated sepsis in trauma (15). The cosegregating *TLR4* p.D299G and *TLR4* p.T399I were studied in trauma patients by two authors (11, 12), both of whom were not able to demonstrate an association between genotype and infection or outcome of sepsis.Chen *et al*. studied the clinical relevance of five single nucleotide polymorphisms in *TLR4* (-2381A>G, -2242T>C, -1892G>A, -1837A>G, and -1418T>C) in patients with major trauma (13). Only *TLR4* -2242T>C polymorphismhigher sepsis morbidity rates and multiple organ dysfunction scores were found. Duan *et al*. prospectively studied the *TLR4* 11367G>C polymorphism in patients with major trauma (14). Patients with the C variant allele had significantly lower sepsis morbidity than those homozygous for the G allele. In addition, MOD scores in the patients with trauma who carry the C allele were also significantly lower than those in the patients carrying the G allele.

Toll-Like Receptor 9 (*TLR9*)

Several SNPs in *TLR9* have been studied in trauma patients by two authors (Table 1) (11, 20).

Chen *et al*. studied the effect of five polymorphisms in TLR9 in 557 consecutive Han Chinese patients with severe multiple blunt trauma injuries (20). Median ISS was 25 and 37.9% of patients developed sepsis. The rs187084 (−1486A>G), rs352140 (2848C>T) and rs352162 (6577T>C) SNPs were significantly associated with TLR9-mediated TNF-α production. Patients with a minor allele of the rs187084, rs352139 or rs352162 polymorphism had a higher sepsis morbidity rate. Of these three SNPs, only the rs352162 polymorphism was significantly associated with MOD score, showing a recessive effect.

Bronkhorst *et al*. studied TLR9 (-1486T>C and -1237T>C) in a cohort of 219 severely injured patients and found -1486T>C to cause a trend toward reduced prevalence of gram-positive bacteria and fungi for this SNP ($p = 0.060$), but no significant association with SIRS, sepsis, or septic shock (11).

Cluster of Differentiation 14 (*CD14*)

The effects of *CD14* -159C>T promoter SNP were studied in burns patients (16-19, 21-23) and in severely injured trauma patients (11, 24-26) in Chinese (22-24, 26) and mixed ethnic populations (Table 1) (11, 16-19, 21, 25). Comparison of results is complicated by the fact that different outcome parameters were used, including wound cultures, SIRS, sepsis, severe sepsis, MODS and mortality. Sepsis and MODS occurred more frequently in both burns and trauma patients with variant genotype in some reports (17, 22-24, 26) but was not influenced by genotype in other reports (11, 16, 19, 25). Remarkably, in some studies sepsis was associated with the C-allele whereas in other studies sepsis was associated with the T allele.(17). (22). One can only speculate about the origin of this contrast which may be explained by differences in ethnicity of the study population. Mortality risk was increased by *CD14* -159C variant genotype in burns patients (18, 21) but this effect was not found in another study (19).

Differences in total body surface area (TBSA) of burns as well as ethnic demographic baseline characteristics may contribute to these opposing findings.

The effects of *CD14* -1145G>A in trauma patients were studied in Chinese trauma patients (24, 26). In both studies, with a total of 211 trauma patients, the -1145G allele conferred an increased risk of sepsis and MODS.

Myeloid differentiation-2/ Lymphocyte antigen 96 *(LY96)*

Zeng et al. studied 726 unrelated Han Chinese patients with major trauma for *MD2* (27). A total of 37 SNPs were identified in *MD2*. Thirty five of them constructed three haplotype blocks. Sepsis developed in around 40% of patients. Only the rs11465996 was shown to be significantly associated with the risk of development of sepsis and MODS in major trauma patients. Patients carrying th variant G allele revealed significantly higher sepsis morbidity rate and MOD scores.

Gu *et al*. studied *MD2* -1625C>G in 105 severely injured patients of whom 40% developed sepsis (28). The MODS scores in trauma patients carrying G allele at position -1625 were significantly higher than those carrying C allele. Moreover, trauma patients carrying G allele appeared to have higher risk of sepsis compared to those carrying C allele. Sepsis morbidity was significantly different between subject with C and G alleles.

Lipopolysaccharide Binding Protein *(LBP)*

Zeng *et al*. used haplotype tagging to study SNPs in *LBP* in two independent cohorts of major trauma patients recruited from southwest and eastern China (29). Of the nine known SNPs in *LBP o*nly the rs2232618 (p.F436L) was significantly associated with higher susceptibility to sepsis and MOD. Patients carrying the variant C allele revealed significantly higher sepsis morbidity rate and MOD scores when compared to patients carrying the T allele.

1.2 Lectin Pathway Proteins

Mannose-Binding Lectin (*MBL2*)

Heterozygosity for the variants in exon 1 (A/0) conferred an increased risk of wound colonization and infection in severely injured patients (30). This had previously only been demonstrated in a murine model of burns (31). Also, the YX promoter genotype increased the risk of fungal colonization and infection in trauma patients (30).

MBL-Associated Serine-Protease 2 (*MASP2*)

MASP2 p.Y371D DD homozygosity increased the risk of SIRS and septic shock in trauma patients significantly (30). Moreover, a trend was noted for an increased risk of Gram-positive infections in patients with DD genotype. For the *MASP2 p.*D120G genotype polymorphism no statistically significant differences were found for all endpoints although, strikingly, fungi, positive blood cultures and septic shock were only found in DD patients (22.2%, 15.5%, and 17.9%, respectively). Another striking, yet non-significant, finding was that only 8.3% of DG patients developed sepsis versus 37.7% in DD patients (p=0.060).

Ficolin 2 (*FCN2*)

The homozygous *FCN2* p.A258S AS genotype increased the risk of developing septic shock in trauma patients (30). Also, wound colonization and infection risks were significantly increased. A trend was noted for Gram-negative infections.

No significant associations between the *FCN2 p.*T236M genotype and infectious events were found. Positive blood cultures developed in 25.0% of patients with a variant MM genotype, versus only 11.3% of patients with the common TT genotype but this difference was not statistically significant in a multivariate model.

1.3 Other Receptors

Receptor for Advanced Glycation Endproducts *(RAGE)*

A total of 728 unrelated patients with major trauma was studied by Zeng *et al*. and genotyped for *RAGE (32)*. Sepsis occurred in around 40% of patients with median time between trauma to sepsis being 6 days. From different genetic variants selected in this study, only the *RAGE* -429T>C (rs1800625) was shown to be significantly associated with the risk of development of sepsis and MODS in major trauma patients. The patients carrying the variant C allele revealed a significantly lower sepsis morbidity rate and MOD scores, when compared with those carrying the T allele. Moreover, *in vitro* LPS-induced TNF-α production was significantly lower in patients with the variant C allele than in those with wild T allele.

NOD-like Receptor Family, Pyrin Domain Containing 3 *(NLRP3)*

Zhang studied six SNPs in the *NLRP3* gene of 718 Chinese patients with major blunt trauma with a mean ISS of 22.5 (33). 40% of patients developed sepsis with a mean time to sepsis of 7 days. The *NLRP3* -1017G>A polymorphism (rs2027432), although it was found in only three patients with AA variant homozygotes in this study cohort, was significantly associated with higher risk of MODS. In addition, the *NLRP3* 5134A>G (rs12048215) polymorphism was significantly associated with a lower sepsis morbidity rate, showing 26.4% in GG versus 44% in AA. Data from multiple logistic regression analyses further indicated that the patients with the rs12048215 polymorphism had a lower risk of developing sepsis after adjusting for possible confounders. The rs2027432 polymorphism was significantly associated with higher IL-1β levels.

Glucocorticoid Receptor (*GR*)

Duan *et al*. studied a cohort of 95 severe trauma patients with a mean ISS of 27 (34). It appeared that the *BclI* mutation in the *GR* gene was not associated with posttraumatic sepsis or organ dysfunction.

2. Signal Transducing Adaptor Proteins

Interleukin-1 Receptor-Associated Kinase 1 (*IRAK1*)

Sperry et al. studied a cohort of 321 patients with a median ISS of 16 for the T>C substitution (rs1059703) at position 1595 in exon 12 of *IRAK1* which results in a non-synonymous mutation (p.L532S) (35). They found this SNP to be a very strong independent predictor of post-trauma multiple organ failure and mortality

Interleukin-1 Receptor-Associated Kinase 3 (*IRAK3*)

Meyer *et al*. genotyped 474 patients with acute lung injury (ALI) from a prospective critically ill trauma patients cohort study for 25 candidate genes using the IBC chip (36). The incidence of ALI their cohort was 30%. *IRAK3* was found to be associated with ALI in patients from African descent.

3. Inflammatory Cytokines

3.1 Interleukins

Interleukin-I *(IL1A, IL1B, IL1RN)*

IL1A

In a cohort of 308 Han Chinese trauma patients with ISS>16 the *IL1A* -889C>T TT genotype had the highest risk of sepsis and produced the lowest serum levels of Il-1α (Table 1) (37).

IL1B

Carrying an *IL1B-Taq-1* 3953C>T CT genotype in combination with the *IL10* -592A>C AC genotype predisposed to acute respiratory failure in Caucasian trauma patients (N=216; ISS>16) (p=0.003) (Table 1) (38).

The *IL1B* -1470G>C was studied in two overlapping cohorts of severely injured Han Chinese patients from the same hospital (37, 39). Chinese trauma patients carrying the major -1470G allele were more likely to develop sepsis than those with the minor -1470C allele in both studies.

The *IL1B* -511T>C (rs16944) was studied in the previously overlapping cohorts of 238 and 308 Han Chinese patients with severe trauma (37, 39). The CC genotype conferred a statistically significant increase in the risk of sepsis. In a Caucasian cohort of 119 multiple trauma patients *IL1B* -511T>C variation was not found to confer any effect on sepsis (38).

The *IL1B* SNP most studied is the -31C>T (16-19, 21, 37, 39). In mixed-ethnic burns patients from the USA (TBSA>15%) this SNP seems to be no relevant risk factor for the development of sepsis nor for mortality (16-19, 21). In Han Chinese multiple trauma patients, however, the *IL1B* -31C>T major CC genotype seemed to protect against sepsis (30.3% and 37.9%) following major trauma (37, 39).

IL1RN

In one study the effect of *IL1RN* variant 2 variable number tandem repeat (VNTR) polymorphism was studied in patients with traumatic brain injury (TBI) (Table 1) (40). *IL1RN* VNTR allele 2 carriers were more likely to have hemorrhagic events after TBI. In another study in severe trauma patients a *IL1RN* SNP 130T>C (rs315952), distinct from the well-described VNTR SNP, was associated with decreased risk of ARDS (41).

Interleukin-4 (*IL4*)

Two studies from the same hospital with overlapping patient cohorts reported the influence of *IL4* -589T>C genotype in a cohort of 308 Chinese severe trauma patients with a mean ISS of 25.5 (Table 1) (37, 42). A total of 48.4% of patients developed sepsis. The frequency of the TC heterozygous genotype in the sepsis group (37.6%) was significantly higher than in nonsepsis group (25.2%). There was a significant influence of the minor C allele. No relationship was observed between *IL4* -589T>C and MODS in these major trauma patients.

Interleukin-6 (*IL6*)

The *IL6* -174G>C (rs1800795) was studied in three cohorts of burns patients (16-19, 43), six cohorts of trauma patients (12, 38, 44-47) and a cohort of traumatic brain injury (TBI) patients (Table 1) (48). Only two out of these articles described an increased risk of sepsis with presence of the minor -174C allele (17, 45). In a cohort of TBI patients the GG genotype was found significantly more frequently in the survivor group than in non-surviving patients (48).

Chinese trauma patients carrying the *IL6* -572G>C CC genotype had significantly more sepsis morbidity than with a CG or GG genotype (37, 46). A small Bosnian cohort however failed to demonstrate any influence of this SNP (47).

Interleukin-8 (*IL8*)

The effect of *IL8* -251A>T on the development of ARDS was studied in one cohort of 97 blunt trauma patients of whom 23 developed ARDS (Table 1) (49). The allele and genotype distribution of the polymorphism in this cohort did not exhibit a significant association with the development of ARDS or mortality. Patients with the AA genotype showed a significantly longer duration of mechanical ventilation compared to patients with the *IL8* -251TT genotype.

Interleukin-10 (*IL10*)

The effects of *IL10* -592A>C in trauma patients have been described in seven studies (Table 1) (12, 37, 38, 50-52). Three studies (12, 50, 51) found conflicting results of genetic variation in this gene on outcome. Schröder *et al*. found an increased risk for MODS in -592AC genotypes. Huebinger *et al*. found that carriage of the minor -592A allele was associated with a decreased risk of mortality. McDaniel *et al.* found that patients carrying the *IL10* ACC/ATA low producing genotypes were at a lower risk of developing sepsis.

IL10 -819C>T was studied in five cohorts of trauma patients (12, 37, 43, 50, 52). Three studies describe an effect on outcome (12, 37, 50). Huebinger *et al*. found that the minor -819T allele was significantly associated with a decreased risk of mortality. McDaniel *et al.* found that patients carrying the *IL10* ACC/ATA low producing genotypes were at a lower risk of developing sepsis. In a cohort of Chinese trauma patients (where C appeared to be the minor allele) it was shown that this C allele conferred a decreased risk of sepsis (37).

IL10 -1082G>A was studied by ten authors (12, 36-38, 43, 51-55). Six authors observed effects on outcome (12, 36, 38, 52-54). McDaniel *et al*. (12) found that patients who carried the *IL10* ACC/ATA low producing genotypes were at a lower risk of developing sepsis. Zeng *et al*., however, found that patients with the major A allele had significantly higher risk of sepsis (52). Jin *et al*. (54) as well as Schroeder *et al.* (38) described a reduced risk of ARDS and acute

respiratory failure in GG genotypes. In contrast, Gong *et al*. found the -1082GG genotype to be associated with an increased risk of ARDS in patients younger than 52 years old.

Interleukin-17F (*IL17F*)

Accardo Palumbo *et al*. studied the effect of 7488T>C (His161Arg)(rs763780) in *IL17* in a cohort of burns patients (Table 1) (43). At the third day, burn patients had a very significant increase in IL-17 plasma levels. However, there were no statistically significant differences in *IL17* genotype distributions among patients that did or did not developed sepsis.

Interleukin-18 (*IL18*)

McDaniel *et al*. were unable to demonstrate a significant effect of SNPs in *IL18* in trauma patients (Table 1) (12). Stassen *et al*. studied *IL18* -137G>C and *IL18* -607C>A in 69 trauma patients (56). Although the individual SNPs were not associated with outcome, patients carrying both the -607CA genotype and a -137GC genotype (CA/GC) had a significantly reduced risk of sepsis. These data suggest that *IL18* genetic variability may play a role in the predisposition for the development of postinjury sepsis.

3.2 Other Inflammatory Cytokines

High-Mobility Group Box 1 (HMGB1)

Three *HMGB1* polymorphisms -1514T>C, 2179C>G and 6850G>A were studied in a cohort of 556 Han Chinese patients with major trauma. A total of 39.7% of patients developed sepsis. The *HMGB1* 2179C>G variant GG genotype predisposed to the occurrence of sepsis (p=0.003) and MODS (P=0.011) in trauma patients (57). With respect to the other 2 SNPs, there were no significant differences in sepsis morbidity rates and MOD scores.

Interferon-γ (*IFNG*)

In a mixed-ethnic cohort of 68 trauma patients (ISS > 15) of whom 42–50% developed sepsis (12) the *IFNG* 841T>A AA genotype protected against sepsis in African American patients, whereas this was not clear for Caucasian patients. The authors suggest that the carriage of the AA genotype could cause faster elimination of the pathogens (12). In an other cohort of 308 Han Chinese trauma patients (ISS>16) the *IFNG* 541T>A polymorphism was unrelated to sepsis or MOD (37).

Tumor Necrosis Factor (*TNF*)

Three SNPs in *TNF* have been studied in trauma and burns patients by nine authors (Table 1) (12, 16-19, 21, 37, 58-62).

The TNF -308G>A (rs1800629) was described in burns patients by two authors in five studies (16-19, 21) and in trauma patients in eight studies (12, 15, 19, 37, 58-62). Increased risk of sepsis and of mortality has been observed by seven authors (16, 17, 19, 37, 58, 61, 62) but was not seen by four authors (12, 15, 18, 21, 60). Moreover, Gill *et al.* demonstrated in a cohort of trauma patients that the A allele was significantly associated with the risk of microchimerism after allogenic transfusion of cells (59).

The *TNF* -238G>A (rs361525) was studied in trauma patients by one author (62). There was no influence of -238G>A variation on sepsis outcome in a cohort of 152 severely injured patients.

Also, the *TNF* -376G>A (rs1800750) was studied in trauma patients by one author (62). There was no influence of -238G>A variation on sepsis outcome outcome in a cohort of 152 severely injured patients.

Lymphotoxin-α (*LTA*)

Effects of variation in lymfotoxin-α *LTA* 252A>G (rs909253) (previously known as *TNF*-β NcO1) was studied in trauma patients in five manuscripts (45, 58, 60, 61, 63). Three authors observed an effect on clinical outcome (60, 61, 63) and two did not (45, 58).

Majetschak *et al*. found that severe posttraumatic sepsis was significantly increased in patients homozygous for the allele *TNFB2* (presently termed the A allele) (63). Three years later, Majetschak again found that patients developing severe sepsis after trauma were significantly more likely to be homozygous for *TNFB2* and this time also homozygous for *TNFB1* (presently termed the G allele) (60). Menges *et al*. also found that carriage of the G allele (*TNFB1*) conferred an increased risk of developing sepsis (61). Hildebrand *et al*. (45) and Duan *et al*. (58) found no effect on sepsis morbidity.

CONCLUSION

Severe injury or multiple trauma (the so-called ‘first hit’) evokes a systemic inflammatory response in trauma patients. In uncomplicated cases this response is temporary and predictable to a certain extent. If the initial hit however is big enough it may produce a Systemic Inflammatory Response Syndrome (SIRS). The following emergency damage-control surgery and later definitive surgical procedures (the ‘second hit’) may further exhaust the immune system potentially leading to immune paralysis causing the Compensatory Anti-inflammatory Response Syndrome (CARS). Several mechanisms contribute to the development of SIRS such as hormonal, metabolic, hemodynamic, immunological, cell-mediated and ischemia/reperfusion processes (64).

The outcome following major trauma is thus determined by many factors of which sequence variation in the human genome may well be one such factor. A number of genes have been studied so far but these studies are generally unique and numbers are often small. Outcome parameters of studies, as shown in this review are sometimes different making pooling of results or comparison complicated. Nevertheless, some single nucleotide polymorphisms clearly appear to exert an effect on the outcome.

Identifying patients at risk of developing infectious complications may improve their outcome by targeted treatments such as antibiotic prophylaxis, substitution therapy or plasma transfusions.

But unfortunately too little information is currently available to draw firm conclusions. Further research in this field is necessary. Since systemic response to trauma is a complex and polygenic phenotype, more genes will have to be studied in larger cohorts to determine their exact influence on outcome in severely injured patients. State-of-the-art techniques like exome sequencing and whole genome SNP arrays should be used in future studies in order to identify relevant sequence variations in other immune response genes and signalling pathways as well.

AUTHOR CONTRIBUTION STATEMENT

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Study design. Data interpretation. Critical revision.

Supplemental Table S1. Summary of SNPs studied in populations of trauma patients

Gene	OMIM	Cytogenetic Location	SNP	dbSNP ID	References
Pattern Recognition Receptors and Complexes					
TLR1	601194	4p14	-7202A>G	rs5743551	(9)
			742A>G	rs4833095	(9)
			1804G>T	rs5743618	(9)
TLR2	603028	4q31	-15607A>G	rs1898830	(10)
			19216T>C	rs3804099	(10)
			22215T/G	rs7656411	(10)
			p.R753Q	rs5743708	(11, 12)
			p.R753Q	rs5743708	(11, 12)
			-16934T>A	rs4696480	(11)
TLR4	603030	9q33	-2381A>G	rs2737190	(13)
			-2242T>C	rs10116253	(13)
			-1892G>A	rs10983755	(13)
			-1837A>G	rs1927914	(13)
			-1418T>C	rs10759932	(13)
			11367G>C	N.A.	(14)
			896A>G	rs4986790	(11, 12, 15-19)
			1196T>C	rs4986791	(11, 12)
TLR9	605474	3p21	-1486T>C	rs187084	(11, 20)
			2848C>T	rs352140	(20)
			6577T>C	rs352162	(20)
			g.6808A>G	rs352139	
			-1237T>C	rs5743836	(11)
CD 14	158120	5q31	-159C>T	rs2569190	(11, 16-19, 21-26)
			-1145G>A	rs2569191	(24, 26)
LY96	605243	8q21	-1625C>G	rs11465996	(27, 28)

LBP	151990	20q11	26877T>C	rs2232618	(29)
MBL2	154545	10q21	Codon 52	rs5030737	(30)
			Codon 54	rs1800450	(30)
			Codon 57	rs1800451	(30)
MASP2	605102		p.Y371D	rs12711521	(30)
			p.D120G	N.A.	(30)
FCN2	601624		p.A258S	rs7851696	(30)
			p.T236M	rs17549193	(30)
RAGE	600214	6p21	-407 to -345	63bp ins/del	(32)
			570G>A	rs2070600	(32)
			-374T>A	rs1800624	(32)
			-429T>C	rs1800625	(32)
NLRP3	606416	1q44	-1017G>A	rs2027432	(33)
			5134A>G	rs12048215	(33)
hGR/NR3C1	138040	5q31	Bcl I C>G	rs41423247	(34)
Signal Transducing Adaptor Proteins					
IRAK1	300283	Xq28	1595 T>C	rs1059703	(35)
IRAK3	604459	12q14	15SNPs	Ht Block 1	(36)
Inflammatory Cytokines					
IL1A	147760	2q13	-889C>T	rs1800587	(37)
IL1B	147720	2q13	3953C>T	rs1143634	(38, 45)
			-1470G>C	N.A.	(37, 39)
			-511T>C	rs16944	(37-40)
			-31C>T	rs1143627	(16-18, 37, 39)
IL1RN	147679	2q13	VNTR	rs315952	(40)
			C>T	rs315952C	(41)
IL4	147780	5q31	-589T>C	rs2243250	(37, 42)
IL6	147620	7p15	-174G>C	rs1800795	(12, 16-19, 36, 38, 43, 44, 46-48)
			-572G>C	rs1800796	(37, 46, 47)

			-597G>A	rs1800797	
IL8	146930	4q13	-251A>T	rs4073	(49)
IL10	124092	1q32	-1082G>A	rs1800896	(12, 36-38, 43, 51-55)
			-819C>T	rs1800871	(12, 37, 43, 50, 52)
			-592C>A	rs1800872	(12, 37, 38, 43, 50-52)
IL17F	606496	6p12	7488T>C	rs763780	(43)
IL18	600953	11q23	-137G>C	rs187238	(12, 56)
			-607C>A	rs1946518	(12, 56)
TNF	191160	6p21	-308G>A	rs1800629	(12, 15-19, 21, 37, 58-62)
			-238G>A	rs361525	(62)
			-376G>A	rs1800750	(62)
LTA	153440	6p21	252A>G	rs909253	(45, 58, 60, 61, 63)
IFNG	147570	12q15	874T>A	rs2430561	(12, 37)
HMGB1	163905	13q12	-1514T>C	rs1412125	77
			2179C>G	rs2249825	(57)
			6850G>A	rs1045411	(57)
Other Genes not belonging to the Innate Immune System					
MYLK	600922	3q21	p.P21H	rs28497577	(65)
			p.S147P	rs9840993	(65)
				rs4678047	(65)
PRDX6	602316	1q25		43 SNPs	(66)
HSPA1B	603012	6p21	1538A>G	N.A.	(67)
HSPA1L	140559	6p21	2437C>T	rs2075800	(67)
HSP90B1	191175	12q23	-144C>A	rs9472238	(68)
SERPINE1	173360	7q22	-688	rs1799768	(18, 69)
VEGFA	192240	6p21		Ht Block 1	(36)
ANGPT2	601922	8p23	127635T>A	rs1868554	(70)

			135709T>C	rs2442598	
mtDNA		mtDNA	T4216C		(71)
MicroRNA		stem-loop 37/5p +22	G>C	rs4919510	(72)

Supplemental Table S2. Detailed overview of association with outcome for SNPs in the *TLR*, *CD14*, *IL*, and *TNF* genes of trauma patients

Gene	SNP	dbSNP ID	Author	Year	Population	N	SIRS	Sepsis	Septic Shock	MODS	Mortality	References
TLR1	-7202A>G	rs5743551	Thompson	2013	Whites	1498	-	+	+	+	↑ G allele	(9)
	742A>G	rs4833095	Thompson	2013	Whites	1498	-	+	+	+	↑ G allele	(9)
	1804G>T	rs5743618	Thompson	2013	Whites	1498	-	+	+	+	↑ T allele	(9)
TLR2	-15607A>G	rs1898830	Chen	2011	Han Chinese	410	-	+	+	+	-	(10)
	19216T>C	rs3804099	Chen	2011	Han Chinese	410	-	↑ C allele	-	↑ C allele	-	(10)
	22215T/G	rs7656411	Chen	2011	Han Chinese	410	-	+	-	+	-	(10)
	p.R753Q	rs5743708	Bronkhorst	2013	Mixed Ethnic	219	+	+	+	+	+	(11)
		rs5743708	McDaniel	2007	Mixed Ethnic	68	-	↑ AG	-	-	-	(12)
	-16934T>A	rs4696480	Bronkhorst	2013	Mixed Ethnic	219	↑ AA	+	+	+	+	(11)
TLR4	-2381A>G	rs2737190	Chen	2010	Han Chinese	303	-	+	-	+	-	(13)
	-2242T>C	rs10116253	Chen	2010	Han Chinese	303	-	↑ C allele	-	↑ C allele	-	(13)
	-1892G>A	rs10983755	Chen	2010	Han Chinese	303	-	+	-	+	-	(13)
	-1837A>G	rs1927914	Chen	2010	Han Chinese	303	-	+	-	+	-	(13)
	-1418T>C	rs10759932	Chen	2010	Han Chinese	303	-	+	-	+	-	(13)
	11367G>C	N.A.	Duan	2009	Han Chinese	132	-	↓ C allele	-	↓ C allele	-	(14)
	896A>G	rs4986790	Bronkhorst	2013	Mixed Ethnic	219	+	+	+	+	+	(11)

			McDaniel	2007	Mixed Ethnic	68	-	+	-	-	-	(12)
			Shalhub	2009	Whites	598	+	↓ A allele	+	+	+	(15)
			Barber	2004	Mixed Ethnic	159	-	+	↑ G allele	-	-	(16)
			Barber	2006	Mixed Ethnic	228		+	↑ G allele			(17)
			Barber	2008	Mixed Ethnic	149	-	+	+	+	+	(18)
			Shalhub	2009	Mixed Ethnic	69	-	+	+	+	+	(19)
	1196T>C	rs4986791	Bronkhorst	2013	Mixed Ethnic	219	+	+	+	+	+	(11)
			McDaniel	2007	Mixed Ethnic	68	-	+	-	-	-	(12)
TLR9	-1486T>C	rs187084	Bronkhorst	2013	Mixed Ethnic	219	+	+	+	+	+	(11)
			Chen	2011	Han Chinese	557	-	↑ G allele	+	+	-	(20)
	2848C>T	rs352140	Chen	2011	Han Chinese	557	-	+	+	+	-	(20)
	6577T>C	rs352162	Chen	2011	Han Chinese	557	-	↑ C allele	+	+	-	(20)
	g.6808A>G	rs352139	Chen	2011	Han Chinese	557	-	↑ G allele	+	+	-	(20)
	-1237T>C	rs5743836	Bronkhorst	2013	Mixed Ethnic	219	+	+	+	+	+	(11)
CD 14	-159C>T	rs2569190	Bronkhorst	2013	Mixed Ethnic	219	+	+	+	+	+	(11)
			Barber	2004	Mixed Ethnic	159	-	+	+	-	-	(16)
			Barber	2006	Mixed Ethnic	228	-	+	↑ C allele	-	-	(17)
			Barber	2008	Mixed Ethnic	149	-	+	+	+	↑ C allele	(18)
			Shalhub	2009	Mixed Ethnic	69	-	+	+	+	+	(19)

			Barber	2007	Mixed Ethnic	223	-	+	+	+	↑ C allele	(21)
			Dong	2010	Chinese	35	-	↑ T allele	+	-	-	(22)
			Dong	2009	Chinese	77	-	↑ T allele	-	↑ T allele	-	(23)
			Gu	2010	Han Chinese	105	-	↑ T allele	-	↑ T allele	-	(24)
			Heesen	2010	Unknown	58	-	+	+	+	+	(25)
			Liu	2011	Chinese	106	-	-	-	↑ T allele	-	(26)
	-1145G>A	rs2569191	Gu	2010	Han Chinese	105	-	↑ G allele	-	↑ G allele	-	(26)
			Liu	2011	Chinese	106	-	+	-	↑ G allele	-	(24)
IL1A	-889C>T	rs1800587	Gu	2010	Han Chinese	308	-	↑ T allele	-	↑ C allele	-	(37)
IL1B	3953C>T	rs1143634	Schroeder	2008	Caucasian	100	-	-	-	-	+	(38)
			Hildebrand	2005	Unknown	97	+	+	+	+	+	(45)
	-1470G>C	N.A.	Gu	2010	Han Chinese	308	-	↑ G allele	-	+	-	(37)
			Wen	2010	Han Chinese	238	_	↑ G allele	+	+	-	(39)
	-511T>C	rs16944	Gu	2010	Han Chinese	308	-	↑ C allele	-	+	-	(37)
			Schroeder	2008	Caucasian	100	-	-	-	-	+	(38)
			Wen	2010	Han Chinese	238	_	↑ C allele	+	+	-	(39)
			Hadjigeorgiou	2005	Greek	183	-	-	-	-	-	(40)
	-31C>T	rs1143627	Barber	2004	Mixed Ethnic	159	-	+	+	-	-	(16)
			Barber	2006	Mixed Ethnic	228		+	+	-	-	(17)

			Barber	2008	Mixed Ethnic	149	-	+	+	+	+	(18)
			Gu	2010	Han Chinese	308	-	↑ T allele	-	+	-	(37)
			Wen	2010	Han Chinese	238	_	↑ T allele	+	+	-	(39)
IL1RN	VNTR	rs315952	Hadjigeorgiou	2005	Greek	183	-	-	-	-	-	(40)
	C>T	rs315952C	Meyer	2013	European	778	-	+	+	-	+	(41)
IL4	-589T>C	rs2243250	Gu	2010	Han Chinese	308	-	↑ C allele	-	+	-	(37)
			Gu	2011	Han Chinese	308	-	↑ C allele	-	-	-	(42)
IL6	-174G>C	rs1800795	McDaniel	2007	Mixed Ethnic	68	-	+	-	-	-	(12)
			Barber	2004	Mixed Ethnic	159	-	+	+	-	-	(16)
			Barber	2006	Mixed Ethnic	228	-	+	+	-	+	(17)
			Barber	2008	Mixed Ethnic	149	-	+	+	+	+	(18)
			Shalhub	2009	Mixed Ethnic	69	-	+	+	+	+	(19)
			Meyer	2012	Mixed Ethnic	474	-	-	-	+	+	(36)
			Schroeder	2008	Caucasian	119	-	-	-	-	+	(38)
			Accardo	2012	Unknown	71	-	+	+	+	+	(43)
			Heesen	2002	Caucasian	57	-	+	+	+	-	(44)
			Gu	2008	Han Chinese	105	-	-	-	+	-	(46)
			Jeremic	2014	Unknown	47	-	+	+	+	+	(47)
			Dalla Libera	2011	Unknown	77	-	-	-	+	↓ G allele	(48)

	-572G>C	rs1800796	Gu	2010	Han Chinese	308	-	↑ C allele	-	+	-	(37)
			Gu	2008	Han Chinese	105	-	↓ G allele	-	+	-	(46)
			Jeremic	2014	Unknown	47	-	+	+	+	+	(47)
	-597G>A	rs1800797	Gu	2008	Han Chinese	105	-	-	-	-	-	(46)
IL8	-251A>T	rs4073	Hildebrand	2007	Unknown	97	-	+	+	+	+	(49)
IL10	-1082G>A	rs1800896	McDaniel	2007	Mixed Ethnic	68	-	↑ G allele	-	-	-	(12)
			Meyer	2012	Mixed Ethnic	474	-	-	-	+	+	(36)
			Gu	2010	Han Chinese	308	-	+	-	+	-	(37)
			Schroeder	2008	Caucasian	100	-	-	-	-	+	(38)
			Accardo	2012	Unknown	71	-	↑ G allele	+	+	+	(43)
			Schröder	2004	Unknown	119	-	+	-	+	+	(51)
			Zeng	2009	Han Chinese	308	-	↑ A allele	+	+	+	(52)
			Gong	2006	Caucasian	211	-	-	-	-	↓ G allele	(53)
			Jin	2012	Chinese	29	-	-	-	-	↓ G allele	(54)
	-819C>T	rs1800871	McDaniel	2007	Mixed Ethnic	68	-	+	-	-	-	(12)
			Gu	2010	Han Chinese	308	-	↑ T allele	-	+	-	(37)
			Accardo	2012	Unknown	71	-	-	+	+	+	(43)
			Huebinger	2010	Mixed Ethnic	265	-	+	-	+	↓ T allele	(50)
			Zeng	2009	Han Chinese	308	-	+	+	+	+	(52)

	-592C>A	rs1800872	McDaniel	2007	Mixed Ethnic	68	-	+	-	-	-	(12)
			Gu	2010	Han Chinese	308	-	+	-	+	-	(37)
			Schroeder	2008	Caucasian	100	-	+	-	-	+	(38)
			Accardo	2012	Unknown	71	-	-	+	+	+	(43)
			Huebinger	2010	Mixed Ethnic	265	-	+	-	+	↓ A allele	(50)
			Schröder	2004	Unknown	119	-	+	-	↑ AC	+	(51)
			Zeng	2009	Han Chinese	308	-	+	+	+	+	(52)
IL17F	7488T>C	rs763780	Accardo	2012	Unknown	71	-	-	+	+	+	(43)
IL18	-137G>C	rs187238	McDaniel	2007	Mixed Ethnic	68	-	+	-	-	-	(12)
			Stassen	2003	Mixed Ethnic	66	-	+	+	+	+	(56)
	-607C>A	rs1946518	McDaniel	2007	Mixed Ethnic	68	-	+	-	-	-	(12)
			Stassen	2003	Mixed Ethnic	66	-	+	+	+	+	(56)
TNF	-308G>A	rs1800629	McDaniel	2007	Mixed Ethnic	68	-	+	-	-	-	(12)
			Barber	2004	Mixed Ethnic	159	-	↑ A allele	+	-	-	(16)
			Barber	2006	Mixed Ethnic	228	-	↑ A allele	+	-	+	(17)
			Barber	2008	Mixed Ethnic	149	-	+	+	+	+	(18)
			Shalhub	2009	Mixed Ethnic	69	-	↑ A allele	+	+	+	(19)
			Shalhub	2009	Mixed Ethnic	598	-	+	+	+	+	(15)
			Barber	2007	Mixed Ethnic	223	-	+	+	+	+	(21)

			Gu	2010	Han Chinese	308	-	↑ A allele	-	+	-	(37)
			Duan	2011	Han Chinese	306	-	↑ A allele	+	+	+	(58)
			Gill	2008	Unknown	59	-	-	-	-	-	(59)
			Majetschak	2002	Unknown	70	-	+	+	-	+	(60)
			Menges	2008	Unknown	159	-	↑ A allele	+	+	↑ A allele	(61)
			O'Keefe	2002	Unknown	152	-	↑ A allele	+	+	↑ A allele	(62)
	-238G>A	rs361525	O'Keefe	2002	Unknown	152	-	+	+	+	+	(62)
	-376G>A	rs1800750	O'Keefe	2002	Unknown	152	-	+	+	+	+	(62)

+: outcome parameter was studied

-: outcome parameter was not studied

↑↓: genotype was positively/negatively associated with outcome parameter

Table 1. Overview of association with outcome for SNPs in the *TLR*, *CD14*, *IL*, and *TNF* genes of trauma patients

Gene	Number of SNPs studied	Number of patients studied	SIRS	Sepsis	Septic Shock	MODS	Mortality	References
TLR1	3	1498	-	+	+	+	↑ (3 SNPs)	(9)
TLR2	5	697	↑ (1 SNP)	↑ (2 SNPs)	+	↑ (1 SNP)	+	(10-12)
TLR4	8	1925	+	↑ (1 SNP) ↓ (2 SNPs)	↑ (1 SNP)	↑ (1 SNP) ↓ (1 SNP)	+	(11-19)
TLR9	5	776	+	↑ (3 SNPs)	+	+	+	(11, 20)
CD 14	2	1428	+	↑ (2 SNPs)	↑ (1 SNP)	↑ (2 SNPs)	↑ (1 SNP)	(11, 16-19, 21-26)
IL1A	1	308	-	↑ (1SNP)	-	↑ (1 SNP)	-	37
IL1B	4	1462	+	↑ (3 SNPs)	+	+	+	(16-18, 37-40, 45)
IL1RN	2	961	-	+	+	-	+	(40, 41)
IL4	1	308	-	↑ (1 SNP)	-	+	-	(37, 42)
IL6	3	1931	-	↑ (1 SNP) ↓ (1 SNPs)	+	+	↓ (1 SNPs)	(12, 16-19, 36-38, 43, 44, 46-48)
IL8	1	97	-	+	+	+	+	(49)
IL10	3	1953	-	↑ (2 SNPs)	+	↑ (1 SNP)	↓ (3 SNPs)	(12, 36-38, 43, 50-55)
IL17F	1	71	-	-	+	+	+	(43)
IL18	2	134	-	+	+	+	+	(12, 56)
TNF	3	2548	-	↑ (1 SNP)	+	+	↑ (1 SNP)	(12, 15-19, 21, 37, 58-62)

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