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PROCEEDINGS

Data Mining FAERS to Analyze Molecular Targets of Drugs Highly Associated with Stevens-Johnson Syndrome

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Abstract Drug features that are associated with Stevens-Johnson syndrome (SJS) have not been fully characterized. A molecular target analysis of the drugs associated with SJS in the FDA Adverse Event Reporting System (FAERS) may contribute to mechanistic insights into SJS pathophysiology. The publicly available version of FAERS was analyzed to identify disproportionality among the molecular targets, metabolizing enzymes, and transporters for drugs associated with SJS. The FAERS in-house version was also analyzed for an internal comparison of the drugs most highly associated with SJS. Cyclooxygenases 1 and 2, carbonic anhydrase 2, and sodium channel 2 alpha were identified as disproportionately associated with SJS. Cytochrome P450 (CYPs) 3A4 and 2C9 are disproportionately represented as metabolizing enzymes of the drugs associated with SJS adverse event reports. Multidrug resistance protein 1 (MRP-1), organic anion transporter 1 (OAT1), and PEPT2 were also identified and are highly associated with the transport of these drugs. A detailed review of the molecular targets identifies important roles for these targets in immune response. The association with CYP metabolizing enzymes suggests that reactive metabolites and oxidative stress may have a contributory role. Drug transporters may enhance intracellular tissue concentrations and also have vital physiologic roles that impact keratinocyte proliferation and survival. Data mining FAERS may be used to hypothesize mechanisms for adverse drug events by identifying molecular targets that are highly associated with drug-induced adverse events. The information gained may contribute to systems biology disease models.

Keywords Adverse drug event · Data mining · Stevens-Johnson syndrome · Pharmacovigilance

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Introduction

Stevens-Johnson syndrome (SJS) is a rare, serious, life-threatening adverse drug event (ADE) often identified as a post-market safety signal. SJS is thought to be an immune-mediated phenomenon, closely linked to the human leucocyte antigens (HLA) of the major histocompatibility complex (MHC). Recent molecular advances have led to improved understanding of this relationship. Histologically, a key feature is keratinocyte apoptosis and death resulting in epidermal necrosis and blistering as seen on physical examination [1]. Evidence indicates that the immune system mediates this toxicity. Cytotoxic T lymphocytes (CD8+) have been isolated from the blister fluid. Cytotoxic T cells secrete granulysin and Fas ligand. These cytokines and immune mediators are found in high levels in the blister fluid. CD8+ T lymphocytes suggest a role for MHC class-I antigen presentation. HLA alleles have been associated with skin hypersensitivity reactions for carbamazepine (HLA-B*15:02) and abacavir (HLA-B*57:01) [2]. Recent investigations have shown that carbamazepine and abacavir can interact directly with these specific

HLA alleles and stimulate T cell immunity [3, 4]. These investigations support what has been termed the pharmacological interaction theory that non-covalent drug binding alters the MHC-peptide interaction with T cell receptors such that a T cell response to self-peptides is elicited [5, 6]. These discoveries raise hope that drugs may be able to be screened for their hypersensitivity potential. While these HLA allele associations are specific, not all individuals with the allele develop the disease. There remains much to be learned about other cellular processes that determine which individuals who have the HLA allele are susceptible.

Allergic skin hypersensitivity reactions including SJS have been documented following exposure to antibiotics including the penicillins, sulfonamides, and macrolides; anticonvulsants; non-steroidal anti-inflammatory; and antiviral agents. Prior reviews had attributed this toxicity to the biotransformation of the drugs in human skin and their active metabolites [7, 8]. Recent investigations have documented the presence of cytochrome P450 (CYP) enzymes in the skin [9]. Of note, the CYP isoenzyme profiles are different between the liver and the skin. The skin CYP isoenzymes may create drug intermediates capable of covalently binding macromolecules by forming haptens, neo-antigens that would be processed and transported to the cell surface to interact with T cells. The direct binding of HLA allele studies suggests that non-covalent binding of the drug is most likely a key mechanism. However, drug biotransformation may have a role, as oxidant stress may contribute to the *danger signal* that stimulates the immune system to react. Similarly, the impaired function of common molecular targets may suggest targets important within a disease model for SJS.

More research is needed to fully understand the pathophysiology of SJS. Data mining as a bioinformatics approach is being applied to the FDA Adverse Event Reporting System (FAERS) database to generate mechanistic hypotheses into drug safety issues. The molecular actions of drugs both on-target and off-target are mapped to adverse events to identify these mechanisms. We performed a molecular target, metabolizing enzyme, and drug transporter analyses of drugs associated with SJS on the publicly available FAERS version. This data mining analysis therefore highlights molecular targets, enzymes, and transporters that may play a contributory role in the pathophysiology of SJS.

Materials and Methods

A software program, Molecular Analysis of Side Effects (MASE), was used for the analysis of the publicly available FAERS. MASE is currently being evaluated under a Research Collaboration Agreement by the FDA. MASE integrates the publicly available FAERS data with various chemical and biological data sources in a drug-centric focused manner. The publicly available FAERS data is from 2004 to present. Within the data integration process, FAERS medication synonyms are mapped to drugs and compounds in DrugBank (http://www.drugbank.ca/) and PubChem (http://pubchem.ncbi.nlm.nih.gov/). Based on this medication-drug mapping, the link to biomolecules and molecular mechanisms involved in pharmacodynamics and pharmacokinetics is established via UniProt (http://www.uniprot.org/) and the pathway resources NCI-Nature (http://pid.nci.nih.gov/), Reactome (http://www.reactome.org), and BioCarta (http://www.biocarta.com/). Literature data is extracted based on co-occurrence of MASE entity names and synonyms in PubMed abstracts (http://www.ncbi.nlm.nih.gov/pubmed/). Drugs are classified according to the Anatomical Therapeutic Chemical (ATC) classification system (http://www.whocc.no/atc/structure_and_principles/). Indications and reactions are classified using the MedDRA dictionary (http://www.meddramsso.com/). Proportional reporting ratios (PRRs) and relative odds ratios (RORs) are calculated using the approach described by van Puijenbroek et al. [10]. MASE contains a de-duplication algorithm. The cases have not been individually reviewed (public FAERS does not contain narratives). Therefore, litigation cases and any missed duplicate reports have not been removed from the total number of reports.

A FAERS search was also performed. The search used the preferred term Stevens-Johnson syndrome. The top 30 US-approved drugs having more than 30 FAERS reports were chosen for a detailed analysis. Note again that the cases have not been individually reviewed, and therefore, duplicate reports and litigation cases have not been removed from the total number of reports. Additionally, cases have not been adjudicated for causality. By selecting an $N>30$ for the detailed analysis, the likelihood of evaluating a drug not associated with SJS should be low. This number correlates to when PRR and empirical Bayesian geometric mean (EBGM) results become similar, and therefore, PRR results more definitely exclude false positives. Subsequently, the labels of these drugs and PubMed were searched to analyze the known biotransformation data for SJS-associated drugs.

Results: Disproportionate Molecular Targets Identified by Data Mining FAERS Reported Drugs Associated with SJS

Table 1 contains the results from the MASE analysis for drugs (primarily cases after 2004) ranked by PRR with greater than 30 reports, while Table 2 contains the FAERS results using the FDA program Empirica Signal for drugs ranked by EBGM. Antibiotics, NSAIDs, and anticonvulsants are heavily represented in both data mining analyses. The major difference between the in-house FAERS and MASE analyses is the lack of many of the NSAIDs. Many of the NSAIDs had marketing

Table 1 CYP metabolism of top drugs associated with SJS in FAERS

Drug	Number	EBGM	CYP metabolism	PubMed ID
Metamizole	117	43.1		
Pyrimethamine and sulfadoxine	50	32.4	2C9 (*N*-oxide)	[11–13]
Allopurinol	489	29.3	Xanthine oxidase	
Valdecoxib	1298	28.2	3A4, 2C9, glucuronidation	Label
Acetylcysteine	64	19.1		
Phenytoin	1115	18.7	2C9, 2C19	[14]
Clemastine	37	18.2	Hydroxylated, *N*-oxide metabolites	[15]
Sulfamethoxazole and trimethoprim	803	15.4	2C9 hydroxylamine	[16]
Loxoprofen	48	15.1	Yes	[17]
Diflunisal	53	14.4		Glucuronidation per label
Zonisamide	112	13.3	3A4 reduction	Label
Lamotrigine	1179	13	2A6, 2C11; aryl epoxide; 2B6, 2D6, 3A4	[18]
Nevirapine	277	11.4	3A4, 2B6	Label
Phenobarbital	103	10.4	2C9, 2C19	[19]
Amifostine	42	9.2	Hydrolyzed by alkaline phosphatase	Label
Carbamazepine	550	7.6	1A2, 2B6, 2C8, 2C9, 3A4	[20]
Meropenem	49	7.3		
Torsemide	32	6.9	2C9	[21]
Ampicillin/sulbactam	50	6.8		
Cefepime	40	6.8	Oxidation to sulfone: reduction to sulfide	Label
Oxaprozin	37	6.7	Hydroxylation	[22]
Cefdinir	47	6.6		
Furosemide	246	6.5	P450 gamma-ketocarboxylic acid	[23]
Sulindac	85	6.2	1A2, 1B1, 3A4, minimal 2C9	[24]
Amoxicillin	206	6.2		
Vancomycin	198	6.2		
Pantoprazole	93	5.8	2C19 demethylation/3A4 oxidation	
Piperacillin and tazobactam	58	5.8		
Fluconazole	153	5.6	2D6, 3A4	[25]
Cefotaxime	56	5.5		
Acetaminophen	249	2.9	1A2, 3A4, 2E1, 2C9	

discontinued prior to 2000, and these reports are likely not contained in the publicly available FAERS used by MASE. A comparison of the two tables finds some antibiotics replacing the NSAIDs in the top drug analysis.

All the SJS reports (N=6473) in the current version of MASE include a total of 906 drugs (considered primary, secondary, or concomitant). These 906 drugs were analyzed for their molecular targets in the publicly available databases. Therefore, if an SJS report has more than one drug associated with the case, then all the molecular targets of all the drugs including the concomitants would be identified for that SJS report.

Table 3 provides the top molecular target analysis of the drugs reported in the SJS FAERS reports. Molecular targets identified in high frequency in SJS cases include cyclooxygenases 1 and 2, carbonic anhydrase 2, and sodium channel protein type 2 subunit alpha. Table 4 lists the drugs and their target analysis, metabolizing enzyme, and transporter actions as noted from the MASE databases and supplemented by PubMed search results. Specific drugs and relationships with these targets are addressed in the "Discussion."

Discussion

One observation that seems to emerge is an association of the protein targets and roles or links to the immune response. Cyclooxygenases 1 and 2 are the highest frequency molecular targets identified. An association with selective COX-2 inhibitors has previously been reported by the FDA [38]. This analysis also noted that the rate was higher for the sulfonamide COX-2 inhibitors including valdecoxib and celecoxib, when

Table 2 MASE analysis for drugs associated with SJS (over 30 reports required)

Drug	Number	PRR
Valdecoxib	1553	39.2
Zonisamide	80	11.1
Lamotrigine	784	10.0
Metamizole	62	8.7
Phenytoin	305	8.1
Nevirapine	126	7.5
Acetylcysteine	52	7.3
Phytonadione	34	6.9
Cefdinir	32	6.4
Flunitrazepam	32	5.8
Carbamazepine	264	5.7
Ceftriaxone	107	5.7
Sulfamethoxazole	280	5.5
Cefuroxime	123	5.2
Trimethoprim	271	5.0
Rifampin	47	5.0
Azithromycin	120	4.9
Cefepime	34	4.9
Vancomycin	118	4.8
Cephalexin	59	4.8
Allopurinol	269	4.4
Amoxicillin	215	4.1
Fluconazole	135	4.0
Clindamycin	55	4.0
Torasemide	63	3.7
Ciprofloxacin	167	3.6
Chlorpheniramine	39	3.5
Clarithromycin	83	3.5
Erythromycin	31	3.5
Amphotericin B	38	3.3
Acetaminophen	313	1.24

PRR proportional reporting ratio

compared to the non-sulfonamide rofecoxib. Another literature review concluded that the oxicam class of NSAIDs was the most prevalent class of NSAIDs associated with SJS [39]. Many other SJS-associated drugs have anti-inflammatory actions, many mediated through prostaglandin inhibition (Table 4).

Carbonic anhydrase inhibition was identified as another highly associated SJS target by MASE. Carbonic anhydrase II (CAII) is involved in the maintenance of cellular pH, water transport, and ion homeostasis. CAII inhibition is a property of many sulfonamide and/or dioxothiazine drugs including antibiotics (ceftriaxone), anticonvulsants (zonisamide), and diuretics, drug classes commonly associated with SJS [40]. Part of the core structure of the oxicam class includes a S–N bond into a ring structure, dioxothiazine ring structurally resembling the sulfonamide bond. COX-2 inhibitors valdecoxib, celecoxib, and metamizole also inhibit CAII [41]. No studies have been done that evaluate the potential role for carbonic anhydrase II inhibition in the pathogenesis of SJS. CAII has been shown to be strongly expressed in normal skin [42, 43]. CAII is upregulated in atopic dermatitis and is inducible in keratinocytes exposed to Th2 cytokines [44]. CAII has been identified as an acute phase protein [45]. These observations highlight a role for CAII in some inflammatory responses. Autoantibodies to CAII have been detected in a number of autoimmune diseases including connective tissue diseases, diabetes mellitus, pancreatitis, and retinopathy. In a study of preeclampsia, anti-CAII antibodies correlated with increased levels of oxidant stress [46]. CAII-deficient mice develop duodenal ulcers secondary to impaired PGE2 signaling [47]. Carbonic anhydrase I has a role in epithelial regeneration in a mouse colitis inflammation model [48]. Further evaluation of a potential role of carbonic anhydrase in the pathophysiology of SJS appears warranted.

Sodium channel 2α (Nav1.2) is another target highly associated with SJS in the MASE analysis. Nav1.2 seems to be specific to the anticonvulsants, while the COX and CAII inhibitors covered multiple classes of drugs. The only drugs known to interact with Nav1.2 are the anticonvulsants. Nav1.2 is expressed on keratinocytes and appears to be associated with pain [49]. Carbamazepine and phenytoin bind the same site on Nav1.2. This fact may highlight a shared structure activity relationship whereby they interact with HLA-B*15:02 in a similar fashion, but the other differences in their structures may result in the stimulation of differing T cell clones [6]. The epithelial sodium channel is important to keratinocyte differentiation, migration, and wound healing [50, 51].

Our analysis identifies CYP3A4 and CYP2C9 as the most common biotransformation enzymes associated with SJS drugs. Greater than two thirds of the SJS reports include a drug that undergoes bioactivation by one or both of these CYPs. This percentage is likely higher as a number of drugs in the table have not been tested for potential CYP metabolism. Many of the most common drugs associated with SJS identified in FAERS have not had metabolic studies performed as elimination is predominantly by the renal route. This includes seven of the antibiotics primarily eliminated by the renal route and other older drug approvals including some drug withdrawals. Thirteen of the drugs have specific CYPs identified that biotransform the parent drug. Another five drugs appear to have evidence for CYP bioactivation, although specific CYPs have not been identified. The 13 drugs where the CYPs are identified all include CYP2C9, CYP3A4, or both of these CYPs in their metabolism. Therefore, both analyses support the hypothesis that drugs that undergo CYP3A4 and/or CYP2C9 metabolism may be at greater risk for the development of SJS. CYP3A4 and CYP2C9 are two of the predominant CYPs in the liver. An oligopeptide microarray

Table 3 MASE-identified molecular targets, metabolizing enzymes, and transporters for Stevens-Johnson syndrome for 6473 SJS reports in the public FAERS Version

Target	AE reports	PRR	CI PRR
CYP3A4	4709	1.34	1.32–1.36
CYP2C9	4371	1.66	1.63–1.69
MRP 1	3638	1.13	1.10–1.15
COX 1	3211	2.01	1.96–2.06
COX 2	2644	2.39	2.32–2.46
UDP-glucuronosyl transferase	2370	6.15	5.95–6.35
Carbonic anhydrase 2	2016	4.9	4.72–5.08
CYP 1A2	2011	0.93	0.89–0.96
SLC 22 member 6 (OAT1)	1467	1.35	1.29–1.41
Na channel protein type 2 subunit alpha	900	8.69	8.17–9.24
Oligopeptide transporter, kidney (PEPT2)	716	2.11	1.97–2.26
CYP2E1	555	0.97	0.89–1.05

Table 4 Reported target, metabolizing enzyme, and transporter actions of drugs highly associated with SJS

Drug	Number	PRR	COX-1/2	CAII	SCN2α	3A4/2C9	UDPG	PEPT2
Valdecoxib	1553	39.2	s/i			s/s, i	s (1–9)	
Zonisamide	80	11.1		i	i	s/		
Lamotrigine	784	10.0	/i		i	s/	(1–4)	
Metamizole	62	8.7	i/i	i [26]		ind/		
Phenytoin	305	8.1			5a i	s, ind/s, i, ind		
Nevirapine	126	7.5				s, i, ind/s, i		
Acetylcysteine	52	7.3						
Phytonadione	34	6.9						
Cefdinir	32	6.4						i
Flunitrazepam	32	5.8				s/s	i (2b7)	
Carbamazepine	264	5.7			5a i	s, ind/ind	s [27] i [28]	
Ceftriaxone	107	5.7		i [29]				i
Sulfamethoxazole	280	5.5	s/			s, i/s, i	s (1–9)	
Cefuroxime	123	5.2						i
Trimethoprim	271	5.0				s/s, i	pigs [30]	
Rifampin	47	5.0				s, ind/s, ind	(1–1)	
Azithromycin	120	4.9				s, i/		
Cefepime	34	4.9						i
Vancomycin	118	4.8						
Cephalexin	59	4.8				s/		i
Allopurinol	269	4.4						
Amoxicillin	215	4.1						i
Fluconazole	135	4.0				i/i	i (2b4, 2b7) [31] s (2b7) [32]	
Clindamycin	55	4.0				si/		
Torasemide	63	3.7	s/			/s		
Ciprofloxacin	167	3.6	Immunsupp Inc COX-2 PGE2 [33]			i/	s [34]	
Chlorpheniramine	39	3.5				s/	s [35]	
Clarithromycin	83	3.5				s, i, ind/		
Erythromycin	31	3.5	Down COX-2 by p38 MAPK [36]			s, i/		
Amphotericin B	38	3.3	Induces COX-2/PGE2 [37]					
Acetaminophen	313	1.24	i/i			s, i, ind/s		

s substrate, *i* inhibitor, *ind* inducer

analysis of human skin samples finds these CYPs to be in much lower concentrations with CYPs 1A1, 1B1, 2B6, 2E1, and 3A5 as the predominant human skin CYP isoforms [9]. It is unknown whether drug exposure induces CYPs in the skin. In addition, our analysis suggests an association with phase II metabolism via glucuronidation. Many drugs associated with SJS also have uridine 5′-diphospho (UDP)-glucuronosyltransferase as a biotransformation enzyme.

A recognized consequence of the biotransformation of many drugs includes the production of reactive metabolites and oxidative stress. This process may represent a danger signal that promotes an enhanced immune response. Further research is needed to determine if biotransformation and oxidant stress is a necessary condition for some drugs to cause SJS.

Our analysis also highlights a number of drug transporters that may also be associated with SJS. Multidrug resistance protein 1 (MRP1) is the most commonly identified transporter, although the PRR score is low. More than half of the reports include drugs with MRP1 as a target. Another transporter common to SJS reports is SLC22A6, also known as organic anion transporter 1 (OAT1). The beta-lactam antibiotics, NSAIDs, and diuretics are transported by OAT1.

Drug transporters can lead to the intracellular accumulation of a drug in a tissue, potentially enhancing toxicity. The presence of 40 solute drug carriers has been identified on human epidermal keratinocytes [52]. Sorafenib is a tyrosine kinase inhibitor (TKI) that produces significant skin toxicity, namely, hand-foot syndrome or palmar-plantar erythrodysesthesia. The intracellular accumulation of sorafenib has been shown to depend upon one of these solute carriers, OATP1B1. In addition, drug transporters have important physiologic roles. Inhibition of their function likely has detrimental effects. Probenecid inhibits the ABCC-type transporters [53]. This inhibition results in decreased keratinocyte proliferation. Other physiological responses to this inhibition that may impact the development of SJS require further investigation.

OAT1 is the most frequent transporter noted for SJS in this study. Substrates for this transporter include many drugs known to be associated with SJS. A partial list includes NSAIDs, β-lactam antibiotics, diuretics, glucuronide conjugates, and cysteine conjugates [54]. OAT1 has a transport role in a number of vital metabolic pathways including the citric acid cycle, pyruvate, nucleotide, polyamine, and fatty acid metabolism [55].

Another transporter identified as highly associated with SJS in our analysis is the oligopeptide transporter, kidney isoform (PEPT2). This drug transporter handles β-lactam antibiotics and antiviral agents [56–58]. PEPT2 is a proton-dependent transporter of di- and tri-peptides. PEPT2 transports intracellularly bacterial peptides that are recognized by the nucleotide-binding oligomerization domain (NOD)-like receptor that results in activation of the innate immune system [59]. PEPT2 appears to have a role in immune activation of macrophages by the transport of *S*-nitrosothiols intracellularly [60]. The consequences of drug and drug-drug interactions on these pathways are the subjects of ongoing research.

Further testing is needed to determine the overall role the targets identified in this analysis may play in SJS. It is likely that some targets contribute to the individual variability that makes some patients more susceptible to SJS than others.

Further in silico, in vitro, and in vivo experiments are needed to test these hypotheses. It is possible that the phenotypic analysis of FAERS may suggest key targets in pathways associated with SJS and other adverse events. One example would be to perform knockout studies to see the importance of the identified targets in the immune response and future development of an SJS disease pathway.

The analysis of FAERS data can also provide input for cheminformatic structure activity response analyses. Computational analyses can be done to look for chemical similarities that are shared by the drugs that cause SJS. An analysis of the drugs that also act upon specific targets will help define the structural properties of drugs that bind a target. It is possible that some of the drugs that cause SJS and were not previously known to interact with carbonic anhydrase may also share this property. This type of in silico analysis has been described previously to show that cyclobenzaprine had serotonergic properties that were then confirmed by receptor in vitro binding studies [61]. QSAR analyses have been developed to predict hepatotoxicity [62]. Similarly, FDA-approved drugs have been searched to identify previously unknown drug target interactions that were confirmed by in vivo testing [63]. These in silico analyses may help identify key chemical ligands associated with SJS. These ligands may prove to represent similar drug structures that bind to HLA complexes. Some targets similar to any drug's adverse event may represent secondary effects, e.g., akin to an off-target drug effect.

Another potential application for this data mining approach is for the analysis of biological plausibility. For example, if a drug shares many of the targets with other drugs clearly associated with an adverse event, it is more likely to also be associated with that adverse event. Acetaminophen was recently labeled for SJS. A PubMed search suggests that acetaminophen may have activity at most of the targets highly associated with SJS. One report suggests acetaminophen inhibits COX-1 and COX-2, but is a more potent COX-2 inhibitor [64]. Acetaminophen is also reported to be a CAII inhibitor [40]. Using human recombinant CYPs, 3A4 was found to be the most efficient in the generation of NAPQI, followed by CYP2E1 [65]. CYP1A1, CYP1A2, CYP2C19, and CYP2D6 had intermediate activity, while CYP2A6, CYP2B6, and CYP2C9 had weak activity. Glucuronidation is a

predominant conjugation pathway for acetaminophen clearance. Antibodies to the acetaminophen-glucuronide conjugate have been detected in acetaminophen-induced thrombocytopenia [66]. Acetaminophen is a substrate and weak inhibitor of OAT1, while most other NSAIDs are strong inhibitors of OAT1 [55]. A PubMed search for PEPT2 or NAV1.2 activity did not identify investigations into activity at these targets.

Conclusions

Growing evidence implicates a role for direct drug interactions with MHC complexes as having an important role in the development of SJS. HLA studies have identified specific alleles that are associated with drug-induced SJS. However, only some patients with the specific allele develop SJS when exposed to the drug. An analysis using FAERS was done to identify drug-related targets, enzymes, and transporters that may play a role in the pathophysiology of SJS. The analysis finds disproportionality for drugs that inhibit cyclooxygenases, carbonic anhydrase, and sodium channel 2 subunit α. CYP3A4, CYP2C9, and UDP-glucuronidation represent the highest frequency biotransformation pathways associated with these drugs, while MRP-1, OAT1, and PEPT2 are highly associated with their transport. These common targets may represent shared structure activity relationships among the drugs associated with SJS. Further research is needed to determine the potential role of these targets in the pathophysiology and development of systems biology disease models for SJS.

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