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ORIGINAL INVESTIGATION

Mutant GNLY is linked to Stevens–Johnson syndrome and toxic epidermal necrolysis

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Abstract

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare severe cutaneous adverse reactions to drugs. Granulysin (GNLY) plays a key role in keratinocyte apoptosis during SJS/TEN pathophysiology. To determine if GNLY-encoding mutations might be related to the protein's functional disturbances, contributing to SJS/TEN pathogenesis, we performed direct sequencing of GNLY's coding region in a group of 19 Colombian SJS/TEN patients. A *GNLY* genetic screening was implemented in a group of 249 healthy individuals. We identified the c.11G > A heterozygous sequence variant in a TEN case, which creates a premature termination codon (PTC) (p.Trp4Ter). We show that a mutant protein is synthesised, possibly due to a PTC-readthrough mechanism. Functional assays demonstrated that the mutant protein was abnormally located in the nuclear compartment, potentially leading to a toxic effect. Our results argue in favour of GNLY non-synonymous sequence variants contributing to SJS/TEN pathophysiology, thereby constituting a promising, clinically useful molecular biomarker.

Introduction

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare severe cutaneous adverse reactions (SCAR) to drugs characterised by exanthema, erosion of the skin and mucous membranes, purpura, blistering, skin detachment secondary to keratinocyte death, and ocular involvement (Duong et al. 2017). Respiratory and gastrointestinal systems can sometimes be affected. SJS and TEN are considered to be variations of the same hypersensitivity disorder. Patients are classified as suffering from SJS and TEN when < 10% and > 30% of the total body surface area (TBSA) are affected, respectively (Roujeau 1997). SJS/TEN overlap has been used for classifying patients suffering 10–30% TBSA injury.

All of the disease's presentations involve high mortality rates, ranging from 1–5% (SJS) to 30–50% (TEN). SJS and TEN are rare disorders since they affect ~ 1.5 to 1.9 individuals per million inhabitants worldwide per year (Harr and French 2010; Letko et al. 2005). At least 200 drugs to date have been related to the disease's onset, such as allopurinol, nonsteroidal anti-inflammatories, antimicrobial sulpha, antiepileptic and antiretroviral molecules (Harr and French 2010). SJS/TEN pathophysiology has not yet been completely understood, but it has been shown that distinct immune system cells and molecules (e.g. CD8-T and NK cells, FasL and granulysin cytolytic proteins) are key actors in the disease's pathogenesis (Schwartz et al. 2013). Specific HLA-B alleles' genetic associations have been linked to the phenotype. For instance, it has been

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suggested that the HLA-B*1502 subtype in Asian populations might be a genetic marker for the disease. Chung et al. observed a strong correlation between this allele and SJS/TEN in Han Chinese individuals who were receiving carbamazepine (Chung et al. 2004). Subsequent studies involving additional southeastern populations have confirmed HLA-B*1502 patients' susceptibility to carbamazepine and further antiepileptic drugs (Fan et al. 2017; Man et al. 2007; Tassaneeyakul et al. 2010). Allopurinol treatment and HLA-B*5801 have been strongly associated with severe adverse drug reactions in Asian (Han Chinese, Japanese and Thai) and (to a lesser extent) Caucasians populations (Harr and French 2010; Pavlos et al. 2012; Yip et al. 2015).

However, these and other HLA associations with specific drug-induced SJS/TEN phenotypes have been limited to specific ethnicities. Furthermore, the exact functional mechanism linking HLA polymorphic molecules and T cells' cytotoxic response has not been completely elucidated. Polymorphisms in a few other genes have been proposed as being potentially related to SJS/TEN, such as *EPHX1, CYP2C9, CYP2B6* and *IKZFI* (Caruso et al. 2014; Ciccacci et al. 2013; He et al. 2014; Ueta et al. 2015).

Several molecular mediators have been described for SJS/TEN pathophysiology, such as granulysin (GNLY), TNF-α, Fas–Fas ligand interaction, TNF-related apoptosis-inducing ligand (TRAIL), and perforin–granzyme B (Harris et al. 2016). GNLY has been shown to play a key role in keratinocyte apoptosis since its expression was higher than that for other cytotoxic proteins in SJS/TEN-affected individuals' blister fluids from skin lesions (Chung et al. 2008). Interestingly, GNLY has also been shown to have proinflammatory activator and chemoattractive properties which may contribute towards the SJS/TEN phenotype (Finn et al. 2011). GNLY has been found to be high in SJS/TEN patients' plasma (before the onset of skin detachment or mucosal lesions) which has enabled it to be used as a potential biomarker for the disease's early phases (Abe et al. 2009; Yang et al. 2017). However, the exact mechanisms underlining GNLY overexpression and toxicity contributing to the SJS/TEN phenotype are yet to be discovered.

The present study has hypothesised that GNLY-encoding mutations might be related to the protein's structural/functional disturbances, thereby contributing to SJS/TEN pathogenesis. We thus sequenced *GNLY*'s coding region in a group of 19 Colombian SJS/TEN patients. We identified the c.11G > A heterozygous sequence variant in a woman affected by TEN, which creates a premature termination codon (PTC) (p.Trp4Ter). We show that a mutant protein is synthesised, possibly due to a PTC-readthrough mechanism. Functional in vitro assays demonstrated that the mutant protein was abnormally located in the nuclear compartment, potentially leading to a toxic effect.

Our results argue in favour of GNLY non-synonymous sequence variants contributing to SJS/TEN pathophysiology, thereby constituting a promising, clinically useful molecular biomarker.

Materials and methods

Patients and controls

The clinical features defined by Roujeau were used for the patients' diagnostic classification (2). Nineteen (SJS-1 through SJS-19) patients were included in the present study: 5 SJS, 2 SJS/TEN and 12 TEN (Table 1). They attended the Hospital Simón Bolívar (Bogotá, Colombia) or the Fundación Valle de Lili (Cali, Colombia). Two patients belonged to the same family (a woman SJS-18 and her son SJS-19). Eleven drugs were associated with the symptoms' onset: carbamazepine (4 cases), lamotrigine (3), contrast media (2), sulfadoxine/pyrimethamine (2, the familial case), codeine/paracetamol (1), cephalexin (1), sulphonamide (1), phenytoin (1), trimethoprim/sulfamethoxazole (1), metoclopramide (2) and *Urtica dioica* (1) (Table 1). Two groups of individuals (GP1 and GP2), lacking antecedents of adverse reactions to drugs, were created to screen the c.11G > A (p.Trp4Ter) variant. GP1 consisted of 99 Colombian individuals who had taken phenytoin for at least 6 months while GP2 included 150 healthy women from the Valledupar area (a northern region of Colombia).

The Universidad del Rosario (Code: DVG-098) and Fundación Valle de Lili's (Code: P-515) Ethical Committees approved the study. The clinical investigation followed Helsinki Declaration guidelines (1975, as revised in 1996). All patients had signed informed consent forms.

GNLY sequencing and in silico analysis

Genomic DNA was isolated from whole-blood samples from all patients and GP1–GP2 individuals, using the standard salting-out procedure.

The complete *GNLY* encoding region (5 exons, ENST00000263863) was amplified by PCR using exon-flanking primers. The amplicons were then purified and directly sequenced using internal oligonucleotides enabling intron-exon boundaries to be studied. Genetic screening of GP1-GP2 individuals was used for c.11G > A (p.Trp4Ter), by implementing an identical protocol to that used with patients. Primer sequences and PCR conditions are available upon request. Sequence variants' minor allele frequencies (MAF) were screened in the Genome Aggregation Database (gnomAD) (gnomad.broadinstitute.org). The c.11G > A (p.Trp4Ter) variant allele frequency identified in the patient was compared to that regarding GP1-GP2

Table 1 Patients' clinical characteristics

ID	Age	Gender	Drug	Symptoms				Phenotype
				Systemic	Mucosal involvement	Rash/flictenas	TBSA injury (%)	
SJS-1	29	Female	Acetaminophen/codeine	Not referred	Oral	Yes/not	10	SJS
SJS-2	59	Male	*Urtica dioica*	Fever	Oral, ocular	Yes/not	20	SJS/TEN
SJS-3	59	Female	Metoclopramide	Fever	Oral, ocular	Yes/yes	95	TEN
SJS-4	46	Female	Lamotrigine	Leukocytosis fever	Oral, genital	Yes/yes	70	TEN
SJS-5	48	Female	Contrast medium	Not referred	Oral, genital	Yes/not	10	SJS
SJS-6	68	Female	Carbamazepine	Fever	Oral, ocular genital	Yes/yes	80	TEN
SJS-7	15	Female	Lamotrigine	Leukocytosis fever	Oral, ocular genital	Yes/yes	80	TEN
SJS-8	37	Male	Cephalexin	Leukocytosis fever	Oral, ocular genital	Yes/yes	90	TEN
SJS-9	30	Male	Sulfonamide	Not referred	Oral	Yes/not	10	SJS
SJS-10	35	Male	Contrast medium	Arthralgias	Oral	Yes/yes	10	SJS
SJS-11	45	Female	Phenytoin	Leukocytosis fever	Oral, ocular	Yes/yes	60	TEN
SJS-12	36	Female	Carbamazepine	Leukocytosis	Ocular	Yes/not	20	SJS/TEN
SJS-13	5	Male	Carbamazepine	Leukocytosis fever	Oral, ocular	Yes/yes	80	TEN
SJS-14	18	Male	Carbamazepine	Leukocytosis fever	Oral, ocular genital	Yes/yes	80	TEN
SJS-15	54	Female	Trimethoprim/sulfamethoxazole	Arthralgias leukocytosis	Oral	Yes/yes	78	TEN
SJS-16	9	Male	Metoclopramide	Fever	Oral, genital	Yes/not	80	TEN
SJS-17	5	Male	Lamotrigine	Leukocytosis fever	Oral, ocular genital	Yes/yes	80	TEN
SJS-18	18	Female	Pyrimethamine sulfadoxine	Fever arthralgias	Oral, ocular	Yes/yes	80	TEN
SJS-19	1	Male	Pyrimethamine sulfadoxine	Not referred	Ocular	Yes/not	10	SJS

individuals. Chi-square tests were used for calculating allele frequencies' significant differences between case (SJS-3) and control individuals. The Clustal Omega tool (https://www.ebi.ac.uk/) was used for aligning GNLY proteins from differing mammalian species (Pan troglodytes, Ovis aries, Bos taurus and Sus scrofa). SIFT and PolyPhen2 computational tools were used for assessing the c.356 C > T (p.Thr119Ile) sequence variant's potentially harmful effect.

Plasmids and constructs

The complete *GNLY* (NM_006433) coding sequence was amplified by PCR and cloned into the pcDNA3.1/CT-GFP-TOPO vector (Thermo Fisher Scientific K482001) (WT construct). This plasmid was subjected to site-directed mutagenesis (GeneArt kit, Thermo Fisher A13282) to introduce the c.11G > A (p.Trp4Ter) sequence change (construct name: M1). The M2 construct, carrying the c.34A > G (p.Met12Val) mutation located at the first downstream in-phase ATG codon (AUG) after the start of the open reading frame (ORF), was then created by a similar approach, using M1 DNA as template. The M2 construct carries, therefore, the p.Trp4Ter and p.Met12Val mutations. Sanger sequencing was used for verifying all constructs to exclude potentially unexpected variants induced during cloning and site-directed mutagenesis.

Cell culture and transfections

Chinese hamster ovary (CHO) cells were grown in DMEM-F12 (Life Technologies) medium supplemented with foetal bovine serum and 1% penicillin/streptomycin (Invitrogen–Gibco). 6×10^6 cells/well were seeded at the bottom of 24-well plates (for Western blot, WB) or on coverslips (for subcellular localisation) previously placed inside wells and transiently transfected, using the FuGENE 6 transfection reagent (Promega), with 300 ng of WT, M1, M2, or pcDNA3.1 (empty vector) plasmids. These experiments enabled performing downstream WB and protein subcellular localisation assays (GFP staining). Non-transfected cells were grown to study GNLY basal expression in CHO cells by WB assays.

GNLY in vitro subcellular localisation

After 48 h' transfection, cover slips were washed with 1X PBS. The cells were fixed with 4% paraformaldehyde for 25 min and washed three times with 1X PBS. The cover slips were mounted on slides using DAPI for nuclei staining. A fluorescent Nikon Eclipse NiE microscope was used for visualising the slides and images were captured with a Photometric (Coolnap EZ) digital camera using NIS-Elements Advanced Research software.

Western blot assays

The transfected cells were directly lysed with 1X RIPA and boiled in 2X Laemmli buffer. Cell lysate proteins were separated on SDS-PAGE using 5–13% acrylamide–bisacrylamide gels. The proteins were then transferred to nitrocellulose membranes (Biorad-162-0115) which were probed using an anti-granulysin monoclonal antibody (sc-27119, Santacruz Biotech), followed by incubation with a horseradish peroxidase-conjugated anti-mouse IgG antibody (Sigma A0545). The SuperSignal West Pico Chemiluminescent substrate system (Thermo Scientific, #34580) was used for analysing the antigens.

Results

GNLY sequencing and bioinformatics analysis

Sequencing analysis of the GNLY-encoding regions in SJS/TEN patients revealed the c.11G > A (p.Trp4Ter, heterozygous) (SJS-3) and c.356 C > T (p.Thr119Ile) non-synonymous variants (Table 2). The gnomAD database MAF frequencies were 0.08464 (c.11G > A, rs.10181739) and 0.3798 (c.356 C > T, rs11127). We did not find the c.11G > A sequence variant in GP1 and GP2 individuals. Statistically significant differences were identified between the SJS-3 patient and the GP control groups regarding c.11G > A variant genotypic frequencies ($p = 0.013$) (Table 2). Multiple protein alignments between species demonstrated that the W^4 residue and several surrounding C-ter and N-ter amino acids had been conserved during mammalian species' evolution (Supplementary Fig. 1); on the contrary, the T^{119} residue had not been strictly conserved during evolution. SIFT and PolyPhen2 predictors' scores were not compatible with potentially harmful effects regarding the p.Thr119Ile variant (SIFT: 0.2219 tolerated, Polyphen2: benign). The *GNLY* c.11G > A variant was submitted to a locus-specfic database (https://databases.lovd.nl/shared/genes/GNLY).

Western blot experiments

Non-transfected cells' WB (and those transfected with the empty vector) was negative, thereby indicating a lack of GNLY basal expression in CHO cells (Fig. 1). Experiments using protein extracts from WT, M1- and M2-transfected cell constructs had bands corresponding to the theoretical molecular weight of GNLY fused to the GFP protein (~42 kDa) (Fig. 1).

Protein subcellular localisation

CHO cells transfected with the WT GNLY version revealed the protein's diffuse cytoplasmatic localisation and no cells showed GFP nuclear staining (Fig. 2). Transfections with the M1 construct led to the GNLY-p.Trp4Ter protein's exclusive nuclear subcellular localisation (Fig. 2).

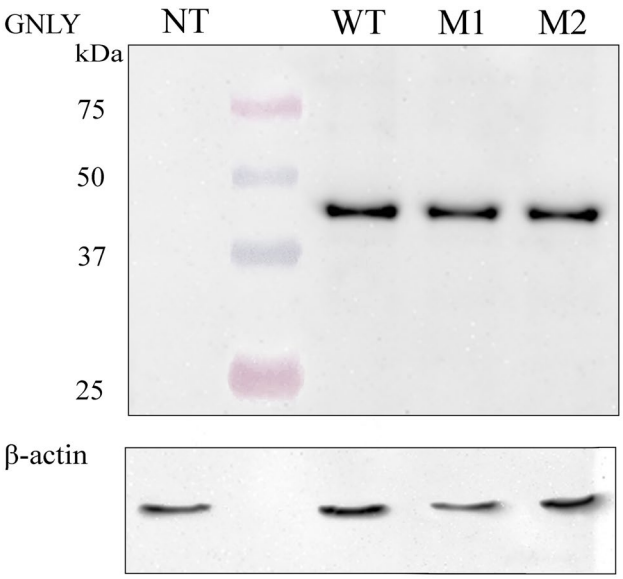

Fig. 1 Western blot experiments from CHO cells transfected with WT and mutant versions of GNLY. *WT* wild type, *M1* GNLY carrying the p.Trp4Ter mutation, *M2* GNLY carrying the p.Trp4Ter and p.Met12Val mutations, *NT* non-transfected cells

Table 2 Allele and genotype frequencies in patients and controls

Sequence variants	c. 11G > A			c.356 C > T
dbSNP	rs.10181739			rs.11127
Protein	p.Trp4Ter			p.Thr119Ile
Allele frequency	Patients	GP1	GP2	Patients
	G: 97.2% A: 2.8%	G: 100% A: 0%	G: 100% A: 0%	C: 50% T: 50%
Genotype frequency	Patients	GP1	GP2	Patients
	GG:94.5% GA:5.5% AA: 0%	GG:100% A: 0% *p value*: 0.0133*	G: 100% A: 0% *p value*: 0.0133*	CC: 33.3% CT: 33.3% TT: 33.3%

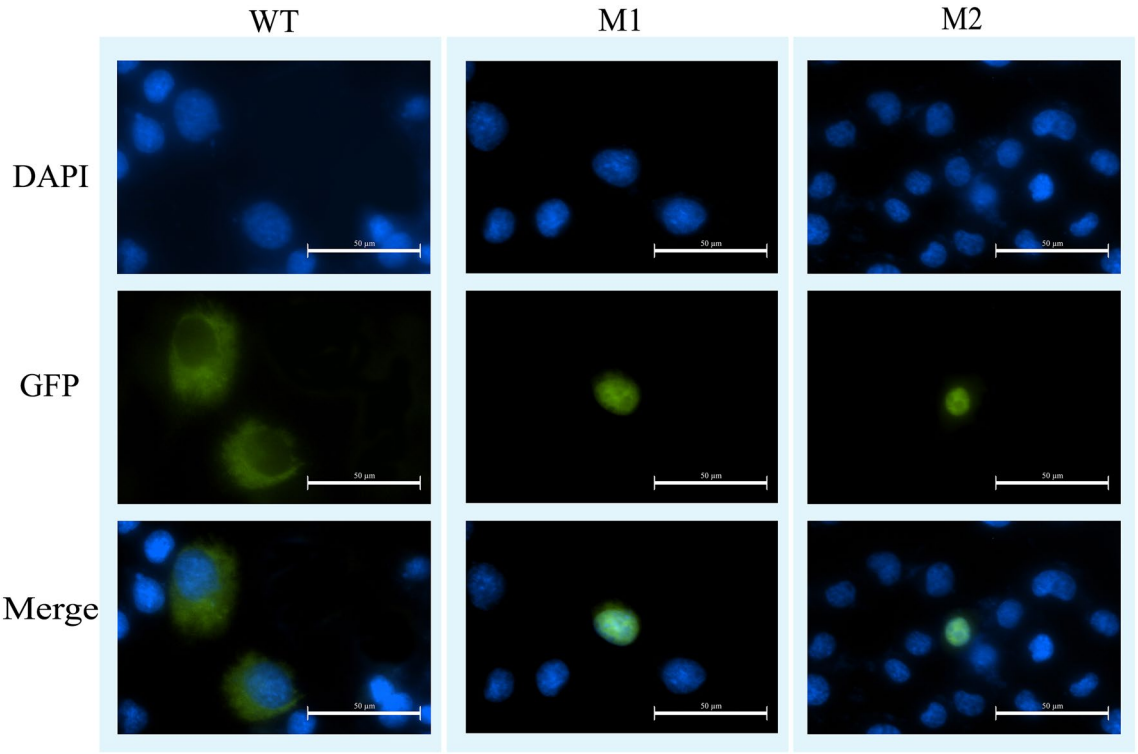

Fig. 2 GNLY subcellular localisation. *WT* wild type, *M1* GNLY carrying the p.Trp4Ter mutation, *M2* GNLY carrying the p.Trp4Ter and p.Met12Val mutations

Discussion

SJS/TEN constitute medical emergencies, as they are potentially fatal. The GNLY protein's central role in these diseases' pathogenesis and previously described specific genetic markers' lack of clear-cut aetiological association, led us to suspect that *GNLY*-encoding variants may contribute to the phenotype. This study has described, for the first time, the molecular analysis of a cohort of Latin-American (Colombian) SJS/TEN patients. The phenotype was suspected to have originated in most of these patients after the administration of molecules belonging to groups previously related to SJS/TEN, such as anticonvulsants (carbamazepine, phenytoin, lamotrigine), antibiotics (sulfadoxine, cephalexin, trimethoprim/sulfamethoxazole), nonsteroidal anti-inflammatory drugs (NSAIDs) (paracetamol) and non-therapeutic agents/chemicals (contrast agent) (Dodiuk-Gad et al. 2015; Fakoya et al. 2018; Rodriguez-Martin et al. 2019).

The SJS/TEN phenotype in individual SJS-2 was probably due to having taken an *Urtica dioica* (stinging or common nettle) extract used here for treating rheumatoid arthritis. Although it has been shown that the leaves of this plant contain molecules typically related to allergic reactions (i.e. histamine, serotonin, acetylcholine and 5-hydroxytryptamine), to the best of our knowledge, this is the first description of a life-threatening response after its consumption (Esposito et al. 2019; Kregiel et al. 2018). Interestingly, we have identified two related individuals (mother, SJS-18, and her son, SJS-19) affected by TEN and SJS. Pt-SJS18 took sulfadoxine/pyrimethamine as toxoplasmosis treatment during breastfeeding, which led to TEN. Shortly afterwards her son displayed the onset of similar signs, probably due to the drug being transmitted through breast milk. This familial case underlines the fact that phenotype in these individuals may have had a common molecular (genetic) origin. However, they did not display *GNLY* sequence variants, suggesting that variants in other genes may contribute towards the disease's onset.

GNLY direct sequencing revealed that patient (SJS-3) was a carrier of the c.11G > A (p.Trp4Ter) non-synonymous variant, and another 12 patients carried c.356 C > T (p.Thr119Ile) (Table 2). We consider that the c.356 C > T variant was not related to the phenotype, as public SNP databases gave 0.3798 MAF, therefore, arguing in favour of a frequently occurring non-functional polymorphism.

On the contrary, the c.11G > A variant (predicted to create a premature stop codon at residue W^4) had low MAF (0.008464). Since the Colombian population consists of a particular ethnical and genetic admixture, we screened this variant in individuals from two groups of control individuals (GP1 and GP2). It is worth noting that although GP2 individuals were not taking any particular medication, they

did come from the same area as patient SJS-3, which helped us to explore whether c.11G > A might have been occurring frequently among a population having a specific genetic background. The lack of this variant in both control groups and its theoretically drastic nature in structural terms led us to implement functional in vitro assays.

Cells have a surveillance system (the nonsense-mediated mRNA decay system—NMD) which degrades mRNAs carrying PTCs. The NMD system starts functioning when the translation machinery encounters a PTC. This mechanism is efficient when the stop signal is located more than 50–55 nucleotides upstream of the last exon–exon junction (Maquat 2004; Nagy and Maquat 1998). However, exceptions to such rule have been reported since transcripts may be degraded even when a PTC is located less than 55 nucleotides from an exon–exon union, or be unaffected when a PTC is located upstream (Brogna and Wen 2009; Lindeboom et al. 2016; Maquat 2004). It has been shown that NMD efficiency decreases when a PTC is located in close proximity to the start codon (< 200 nucleotides); remarkably, PTCs located in an ORF's first 100 nucleotides are rarely targeted by the NMD system (Lindeboom et al. 2016). Different mechanisms have been evoked in such cases, leading to NMD insensitivity, such as RNA stabilisation and translation re-initiation at a downstream AUG codon (Lindeboom et al. 2016; Silva et al. 2008; Zhang and Maquat 1997).

The PTC was located near (fourth codon) the first AUG codon in the present case, 42 base pairs upstream of the first exon–exon junction, suggesting a downstream re-initiation codon potentially leading to the synthesis of a shorter version of the GNLY protein lacking the N-terminal region. The GNLY sequence carries three in-phase AUG codons downstream from the beginning of the ORF, located at positions 34 (codon 12; CD-12), 235 (codon 79; CD-79) and 331 (codon 111; CD-111) (Supplementary Fig. 2). Our WB experiments did not reveal weight differences of GNLY from cells transfected with WT or M1 constructs, which lead us to discard that the CD-79 and CD-111 3´ in-phase AUG codons could have led to translation re-initiation (Fig. 1). Since the first in-phase AUG codon (CD-12) was located very near (34 nucleotides) to the start of the ORF, potential differences in protein weight compared to the WT version may be difficult to detect by WB (Supplementary Fig. 1). We thus created the M2 construct carrying the c.11G > A (p.Trp4Ter) mutation and replaced the AUG (Met^{12}) codon in position 34 with a GUG codon (Val^{12}). Intriguingly, we did not observe differences in GNLY molecular weight regarding cells transfected with M1 or M2 plasmids, which led us to discard a translation restart at the first downstream AUG codon (Fig. 1). These findings suggested that PTC-readthrough could have been involved in the translation of the GNLY-p.Trp4Ter mutant form. This mechanism enables the ribosome to pass through the stop codon and continue translation in the same phase (Dabrowski et al. 2015).

It should be noted that some rules regarding translation termination efficiency have linked the stop codon nucleotide environment to readthrough occurrence. An example would be particular motifs located immediately at 5´ (adenine at 2 positions upstream of the UAG codon) and 3´ (e.g. CAA, CUAG, CARYYA, CARNBA, CAAUUA) from the stop codon (Cridge et al. 2018; Dabrowski et al. 2015; Loughran et al. 2018; Pacho et al. 2011). Although the c.11G > A (p.Trp4Ter) mutation was not located in such a genomic context, PTC-readthrough was plausible because several exceptions to these rules have been described (Namy et al. 2003; Williams et al. 2004). Molecular stop codon readthrough is based on termination codon recognition via eukaryotic release factor 1 (eRF1) and near-cognate tRNA (nc-tRNA) location in the ribosome. This mechanism leads to inaccurate stop codon decoding; nc-tRNA usage seems to be non-stochastic, but the challenge lies in accurately predicting the nature of the residue which will become incorporated during a termination codon readthrough. Glutamine, tryptophan, tyrosine and lysine incorporation at the stop codon has been described for UAG codons (as it is the case in the present study) (Blanchet et al. 2015; Fearon et al. 1994; Feng et al. 1990; Nilsson and Ryden-Aulin 2003).

Theoretically, introducing one of these residues at the protein's N-terminal region may change its local (and global) physicochemical properties, leading to functional disturbances. Interestingly, our in vitro functional experiments highlighted the fact that the GNLY-p.Trp4Ter mutation led to the protein's abnormal subcellular mislocalisation, which became translocated to the nucleus (Fig. 2). This phenomenon has been described previously for an alternative form of GNLY (a 9 kDa version lacking the N-terminal region) which had apoptotic properties (Takamori et al. 2005). Indeed, GNLY is synthesised as a 15-kDa precursor and processed to an active 9 kDa cytotoxic form (Chung et al. 2008; Hanson et al. 1999). Our results have thereby argued strongly in favour of changes in the GNLY N-terminal region do lead to protein conformational/functional disturbances, contributing to SJS/TEN's molecular origin. Indeed, the *GNLY* c.11G > A mutation could be a susceptibility-related genetic factor triggering severe reactions to drugs or defence against viruses, such as SJS/TEN. Although many genetic, epigenetic and environmental factors might interact to originate these complex phenotypes, we consider that other yet-to-be-discovered *GNLY* sequence variants might contribute to the disease's aetiology.

We consider that future sequencing studies of *GNLY*-encoding regions in SJS/TEN patients should provide new insights into these diseases' aetiology and enable new, potential predictive biomarkers to be described.

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Author contributions LC, CS, HM, AR, and AD performed the clinical study. DF, DS, OL, YS, and DB performed the in vitro assays. PL directed the study and wrote the paper. All authors revised and approved the final version of the manuscript.

Data availability statement The data and the *GNLY* c.11G > A variant that support the findings of this study were submitted to a locus-specific database (https://databases.lovd.nl/shared/genes/GNLY).

Compliance with ethical standards

Conflict of interest The authors declare that conflict of interest does not exist.

References

Abe R, Yoshioka N, Murata J, Fujita Y, Shimizu H (2009) Granulysin as a marker for early diagnosis of the Stevens-Johnson syndrome. Ann Intern Med 151:514–515

Blanchet S, Rowe M, Von der Haar T, Fabret C, Demais S, Howard MJ, Namy O (2015) New insights into stop codon recognition by eRF1. Nucleic Acids Res 43:3298–3308. https://doi.org/10.1093/nar/gkv154

Brogna S, Wen J (2009) Nonsense-mediated mRNA decay (NMD) mechanisms. Nat Struct Mol Biol 16:107–113. https://doi.org/10.1038/nsmb.1550

Caruso A, Bellia C, Pivetti A, Agnello L, Bazza F, Scazzone C, Bivona G, Lo Sasso B, Ciaccio M (2014) Effects of EPHX1 and CYP3A4 polymorphisms on carbamazepine metabolism in epileptic patients. Pharmgenomics Pers Med 7:117–120. https://doi.org/10.2147/pgpm.S55548

Chung WH, Hung SI, Hong HS, Hsih MS, Yang LC, Ho HC, Wu JY, Chen YT (2004) Medical genetics: a marker for Stevens–Johnson syndrome. Nature 428:486. https://doi.org/10.1038/428486a

Chung WH, Hung SI, Yang JY, Su SC, Huang SP, Wei CY, Chin SW, Chiou CC, Chu SC, Ho HC, Yang CH, Lu CF, Wu JY, Liao YD, Chen YT (2008) Granulysin is a key mediator for disseminated keratinocyte death in Stevens–Johnson syndrome and toxic epidermal necrolysis. Nat Med 14:1343–1350. https://doi.org/10.1038/nm.1884

Ciccacci C, Di Fusco D, Marazzi MC, Zimba I, Erba F, Novelli G, Palombi L, Borgiani P, Liotta G (2013) Association between CYP2B6 polymorphisms and Nevirapine-induced SJS/TEN: a pharmacogenetics study. Eur J Clin Pharmacol 69:1909–1916. https://doi.org/10.1007/s00228-013-1549-x

Cridge AG, Crowe-McAuliffe C, Mathew SF, Tate WP (2018) Eukaryotic translational termination efficiency is influenced by the 3′ nucleotides within the ribosomal mRNA channel. Nucleic Acids Res 46:1927–1944. https://doi.org/10.1093/nar/gkx1315

Dabrowski M, Bukowy-Bieryllo Z, Zietkiewicz E (2015) Translational readthrough potential of natural termination codons in eucaryotes—the impact of RNA sequence. RNA Biol 12:950–958. https://doi.org/10.1080/15476286.2015.1068497

Dodiuk-Gad RP, Chung WH, Valeyrie-Allanore L, Shear NH (2015) Stevens–Johnson syndrome and toxic epidermal necrolysis: an update. Am J Clin Dermatol 16:475–493. https://doi.org/10.1007/s40257-015-0158-0

Duong TA, Valeyrie-Allanore L, Wolkenstein P, Chosidow O (2017) Severe cutaneous adverse reactions to drugs. Lancet 390:1996–2011. https://doi.org/10.1016/s0140-6736(16)30378-6

Esposito S, Bianco A, Russo R, Di Maro A, Isernia C, Pedone PV (2019) Therapeutic perspectives of molecules from *Urtica dioica* extracts for cancer treatment. Molecules. https://doi.org/10.3390/molecules24152753

Fakoya AOJ, Omenyi P, Anthony P, Anthony F, Etti P, Otohinoyi DA, Olunu E (2018) Stevens–Johnson syndrome and toxic epidermal necrolysis; extensive review of reports of drug-induced etiologies, and possible therapeutic modalities. Open Access Maced J Med Sci 6:730–738. https://doi.org/10.3889/oamjms.2018.148

Fan WL, Shiao MS, Hui RC, Su SC, Wang CW, Chang YC, Chung WH (2017) HLA association with drug-induced adverse reactions. J Immunol Res 2017:3186328. https://doi.org/10.1155/2017/3186328

Fearon K, McClendon V, Bonetti B, Bedwell DM (1994) Premature translation termination mutations are efficiently suppressed in a highly conserved region of yeast Ste6p, a member of the ATP-binding cassette (ABC) transporter family. J Biol Chem 269:17802–17808

Feng YX, Copeland TD, Oroszlan S, Rein A, Levin JG (1990) Identification of amino acids inserted during suppression of UAA and UGA termination codons at the gag-pol junction of Moloney murine leukemia virus. Proc Natl Acad Sci USA 87:8860–8863. https://doi.org/10.1073/pnas.87.22.8860

Finn MW, Clayberger C, Krensky AM (2011) Expression and purification of 15 kDa granulysin utilizing an insect cell secretion system. Protein Expr Purif 75:70–74. https://doi.org/10.1016/j.pep.2010.07.009

Hanson DA, Kaspar AA, Poulain FR, Krensky AM (1999) Biosynthesis of granulysin, a novel cytolytic molecule. Mol Immunol 36:413–422

Harr T, French LE (2010) Toxic epidermal necrolysis and Stevens–Johnson syndrome. Orphanet J Rare Dis 5:39. https://doi.org/10.1186/1750-1172-5-39

Harris V, Jackson C, Cooper A (2016) Review of toxic epidermal necrolysis. Int J Mol Sci. https://doi.org/10.3390/ijms17122135

He XJ, Jian LY, He XL, Tang M, Wu Y, Xu YY, Sun XJ, Zhao LM (2014) Association of ABCB1, CYP3A4, EPHX1, FAS, SCN1A, MICA, and BAG6 polymorphisms with the risk of carbamazepine-induced Stevens–Johnson syndrome/toxic epidermal necrolysis in Chinese Han patients with epilepsy. Epilepsia 55:1301–1306. https://doi.org/10.1111/epi.12655

Kregiel D, Pawlikowska E, Antolak H (2018) Urtica spp.: ordinary plants with extraordinary properties. Molecules. https://doi.org/10.3390/molecules23071664

Letko E, Papaliodis DN, Papaliodis GN, Daoud YJ, Ahmed AR, Foster CS (2005) Stevens–Johnson syndrome and toxic epidermal necrolysis: a review of the literature. Ann Allergy Asthma Immunol 94:419–436. https://doi.org/10.1016/s1081-1206(10)61112-x **(quiz 436-8, 456)**

Lindeboom RG, Supek F, Lehner B (2016) The rules and impact of nonsense-mediated mRNA decay in human cancers. Nat Genet 48:1112–1118. https://doi.org/10.1038/ng.3664

Loughran G, Jungreis I, Tzani I, Power M, Dmitriev RI, Ivanov IP, Kellis M, Atkins JF (2018) Stop codon readthrough generates a C-terminally extended variant of the human vitamin D receptor with reduced calcitriol response. J Biol Chem 293:4434–4444. https://doi.org/10.1074/jbc.M117.818526

Man CB, Kwan P, Baum L, Yu E, Lau KM, Cheng AS, Ng MH (2007) Association between HLA-B*1502 allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. Epilepsia 48:1015–1018. https://doi.org/10.1111/j.1528-1167.2007.01022.x

Maquat LE (2004) Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. Nat Rev Mol Cell Biol 5:89–99. https://doi.org/10.1038/nrm1310

Nagy E, Maquat LE (1998) A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. Trends Biochem Sci 23:198–199

Namy O, Duchateau-Nguyen G, Hatin I, Hermann-Le Denmat S, Termier M, Rousset JP (2003) Identification of stop codon readthrough genes in *Saccharomyces cerevisiae*. Nucleic Acids Res 31:2289–2296. https://doi.org/10.1093/nar/gkg330

Nilsson M, Ryden-Aulin M (2003) Glutamine is incorporated at the nonsense codons UAG and UAA in a suppressor-free *Escherichia coli* strain. Biochim Biophys Acta 1627:1–6

Pacho F, Zambruno G, Calabresi V, Kiritsi D, Schneider H (2011) Efficiency of translation termination in humans is highly dependent upon nucleotides in the neighbourhood of a (premature) termination codon. J Med Genet 48:640–644. https://doi.org/10.1136/jmg.2011.089615

Pavlos R, Mallal S, Phillips E (2012) HLA and pharmacogenetics of drug hypersensitivity. Pharmacogenomics 13:1285–1306. https://doi.org/10.2217/pgs.12.108

Rodriguez-Martin S, Martin-Merino E, Lerma V, Rodriguez-Miguel A, Gonzalez O, Gonzalez-Herrada C, Ramirez E, Bellon T, de Abajo FJ (2019) Incidence of Stevens–Johnson syndrome/toxic epidermal necrolysis among new users of different individual drugs in a European population: a case-population study. Eur J Clin Pharmacol 75:237–246. https://doi.org/10.1007/s00228-018-2569-3

Roujeau JC (1997) Stevens–Johnson syndrome and toxic epidermal necrolysis are severity variants of the same disease which differs from erythema multiforme. J Dermatol 24:726–729

Schwartz RA, McDonough PH, Lee BW (2013) Toxic epidermal necrolysis: Part I Introduction, history, classification, clinical features, systemic manifestations, etiology, and immunopathogenesis. J Am Acad Dermatol 69:173.e1–13. https://doi.org/10.1016/j.jaad.2013.05.003 **(quiz 185–6)**

Silva AL, Ribeiro P, Inacio A, Liebhaber SA, Romao L (2008) Proximity of the poly(A)-binding protein to a premature termination codon inhibits mammalian nonsense-mediated mRNA decay. RNA 14:563–576. https://doi.org/10.1261/rna.815108

Takamori Y, Ogawa K, Nagata K, Takano S, Nakamura M (2005) Granulysin induces cell death with nuclear accumulation. J Med Dent Sci 52:1–7

Tassaneeyakul W, Tiamkao S, Jantararoungtong T, Chen P, Lin SY, Chen WH, Konyoung P, Khunarkornsiri U, Auvichayapat N, Pavakul K, Kulkantrakorn K, Choonhakarn C, Phonhiamhan S, Piyatrakul N, Aungaree T, Pongpakdee S, Yodnopaglaw P (2010) Association between HLA-B*1502 and carbamazepine-induced severe cutaneous adverse drug reactions in a Thai population. Epilepsia 51:926–930. https://doi.org/10.1111/j.1528-1167.2010.02533.x

Ueta M, Sawai H, Sotozono C, Hitomi Y, Kaniwa N, Kim MK, Seo KY, Yoon KC, Joo CK, Kannabiran C, Wakamatsu TH, Sangwan V, Rathi V, Basu S, Ozeki T, Mushiroda T, Sugiyama E, Maekawa K, Nakamura R, Aihara M, Matsunaga K, Sekine A, Gomes JA, Hamuro J, Saito Y, Kubo M, Kinoshita S, Tokunaga K (2015) IKZF1, a new susceptibility gene for cold medicine-related Stevens-Johnson syndrome/toxic epidermal necrolysis with severe mucosal involvement. J Allergy Clin Immunol 135:1538–45.e17. https://doi.org/10.1016/j.jaci.2014.12.1916

Williams I, Richardson J, Starkey A, Stansfield I (2004) Genome-wide prediction of stop codon readthrough during translation in the yeast *Saccharomyces cerevisiae*. Nucleic Acids Res 32:6605–6616. https://doi.org/10.1093/nar/gkh1004

Yang Y, Li F, Du J, Shen Y, Lin J, Zhu X, Luo X, Liang J, Xu J (2017) Variable levels of apoptotic signal-associated cytokines in the disease course of patients with Stevens–Johnson syndrome and toxic epidermal necrolysis. Australas J Dermatol 58:e61–e67. https://doi.org/10.1111/ajd.12462

Yip VL, Alfirevic A, Pirmohamed M (2015) Genetics of immune-mediated adverse drug reactions: a comprehensive and clinical review. Clin Rev Allergy Immunol 48:165–175. https://doi.org/10.1007/s12016-014-8418-y

Zhang J, Maquat LE (1997) Evidence that translation reinitiation abrogates nonsense-mediated mRNA decay in mammalian cells. EMBO J 16:826–833. https://doi.org/10.1093/emboj/16.4.826