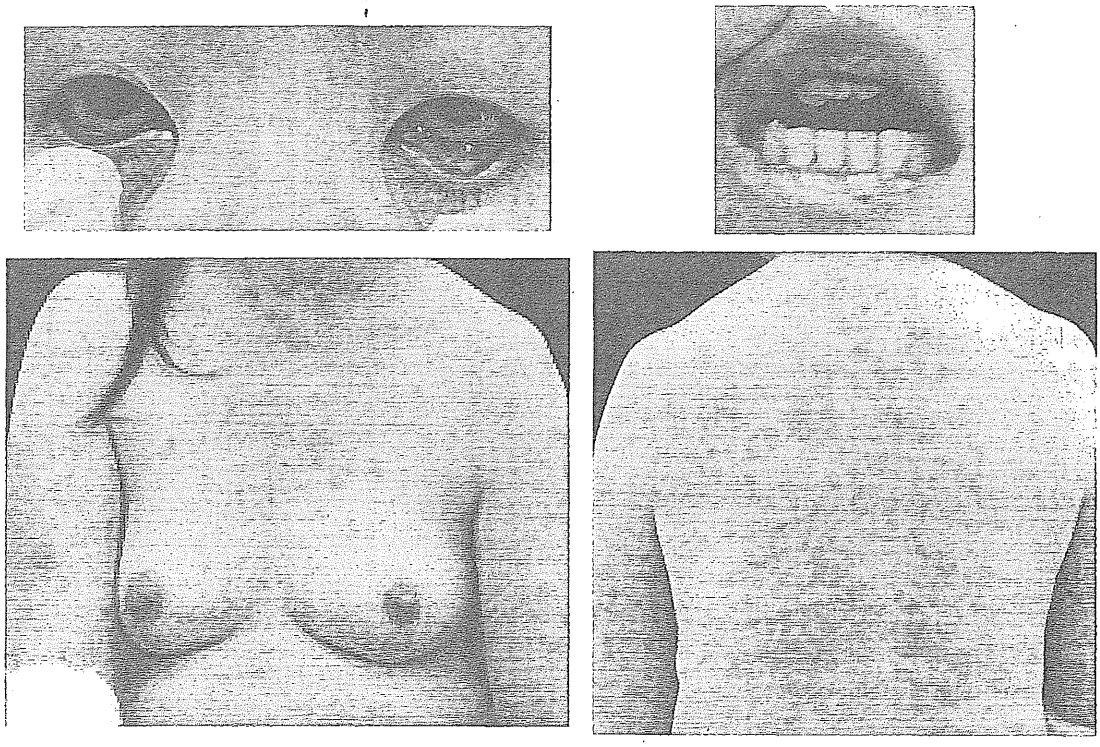


Fig. 5：Case 3；Clinical examination revealed conjunctival hyperemia, erosions on the lips, and erythema of the trunk.

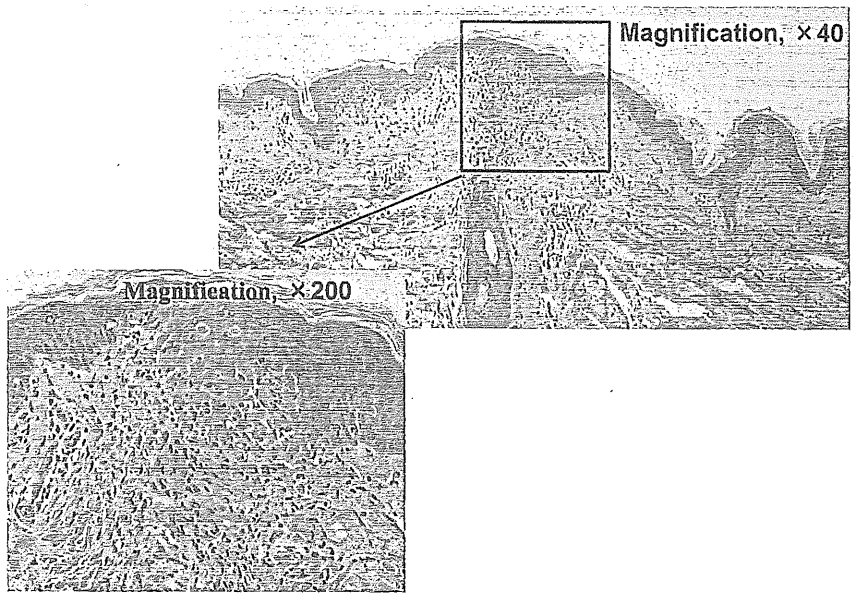


Fig. 6：Histopathology showed apoptosis in the epidermis, liquefaction in the basal cell layer, and lymphocyte infiltration in the upper dermis.

Table 1 に自験4例の特徴のまとめを示す。

考　察

ラモトリギンは Na^+ チャネルを頻度依存的かつ電位依存的に抑制することで神経膜を安定化させ，グルタミン酸等の興奮性神経伝達物質の遊離を抑制させることで抗痙攣作用を示す。ドパミンの上昇，セロトニン代謝を促進し，神経伝達物質の作用を介して脳内の抑制系の賦活作用に基づく既存の抗てんかん薬とは異なるまったく新しい作用機序を有し，他の抗てんかん薬で効果の認められないてんかん発作に対する併用療法に用いられる。VPA との併用

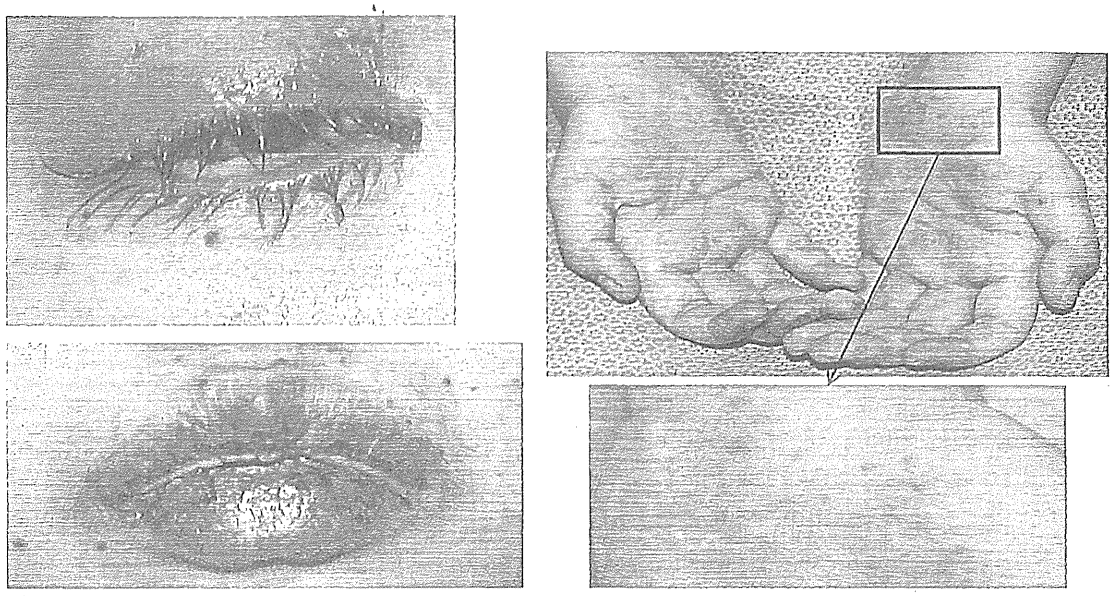


Fig. 7：Case 4；Clinical test showed conjunctival hyperemia in both eyelids, and bleeding erosive lesions on the upper and lower lips and in the oral cavity. Ophthalmologic examination showed conjunctivitis and keratitis with a pseudomembrane on the eyes.

では肝におけるグルクロン酸抱合が競合するため，半減期が約2倍延長するとの報告がある。そのため，単剤療法の場合，最初の2週間は1日25 mgを1回経口服用するのに対し，VPA併用では，その半量からの投与が推奨されている（ラクミタール錠100 mg，デパケン®錠200 mg添付情報，薬効薬理）。

当院で経験した症例では，4例中全例でVPAが併用され，3例で推奨初期量をこえていた（Table 1）。全例が30代の女性で，発症はラモトリギン投与から9～28日（平均19日目）であった。原因薬剤検査では，DLSTは2例で，パッチテストは1例で陽性であった。

SJSで認められた眼症状はいずれの症例も後遺症なく治癒した。ラモトリギンによる重症薬疹は自験4例ともステロイドによく反応し，すみやかなステロイドの全身投与が有効と思われた。

わが国では，会議録などを含めると58例の報告があり，DIHSが22例（37.9%）と最多で，SJS 7例（12.0%），TEN 6例（10.3%）であった[2~35]。そのうち原著として報告のあるものはわれわれが経験した4例を含めて12例あり，DIHS 3例（25%），SJS 6例（50%），TEN 1例（8.3%）であった[2~9]。海外報告では，2012年までに33例の報告があり，TEN 18例（54.5%），SJS 10例（30.3%），DIHS 5例（15.5%）（drug rash with eosinophilia and systemic symptoms（以下DRESS）2例を含む）であり，わが国とくらべTENの発症が多く認められた[4,33~57]（Table 2）。

初期投与量は本邦報告例において明記されているもののうち14例中7例で推奨量より多く投与されており，投与量が本剤の感作に影響することが推察された。

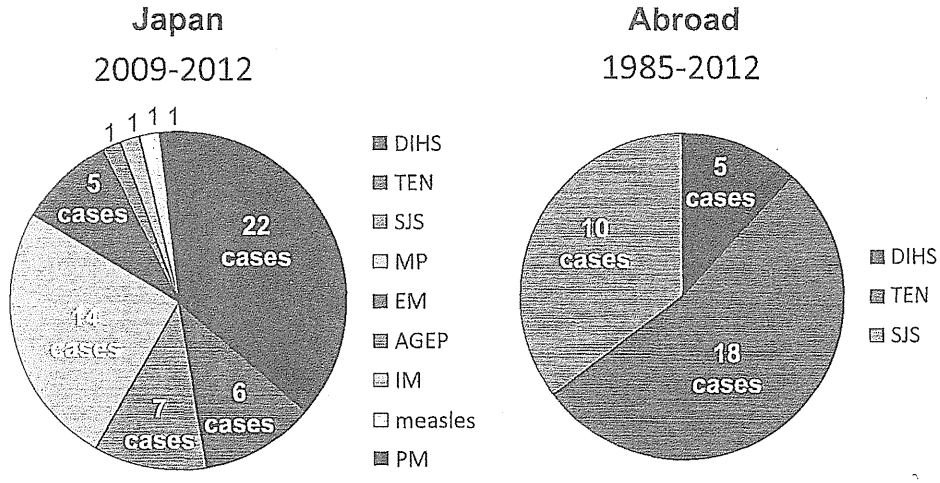
パッチテストに関して，本邦報告例で記載のあるものは自験例含めて10例あり，陽性は3例（約30%）と低かった。抗痙攣薬では一般的にパッチテスト陽性率が高いことが知られているが，85.6%のカルバマゼピンから23.1%のゾニサミドまで個々の薬剤によってばらつきがある[58]。ラモトリギンにおいても，今後の集計が待たれる。

ラモトリギンによるDLSTの陽性率は海外の報告においては高いとするものと低いとするものがある。SachsらはDLSTがラモトリギンに伴う薬疹に有用であり，症状出現3週間以内の早期1ヵ月以降でもDLSTは陽性反応を示したとしている[35]。一方Tangらは，ラモトリギンに伴うと考えられる薬疹患者は急性期，回復後にかかわらずDLSTの陽性率は低かったことを報告している[36]。本邦報告では，紅斑丘疹型の薬疹で3例全例が発症早期にDLSTが陽性を示した[17]。重症薬疹ではDLSTを施行されたもののうち，DIHSで15例中14例，TENで4例中4例，SJSは5例中3例で陽性であり，検査された症例の陽性率は87.5%と高かった。これらから本剤による薬疹では重症薬疹を含めDLST

Table 1：Summary of case characteristics

Case No.	Case 1	Case 2	Case 3	Case 4
Age/sex	38 years/o.F	38 years/o.F	38 years/o.F	32 years/o.F
Rash type	SJS	DIHS	SJS	SJS
Length of LTG treatment	15 days	26 days	9 days	28 days
Sodium valproate therapy (initial dose of LTG)	yes (50 mg/day)	yes (25 mg/day)	yes (100 mg/day)	yes (25 mg/2 days)
Treatment	Steroid pulse therapy	Systemic steroids	Systemic steroids	Systemic steroids
Patch test of LTG	(−)	(+)	(−)	(−)
Stimulation Index of DLST to LTG (examination day after disease onset)	264 (day 46)	1,426 (day 136)	92.6 (day 10)	74.5 (day 59)
HLA	A0206, A2402, B5401, B4001, Cw0102Cw0304, DRB1*0405, 1501	A2402, A3303 B4403, B5401	A0206, B0702, B4006	A0201, A0207, B3802, B4601

Table 2：Distributions of rashes caused by LTG
Drug-induced hypersensitivity syndrome (DIHS) was most frequently reported in Japan, whereas toxic epidermal necrolysis (TEN) was most frequently reported abroad.



が診断に有用であることが示唆された。陽性となった時期については他施設の報告例で明らかなものはSJSの1例であり2週間以内であった[3]。自験例ではSJSは発症から46日目に，DIHSでは135日目に陽性となったが，いずれも発症1ヵ月以内は陰性でその後の再検査で陽性となった。抗てんかん薬によるDIHSにおいてDLSTは発症時に陰性でも数週間の経過中に陽性となることはよく知られている。DIHS同様SJSで陰性であっても，回復期の再検査が重要と考えられた。またステロイドのDLSTにおける影響は少ないとされているが[59]，自験例のようにステロイド中止後に陽性となる場合もあり，再検査は必要と考えられた。

また，抗てんかん薬による重症薬疹と特定のHLAとの相関について多くの研究がすすめられており，自験4例においてもHLAをHLA研究所に依頼し測定した（Table 1）。わが国ではHLA-B*54：01（日本人でのアリル頻度7.59%），HLA-Cw*01：02（同17.93%）およびHLA-DRB1*12：01（同3.74%）とラモトリギン誘因性重症薬疹との相関が提唱され，なかでもHLA-Cw*01：02は特に感度が高い可能性が報告されている[60]。自験例においてもSJS，DIHSの2例でHLA-B*54：01が検出され，SJSの1例でHLA-Cw*01：02が検出され

たことから，それらとの相関が推察された。

ラモトリギンは，今後てんかんのみならず，双極性障害で使用頻度の増加が予想される。重症薬疹発症例に初期投与量が推奨量より多い症例が多かったことから，重症薬疹の発生予防には適切な初期投与量の厳守が必要と考えた。被疑薬の確定のためのラモトリギンによるDLSTの有用性，重症薬疹発症とHLAとの関連性については今後の症例のさらなる蓄積が待たれる。

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Four Cases of Severe Drug Eruption Due to Lamotrigine

Kyoko HISATA[1], Setsuko MATSUKURA[1], Marie ONO[1], Yuji ISODA[1], Yuko WATANABE[1], Akiko MORITA[1], Michiko AIHARA[2], Takeshi KAMBARA[1]

[1] *Department of Dermatology, Yokohama City University Medical Center*
4-57, Urafune-cho, Minami-ku, Yokohama-shi, Kanagawa-ken 232-0024, Japan
[2] *Department of Dermatology, Yokohama City University Hospital*

Lamotrigine (LTG) is an antiepileptic drug with a novel mechanism of action, which was approved for use in Japan in 2008. However, a black box warning regarding life-threatening skin reactions accompanies the prescribing information for LTG. Here, we report 3 cases of Stevens-Johnson syndrome (SJS) and a case of drug-induced hypersensitivity syndrome (DIHS) due to LTG. All patients were treated with systemic corticosteroids and recovered without optic sequelae. A review of the literature revealed 58 cases of LTG-induced rash reported between 2009 and 2012 in Japan, of which 22 were of DIHS and 13 of SJS and toxic epidermal necrolysis (TEN). TEN was most frequently reported abroad from 1985 to 2012 (18 cases), followed by SJS (10 cases). Caution should be exercised when prescribing LTG because of its ability to cause serious drug-induced skin reactions.

(J Environ Dermatol Cutan Allergol, 8 (2)：114-123, 2014)

Key words：lamotrigine, stevens-johnson syndrome, drug-induced hypersensitivity syndrome, drug-induced lymphocyte stimulation test

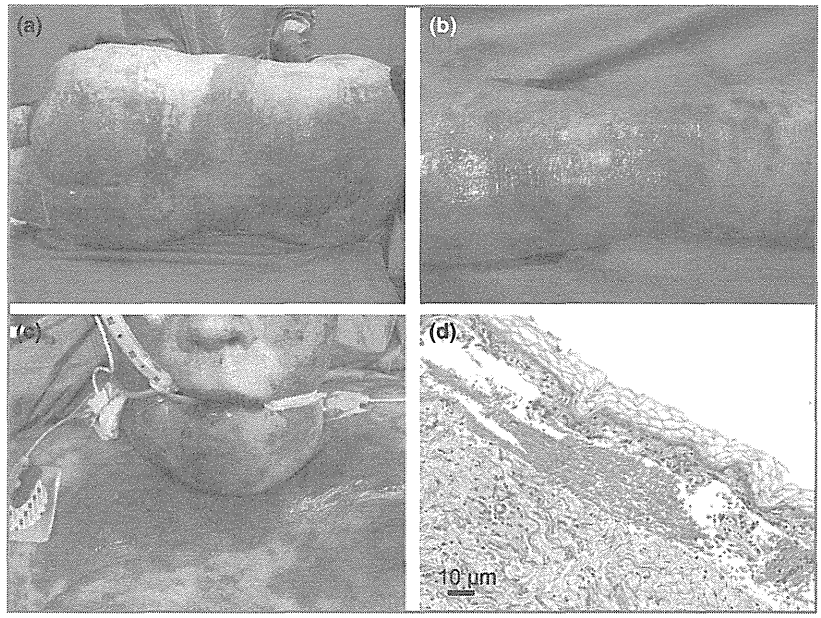

Fig 2. Photographs of skin manifestations in patient 2 following wound debridement, showing (a) the patient's back and (b) a lower leg with multiple macules, papules, blisters and erythematous and epidermolytic areas, typical of toxic epidermal necrolysis (TEN). (c) Involvement of the inner lip mucosa in this patient. (d) Haematoxylin and eosin-stained sections of a skin biopsy specimen of patient 2, with typical epidermolysis and leucocyte infiltrate of TEN.

taking herbal preparations in capsules, an imaginable common denominator of TEN development.

A single or multiplier effect by idiosyncratic, dose-related or drug-interactive reactions of phytochemicals or contaminants might be involved in the development of TEN in these patients. The objective evaluation by the Naranjo adverse drug reaction (ADR) probability scale[9] calculated a possible ADR by the herbal remedy in cases 1 and 3 and a probable cause in case 2. In all cases, the TEN-specific algorithm for epidermal necrolysis (ALDEN) confirmed a possible cause of herbal remedies in TEN developement.[10]

[1]*Department of Plastic, Hand and Reconstructive Surgery and* [3]*Interdisciplinary Emergency and Disaster Medicine, Hannover Medical School, Hannover, Lower Saxony 30625, Germany*
[2]*Department of Plastic Surgery, Frenchay Hospital, Bristol, U.K.*
E-mail: *limbourg.anne@mh-hannover.de*

A. Limbourg[1]
A. Steiert[1]
A. Jokuszies[1]
K. Young[2]
H.-A. Adams[3]
P.M. Vogt[1]

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The serum level of HMGB1 (high mobility group box 1 protein) is preferentially high in drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms

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Dear Editor, Drug-induced hypersensitivity syndrome (DIHS), also known as drug reaction with eosinophilia and systemic symptoms (DRESS), is characterized by high fever, multiple

organ involvement and haematological disorders, essentially without severe erythema or epidermal apoptosis.[1] Sequential reactivation of human herpes virus (HHV)-6 is deeply involved in the pathophysiology and persistence of DIHS/DRESS. A preceding increase in proinflammatory cytokines such as interleukin (IL)-6 and tumour necrosis factor (TNF)-α seems to be relevant to the viral reactivation in DIHS/DRESS, while the exact mechanism is still unclear.[2]

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), other severe cutaneous adverse drug reactions (cADRs), are characterized by high fever, severe erythema and widespread epidermal damage due to keratinocyte apoptosis. Activated cytotoxic T cells and natural killer cells are involved in SJS/TEN.[3] The molecular cytotoxicity of Fas and cytotoxic proteins, including perforin/granzyme B and granulysin, are thought to contribute to induction of keratinocyte apoptosis.[3] High mobility group box 1 protein (HMGB1) is a nonhistone nuclear protein that is released from severely damaged cells. HMGB1 plays a role in transcriptional regulation in the nucleus, while outside of the cell it serves as an activator of the inflammatory cascade.[4] It was recently reported that HMGB1 levels are increased during the acute stage of SJS/TEN and can serve as an early diagnostic marker for SJS/TEN.[5] However, the level of HMGB1 at the onset of other severe cADRs such as DIHS/DRESS has not been investigated. In addition, although there are limited reports on serum cytokine levels in cADRs,[6] these cytokines have not been analysed with regards to HMGB1, which may induce aberrant cytokine production. To clarify the relationship between aberrant HMGB1 and cytokine production at disease onset, and the clinical manifestations elicited, we investigated serum HMGB1 and cytokine profiles in various cADRs.

Peripheral blood was taken from healthy controls and patients with various types of cADR including maculopapular (MP) type, erythema multiforme (EM), SJS, TEN and DIHS/DRESS at the time of onset and recovery. Onset is an acute exacerbation phase (< 7 days) and recovery is a remission phase of cADRs. Serum was stored at −80 °C and cytokine levels were measured by luminometric bead array using the Bio-Plex Suspension Array System (BioRad, Hemel Hempstead, U.K.). HMGB1 was measured by enzyme-linked immunosorbent assay. The groups consisted of the following subjects (full details in Table 1): healthy controls, 14 cases; MP/EM, 11 cases; SJS/TEN, 17 cases and DIHS/DRESS, 17 cases. For comparison of cytokine levels between healthy controls and each cADR group at onset, and between onset and recovery in each cADR group, the Mann–Whitney test and Wilcoxon matched-pairs tests were used, respectively. Statistical significance was established at $P < 0{\cdot}05$ and $P < 0{\cdot}01$.

Table 1 Profile of each group

Group	Number	Age (years), mean ± SD	Sex, n (male/female)	Type
Healthy controls	14	53·1 ± 15·3	8/6	–
MP/EM	11	65·3 ± 8·9	6/5	MP 6/EM 5
SJS/TEN	17	56·5 ± 19·1	7/10	SJS 13/TEN 4
DIHS/DRESS	17	53·5 ± 14·0	10/7	Typical 13/atypical 4[a]

MP, maculopapular; EM, erythema multiforme; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis; DIHS, drug-induced hypersensitivity syndrome; DRESS, drug reaction with eosinophilia and systemic symptoms. [a]Typical, with reactivation of human herpesvirus (HHV)-6; atypical, without reactivation of HHV-6.

HMGB1 was high in both SJS/TEN and DIHS/DRESS compared with healthy controls and other cADRs, but the level was significantly higher in DIHS/DRESS than in SJS/TEN. Comparison of cytokine levels between SJS/TEN and DIHS/DRESS revealed a prominent increase in T helper (Th)2 cytokines/chemokines such as IL-5, IL-9 and IL-13 in DIHS/DRESS. Additionally, IL-10 (an anti-inflammatory cytokine) and IL-12 were elevated in DIHS/DRESS (Fig. 1a). Concerning the serum cytokine levels at the time of onset in each group, the following were significantly increased compared with healthy controls: IL-5, IL-6, chemokine (C-X-C) motif ligand (CXCL)-8, IL-9, IL-12, eotaxin, granulocyte macrophage colony-stimulating factor (GM-CSF), CXCL-10 and vascular endothelial growth factor (VEGF) in MP/EM; IL-6, IL-12 and CXCL-10 in SJS/TEN; and IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, IL-15, eotaxin, GM-CSF, interferon (IFN)-γ, CXCL-10 and VEGF in DIHS/DRESS. Proinflammatory cytokines such as TNF-α and IFN-γ were not necessarily high in severe cADRs. Most, but not all, cytokines returned to normal levels with treatment at the time of recovery (Fig. 1).

Although the levels of various types of serum cytokines were elevated at cADR onset, the levels of proinflammatory cytokines did not correlate with the types of cADR or disease severity. These results suggest that the overproduction of these cytokines contributes to promoting inflammation, but that mechanisms other than an increase of proinflammatory cytokines are essential for inducing the massive keratinocyte apoptosis observed in SJS/TEN.

In DIHS/DRESS, Th2 cytokines, HMGB1 and IL-10, were increased. Recent studies have reported that not only Th2 cytokines, but also Th2 chemokines such as thymus and activation-regulated chemokine, were elevated in serum in DIHS/DRESS.[6,7] In addition, HMGB1 was more highly elevated than in SJS/TEN. HMGB1 has been shown to induce the differentiation of dendritic cells (DCs) to $CD11c^{low}CD45RB^{high}$ DCs followed by shifting of Th1 to Th2 *in vitro*.[8] Furthermore, high expression of HMGB1 in DIHS/DRESS skin has been reported.[9] The area of expression of HMGB1 was larger in DIHS/DRESS lesions than in SJS lesions regardless of keratinocyte damage. Translocation of HMGB1 occurred in DIHS epidermal cells, and this HMGB1 attracted monomyeloid precursors harbouring HHV-6, resulting in HHV-6 transmission to skin-infiltrating $CD4^+$ T cells, which is essential for HHV-6 replication in DIHS/DRESS. On the other hand, IL-10, which is an anti-inflammatory cytokine, was also highly elevated in DIHS/DRESS. It has been reported that expansion of $Foxp3^+CD25^+$ T regulatory cells (Tregs) was observed

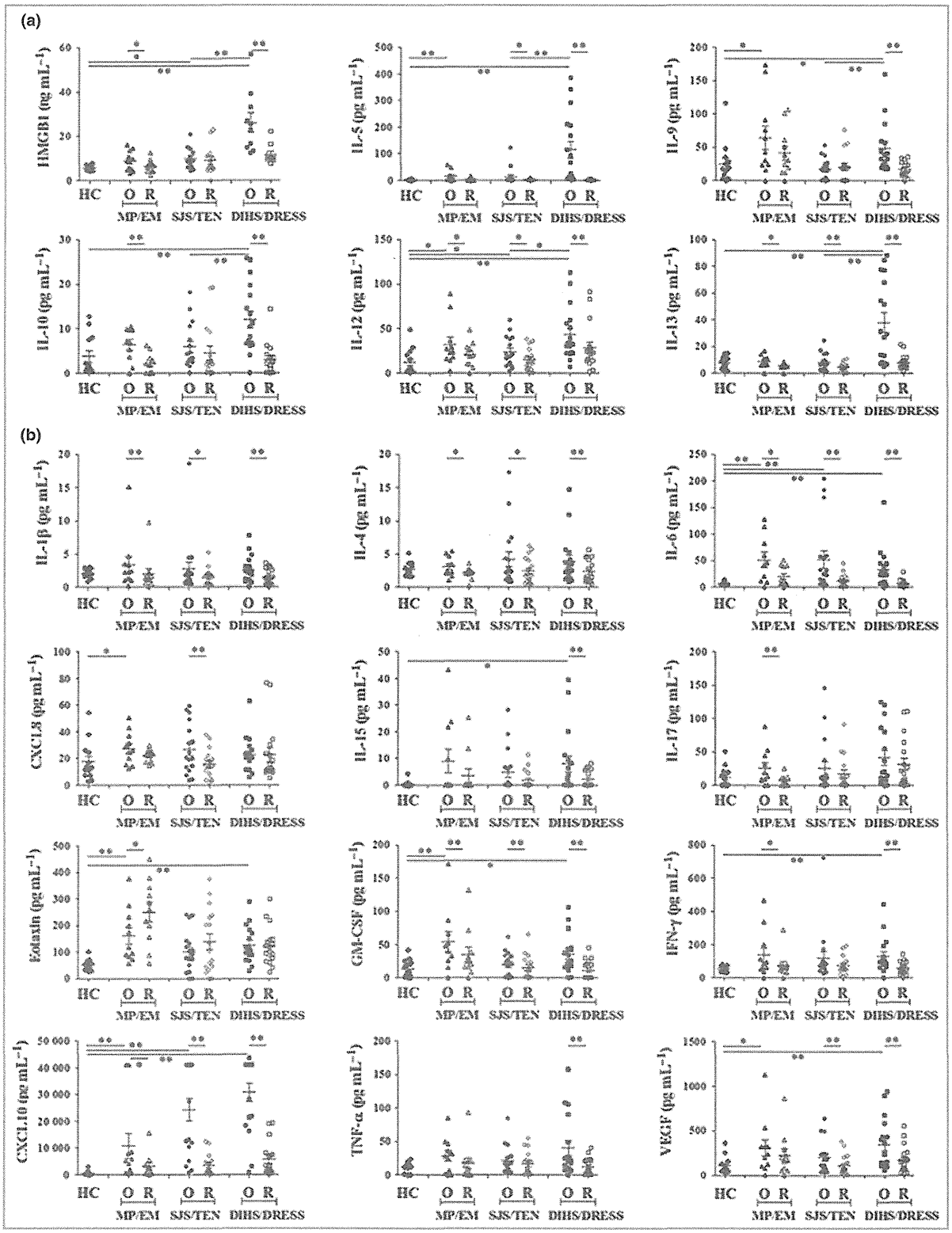

Fig 1. Serum high mobility group box 1 protein (HMGB1) and cytokine levels were analysed by enzyme-linked immunosorbent assay and luminometric bead array. To compare cytokine levels between healthy controls (HC) and each cutaneous adverse drug reaction (cADR) group at onset and between onset and recovery in each cADR group, the Mann–Whitney test and Wilcoxon matched-pairs tests were used, respectively. Significantly higher levels of (a) cytokines and (b) other proinflammatory cytokines in drug-induced hypersensitivity syndrome (DIHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) than in Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN). CXCL, chemokine (C-X-C) motif ligand; EM, erythema multiforme; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; MP, maculopapular; O, onset of disease; R, recovery from disease; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$.

during the acute stage of DIHS but not of TEN, whereas Tregs decrease dramatically in the late stage of DIHS.[10] Taken together, HMGB1 released during the acute phase of DIHS/DRESS might facilitate Th2 cell activation induced by the causative drug, resulting in exacerbation. In this context, Th2 cells and Tregs, both producing IL-10, along with other activated cells producing proinflammatory cytokines, characterize the pathophysiology of DIHS/DRESS in the early stage.

In conclusion, cytokine storm occurs in various types of cADRs, but factors other than cytokines are required for the onset of severe cADR. HMGB1 may contribute to the development of DIHS/DRESS through Th2 cell activation, which plays a key role together with Tregs in the disease. The involvement of HMGB1 in cADRs therefore requires further investigation.

[1]Department of Environmental Immuno-Dermatology, Yokohama City University Graduate School of Medicine, Yokohama, Japan
[2]Department of Dermatology, Yokohama City University Medical Center, Yokohama, Japan
[3]National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan
[4]Department of Dermatology, International University of Health and Welfare, Atami, Japan
E-mail: fuji_fuji_fuji_0101@yahoo.co.jp

H. Fujita[1]
S. Matsukura[2]
T. Watanabe[1]
N. Komitsu[1]
Y. Watanabe[1]
Y. Takahashi[3]
T. Kambara[2]
Z. Ikezawa[4]
M. Aihara[1]

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Conflicts of interest: none declared.

A case of pemphigus herpetiformis-like atypical pemphigus with IgG anti-desmocollin 3 antibodies

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Dear Editor, Pemphigus is an autoimmune blistering skin disease characterized by autoantibodies to keratinocyte cell surface antigens.[1] Major autoantigens for pemphigus are desmogleins (Dsgs), transmembrane cell–cell adhesion proteins belonging to the cadherin family. Dsg1 and Dsg3 are antigens for pemphigus foliaceus and pemphigus vulgaris, respectively. In addition to the four Dsg isoforms (Dsg1–4), there is another group of desmosomal cadherins, the desmocollins (Dsc), which is composed of three isoforms (Dsc1–3).

Pemphigus herpetiformis (PH) is a distinct variant of pemphigus; clinically it shows dermatitis herpetiformis-like features characterized by pruritic annular erythemas with vesicles on the periphery, histopathologically, eosinophilic spongiosis and immunologically, IgG antibodies to keratinocyte cell surfaces.[2] Ishii *et al.* reported that the targets of IgG autoantibodies in PH were Dsgs.[3] Anti-Dsg1 antibodies were detected in the majority of patients, while anti-Dsg3 antibodies were detected in some cases. In this study, we report a case of PH-like atypical pemphigus with IgG antibodies to Dsc3, but without antibodies to Dsgs.

A 57-year-old Japanese man visited us complaining of a 1-year history of erosive skin lesions. He was otherwise healthy with no particular medical history. Physical examination revealed pruritic, urticarial, annular erythemas on the trunk and extremities, with some showing small vesicles at the periphery (Fig. 1a). No mucosal involvement of the oral cavity was present. Blood tests and computed tomography showed no abnormalities.

皮膚病診療：36(5)；445～448, 2014

◇生物学的製剤・副作用＜臨床例＞——⑪

分子標的薬ソラフェニブによる多形紅斑型薬疹

鈴木　亜希*　陳　慧芝*　内田　敬久*　相原　道子*

Key words
ソラフェニブ，多形紅斑型薬疹，肝細胞癌

症例のポイント

- 近年，癌に対する分子標的薬は日常的に使用されるようになり，多彩な皮膚反応が報告されている．
- 分子標的薬による皮膚症状の中で手足症候群や痤瘡様皮疹などは薬理作用によるものと考えられ，中等度以下のものはできる限り薬剤を継続し治療を続けることが望まれる．
- ソラフェニブによる皮膚症状としては手足症候群が知られているが，多形紅斑型薬疹やStevens-Johnson症候群，中毒性表皮壊死症などの重症薬疹の報告もみられる．
- ソラフェニブによる多形紅斑の発症機序についてはアレルギーの関与が疑われる症例もあり，再投与に関しては慎重な対応が必要と思われる．

施行されるも寛解には至らなかった．

現病歴　多発性肝細胞癌に対し2010年8月にソラフェニブ(ネクサバール)800 mg内服を開始した．内服開始5日目に肝機能障害が出現し，7日目から38℃台の発熱が持続した．10日目には肝機能障害の悪化と高熱，嘔気や腹痛，下痢などの腹部症状を認めたため，翌日からソラフェニブを半量の400 mgに減量された．しかし，内服開始12日目には両前腕，胸部に紅斑が出現し，ソラフェニブは中止された．さらに，その翌日には皮疹が全身へ拡大したため，当科併診となった．

現症　体温37.5℃．顔面，頸部，体幹，四肢に大豆大から拇指頭大までの浮腫性紅斑が多発しており，一部で紅斑は癒合していた．また，浮腫性紅斑上に暗赤色の紅斑を伴うtarget lesionがみられた(図1，2)．粘膜疹は認めず，瘙痒などの自覚症状はなかった．

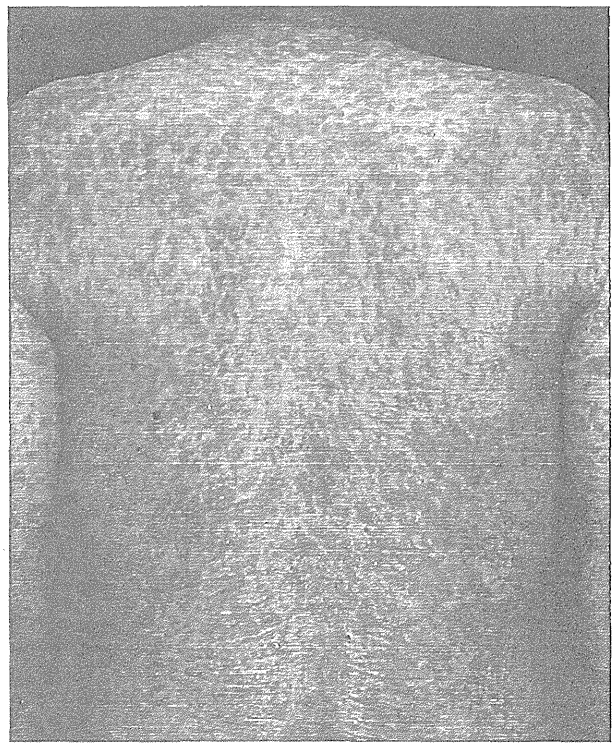
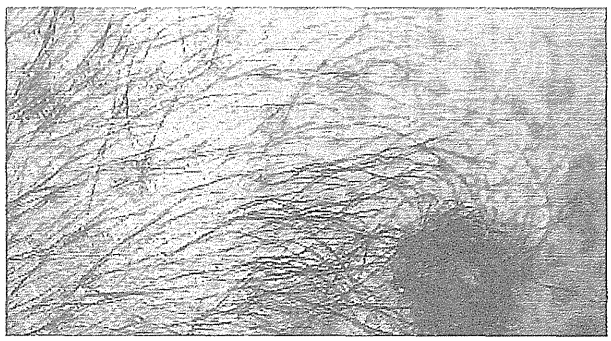


図1　臨床像．体幹、四肢に浮腫性紅斑が多発し、一部は癒合していた．



病理組織学的所見

腹部の浮腫性紅斑より皮膚生検を施行した．表皮には空胞変性と表皮内へのリンパ球浸潤を認め，角化細胞の好酸性壊死が散見された．真皮上層には血管周囲性にリンパ球を主体とする炎症細胞の浸潤があり，一部で好酸球も混在した(図3，4)．

診断確定

臨床経過や皮疹の特徴，病理組織学的所見より，ソラフェニブによる多形紅斑型薬疹と診断した．

治療と経過

ソラフェニブはすでに当科初診前日に中止されており，同薬中止後解熱傾向を認め，倦怠感などの自覚症状も改善していたことから経過観察とした．紅斑は入院翌日には消褪傾向を示し，内服中止12日目にはすべて消失した．アレルギー性機序も考えられたことから，後日ソラフェニブによるパッチテスト(PT)，薬剤添加リンパ球刺激試験(drug-induced lymphocyte stimulation test，以下，DLST)を施行したが，ともに陰性であった．再投与に関しては患者の同意が得られず施行し得なかった．

考　按

ソラフェニブは腫瘍細胞のマイトジェン活性化蛋白質(mitogen-activated　protein，以下，MAP)キナーゼ経路にあるRaf-1キナ

が1,931例(58.77%) にみられたのに対し，多形紅斑が105例(3.23%)，Stevens-Johnson症候群が6例(0.18%) と報告されている[3]．ソラフェニブによる多形紅斑型薬疹の本邦報告例は現在まで自験例含め16例あり，その特徴についてまとめた(表)．年齢は平均63.5歳と基礎疾患の好発年齢を反映しており，男女比は6：10と女性の割合が多かった．発症までの投与期間は3～14日間，平均9日間であり，すべての症例が内服2週間以内と，比較的早期の発症であった．

DLSTは施行した6例中4例で陽性であり，PTは2例のみで施行しており陰性であった．治療に関してはステロイド内服療法を行っている症例が多いが，ステロイド外用のみ，また自験例のように経過観察のみで改善した症例もみられた．このうち5例はいったん中止後減量にて再投与とその後の継続投与が可能であったと報告されている[4～14]．

ソラフェニブによる手足症候群に関しては用量依存性に症状の増悪，軽快を認めることから，薬剤の直接毒性によるものと考えられている[15]．手足症候群の機序については現在明らかにされていないが，基底細胞の増殖抑制や，抗癌剤の汗管からの排出による角化細胞の障害などが推測されており[16,17]，ソラフェニブにおいてはVEGFRとPDGFRが同時に阻害されることでおこるのではないかと考えられている[18]．

多形紅斑型薬疹においては中止後の再投与が可能な例があることから中毒性反応が

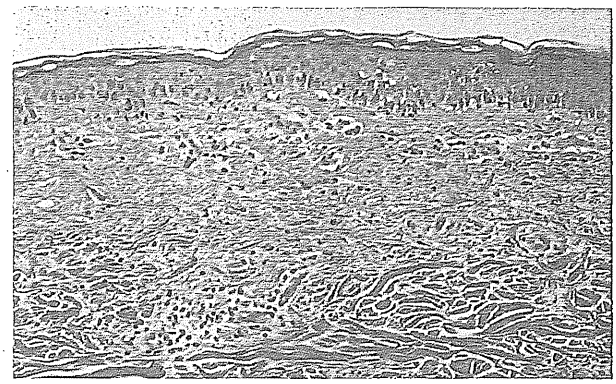


図3　病理組織像．真皮上層の血管周囲性の炎症細胞浸潤(H-E染色，×20)．

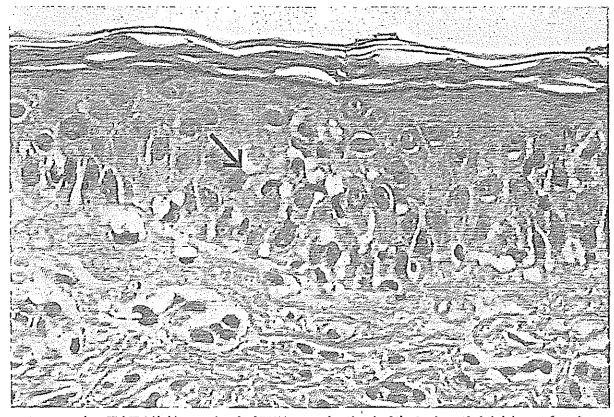


図4　病理組織像．表皮細胞の空胞変性と好酸性壊死(→)および表皮内へのリンパ球浸潤(H-E染色，×100)．

表　ソラフェニブによる多形紅斑の報告例
(自験例含む：2008年3月～2013年11月)

年齢	平均63.5歳(42～79歳)
男女比	6：10
原疾患	腎細胞癌11例　肝細胞癌5例
発症までの投与期間	平均9.0日間(3～14日間)
皮疹以外の症状	発熱2例(肝障害の合併2例)

はHLA-A24，ABCC2-24CC遺伝子がソラフェニブによる多形紅斑のリスクに関連している可能性が示唆されている[19]．今後さらに症例を集積し，ソラフェニブによる多形紅斑と遺伝子多型についての検討が進むことが期待される．

進行性腎細胞癌，肝細胞癌患者においてソラフェニブ中止は予後に大きく影響し，中止時には代替治療がないのが現状である．多形紅斑出現後の再投与については，十分な注意をしながら個々の症例で検討していく必要があると考えた．

本論文の要旨は，日本皮膚科学会第835回東京地方会(神奈川地区)で報告した．

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ABCA1 gene variation and heart disease risk reduction in the elderly during pravastatin treatment☆

Hironobu Akao [a,b,1], Eliana Polisecki [a,c,1], Ernst J. Schaefer [a,c], Stella Trompet [d,e], Michele Robertson [f], Ian Ford [f], J. Wouter Jukema [d,e], Anton J.M. de Craen [d,e], Christopher Packard [g], Brendan M. Buckley [h], Kouji Kajinami [b,*], on behalf of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) Investigator

[a] *Lipid Metabolism Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University and Tufts University School of Medicine, Boston, MA, USA*
[b] *Department of Cardiology, Kanazawa Medical University, Uchinada, Japan*
[c] *Boston Heart Diagnostics, Framingham, MA, USA*
[d] *Department of Cardiology, Leiden University Medical Centre, Leiden, The Netherlands*
[e] *Department of Gerontology, and Geriatrics, Leiden University Medical Centre, Leiden, The Netherlands*
[f] *Robertson Centre of Biostatistics, University of Glasgow, Glasgow, Scotland, UK*
[g] *Department of Vascular Biochemistry, University of Glasgow, Glasgow, Scotland, UK*
[h] *Department of Pharmacology and Therapeutics, University College Cork, Cork, Ireland*

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ABSTRACT

Aims: Our goals were to examine the relationships of a specific ATP-binding cassette transporter A1 (*ABCA1*) variant, rs2230806 (R219K), on baseline lipids, low-density lipoprotein cholesterol (LDL-C) lowering due to pravastatin, baseline heart disease, and cardiac endpoints on trial.
Methods and results: The *ABCA1* R219K variant was assessed in 5414 participants in PROSPER (PROspective Study of Pravastatin in the Elderly at Risk) (mean age 75.3 years), who had been randomized to pravastatin 40 mg/day or placebo and followed for a mean of 3.2 years. Of these subjects 47.6% carried the variant, with 40.0% carrying one allele, and 7.6% carrying both alleles. No effects on baseline LDL-C levels were noted, but mean HDL-C increased modestly according to the number of variant alleles being present (1.27 vs 1.28 vs 1.30 mmol/L, $p = 0.024$). No relationships between the presence or absence of this variant and statin induced LDL-C lowering response or CHD at baseline were noted. However within trial those with the variant as compared to those without the variant, the overall adjusted hazard ratio for new cardiovascular disease (fatal CHD, non-fatal myocardial infarction, or fatal or non-fatal stroke) was 1.22 (95% CI 1.06–1.40, $p = 0.006$), while for those in the pravastatin group it was 1.41 (1.15–1.73, $p = 0.001$), and for those in the placebo group it was 1.08 (0.89–1.30, $p = 0.447$) (p for interaction 0.058).
Conclusion: Our data indicate that subjects with the *ABCA1* R219K variant may get significantly less heart disease risk reduction from pravastatin treatment than those without the variant.

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* Corresponding author. Department of Cardiology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan. Tel.: +81 76 286 2211; fax: +81 76 286 3780.
E-mail address: kajinami@kanazawa-med.ac.jp (K. Kajinami).
[1] Drs. Akao and Polisecki contributed equally to this manuscript.

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1. Introduction

Elevated low-density lipoprotein cholesterol (LDL-C) and reduced high-density lipoprotein cholesterol (HDL-C) levels independently predict the risk of developing coronary heart disease (CHD) [1,2]. Statins reduce LDL-C effectively, but considerable inter-individual variation exists in treatment responses. Previous pharmacogenetic studies have explored genetic variation as determinants of statin response. We have previously documented that genetic variation at the *APOE*, low density lipoprotein receptor (*LDLR*), proprotein convertase subtilisin/kexin type 9 (*PCSK9*), the Niemann-Pick C1-like protein (*NPC1L1*), and the solute carrier organic anion transporter (*SLCO1B1*) and the kinesin like protein (*KIF6*) gene loci affects the degree of statin induced LDL-C lowering response in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) [3–7]. Moreover in this same study we have documented that genetic variation at the *APOE*, *LDLR*, *NPC1L1*, *KIF6*, and the taste receptor type 2, member 50 (*TAS2R50*) gene loci affected on trial CHD risk [3,5,7].

In PROSPER, there was a significant interaction ($p = 0.0069$) between baseline HDL-C levels and treatment effect. Subjects in the lowest HDL-C tertile (with baseline HDL-C < 1.11 mmol/L or <43 mg/dl) experienced apparently a greater benefit in terms of CHD risk reduction from pravastatin (hazards ratio 0.64) than did subjects with higher HDL-C levels [8]. The ATP-binding cassette transporter A1 (*ABCA1*) has been found to be critical in promoting the efflux of cellular cholesterol and phospholipid onto small pre-beta 1 HDL particles and in the process converting them to larger alpha migrating HDL particles [9]. Patients with significant defects in *ABCA1* have marked HDL deficiency and Tangier disease, characterized by cholesterol deposition in the tonsils, liver, spleen, and neurologic tissue, as well as premature CHD [10]. The most studied variant at the *ABCA1* gene locus is the R219K variant which causes an amino substitution in which an arginine is replaced by a lysine at amino acid position 219 in the *ABCA1* protein [11]. It has been previously reported that individuals carrying the R219K variant (found in 46% of a population of 790 subjects) had significantly lower triglyceride levels, slightly higher HDL-C levels, and significantly reduced severity of CHD as compared to non-carriers [12]. Moreover these authors suggested that this variant may be associated with a modest gain of *ABCA1* function [11,12]. Subsequently it was reported that the presence of the R219K *ABCA1* variant in patients with familial hypercholesterolemia ($n = 374$) was associated with a markedly reduced risk of CHD (hazards ratio 0.32, $p < 0.001$) as compared to non-carriers [13]. A recent meta-analysis based on an examination of 6597 CHD cases and 15,369 controls concluded that the presence of the R219K *ABCA1* variant was associated with a lower risk of CHD (hazards ratio 0.76, $p < 0.0001$), and modestly higher HDL-C levels [14].

However, there are no studies to our knowledge that have assessed whether the R219K *ABCA1* genetic variant affects responses to statin treatment in terms of lipid modification or CHD risk reduction. Our goals in this study were to determine whether the *ABCA1* variant rs2230806 or R219K in PROSPER participants would affect baseline lipids, CHD prevalence at baseline, pravastatin induced LDL-C lowering, and pravastatin mediated CHD risk reduction.

2. Materials and methods

2.1. Study subjects

The results and the methodology used in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) study have been previously described [8,15]. In this study 2804 men and 3000 women, aged 70–82 years, with pre-existing vascular disease ($n = 2404$) or at least one of three major vascular risk factors (diabetes $n = 575$, smoking $n = 1433$, or hypertension $n = 3360$) were randomized to pravastatin 40 mg/day ($n = 2891$) or placebo ($n = 2913$) and followed for an average of 3.2 years. Over this time period, the mean LDL-C reduction in the active treatment group was 32%, and the risk of developing CHD was decreased by 19%, which was statistically significant [8].

2.2. Biochemical and DNA analysis

Total cholesterol (TC), HDL-C, and triglycerides were assessed after an overnight fast, at 6 months, and at 12 months, and LDL-C was calculated by the Friedewald formula, as previously described [8]. Apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB) were measured only at baseline as described. DNA was isolated from cells from this cohort and DNA from 5783 subjects participating in this study were available for this study. ApoE phenotype was determined on plasma samples by western blotting, using the method of Havekes et al. in the central laboratory of the Royal Infirmary in Glasgow, Scotland. Subjects were classified according to the presence of apoE2, apoE3, or apoE4 bands on gel blotting [16]. This gel phenotyping method has been shown to have 99% concordance with genotyping [17].

For DNA analysis the single nucleotide polymorphism (SNP), R219K (rs2230806) of the *ABCA1* gene was genotyped using Taq Man® SNPs genotyping assays (Applied Biosystems, Foster City, CA). The custom assay identification number was C_2741051_1. The endpoint was read after PCR amplification was performed using an Applied Biosystems 7900 HT Sequence Detection System. Genotypes with quality scores below the 95% were repeated ($n = 13$) and 5% blinded replicates for genotype determinations were performed. In addition, a total of 119 subjects or 2.2% who had the apoE4/2 phenotype were excluded from these analyses, as well as 246 subjects who had missing apoE phenotypes. These exclusions were carried out because apoE phenotype or genotype can affect statin-induced LDL-C lowering response, as well as CHD risk. Subjects carrying the apoE4 allele have the lowest statin-induced LDL-C lowering response and the highest CHD risk, while the converse is true for carriers of the apoE2 allele [18–21]. The subject characteristics for these individuals representing the 5414 subjects studied are shown in Table 1.

2.3. Statistical analysis

Observed genotype frequencies were compared with those expected under Hardy–Weinberg equilibrium using a χ^2 test. For data analysis, multivariate analysis of covariance (ANCOVA) was performed to detect associations between lipoprotein levels at baseline as well as changes in response to treatment with pravastatin at 6 months and with *ABCA1* genotypes adjusted for gender, body mass index, age, alcohol, smoking, diabetes, apoE phenotype, baseline HDL-C levels, and country of origin, since subjects participating in PROSPER were either from Scotland, Ireland, or the Netherlands. Prevalence at baseline of myocardial infarction (MI) and all types of vascular disease (history of angina, claudication, MI, stroke, transient ischemic attack, peripheral arterial disease surgery, or amputation for vascular disease more than 6 months before study entry) at baseline, as well as incidence of primary endpoints (CHD death or nonfatal MI or fatal non-fatal stroke), and all cardiovascular events (primary endpoints and coronary artery bypass grafting, coronary angioplasty, and peripheral artery surgery or angioplasty), were compared between carriers of different *ABCA1* SNP genotypes using multivariable logistic regression analysis in all subjects, and also with stratification by gender and treatment. All

Table 1
Study subjects ($n = 5414$).

Study characteristics	Placebo	Pravastatin
Mean (SD)[a]	($n = 2732$)	($n = 2682$)
Age (years)	75.3 (3.3)	75.4 (3.3)
BMI (kg/m^2)	26.9 (4.3)	26.8 (4.1)
Females, *n* (%)	1413 (51.7)	1382 (51.5)
History diabetes mellitus, *n* (%)	294 (10.8)	281 (10.5)
History hypertension, *n* (%)	1694 (62.0)	1664 (62.0)
History vascular disease, *n* (%)	1191 (43.6)	1211 (45.2)
History of MI, *n* (%)	374 (13.7)	356 (13.3)
Current smoking, *n* (%)	740 (27.1)	693 (25.8)
Alcohol consumption (units per week)	5.0 (8.6)	5.3 (9.5)
Total cholesterol (mmol/L)	5.67 (0.88)	5.70 (0.93)
LDL cholesterol (mmol/L)	3.79 (0.78)	3.81 (0.81)
HDL cholesterol (mmol/L)	1.27 (0.34)	1.28 (0.35)
Triglyceride (mmol/L)	1.53 (0.69)	1.55 (0.71)
apoA-I (g/L)	1.32 (0.24)	1.33 (0.25)
apoB (g/L)	1.15 (0.22)	1.15 (0.23)
apoE 2/2 + 2/3 (%)	12.1	12.1
apoE 3/3 (%)	64.7	64.3
apoE 3/4 + 4/4 (%)	23.2	23.6
*ABCA1*_R219K-rs2230806	MAF A:0.28	

BMI: body mass index. MAF: minor allele frequency. MI: myocardial infarction.
[a] Means (S.D.) unless otherwise specified; apoE 2/4 carriers were excluded (see Materials and Methods section).

analyses were fully adjusted for age, gender, country, history of vascular disease, body mass index, history of diabetes, as well as history of hypertension, alcohol use, current smoking, apoE phenotype, and baseline HDL-C levels. We estimated the power of the study to detect genotypic differences in the incidence of cardiovascular events by assuming an alpha-level of 0.05, a dominant inheritance model, an assumed prevalence of events of 15% and a power of 80% for a balanced case–control study (1:1) for minor allele frequency 0.30, and genetic relative risk may around 1.2, which indicated approximately 2000–2500 as the number of cases per group required.

To evaluate the modifying effects of genotypes and gender on the response to treatment, gene-treatment and gene–gender interaction terms were added to the regression models. There was no interaction between R219K and apoE phenotype. All analyses were performed using SAS/STAT and SAS/Genetics (SAS Version 9.1, SAS Institute Inc., Cary, NC). A two-sided $p < 0.05$ was considered statistically significant. As for correction of multiple testing, we applied Bonferroni correction and the *p*-value threshold of 0.05 was divided by the number of independent tests.

3. Results

As summarized in Table 1, the participating subjects were elderly, with a median age of 75 ± 3 years at baseline. Mean LDL-C levels were in the moderate-risk category (3.36–4.14 mmol/L or 130-160 mg/dl), as defined by the United States National Cholesterol Education Program [22]. Approximately half of the men and about one third of the women reported a history of any type of vascular disease. Data on apoE phenotype distribution in this population are also shown in Table 1. Genotype frequencies for the *ABCA1* SNP examined conformed to Hardy–Weinberg equilibrium ($p > 0.05$, data not shown).

3.1. Association with baseline lipid levels

Neither baseline LDL-C or triglyceride levels differed significantly among the three genotypic groups. However mean HDL-C levels increased modestly according to the number of variant allele present (1.27 versus 1.28 versus 1.30 mmol/L, $p = 0.024$) (Table 2). After gender separation, similar trend in terms of HDL-C levels was observed although statistical significance was no longer present (Table 2).

3.2. Associations with lipid response to treatment

To determine whether the R219K variant affected lipid responses to pravastatin, the association between the R219K variant under study and 6 month and 12 month changes in TC, LDL-C, HDL-C, and triglyceride levels in individuals treated with pravastatin or placebo were examined. No significant relationships between the presence or absence of this variant and statin induced LDL-C response (Table 3) or changes in other lipid parameters including triglycerides or HDL-C levels (data not shown) were noted.

3.3. Associations with history and within trial incidence of cardiovascular disease

There were no significant associations between the prevalence of various forms of vascular disease at baseline and the presence of the R219K variant (data not shown). As shown in Tables 2 and 3A and B, the number of AA homozygotes in placebo or pravastatin group was around 100, and cases with primary endpoint were 10–20 in each gender. Therefore, we examined variant effects in a dominant model, not additive model, to avoid false positive or negative association. In Table 4, we show the data on the relationships between *ABCA1* genotypes and cardiovascular endpoints within trial. For all subjects, there was a significantly increased risk of new cardiovascular disease (fatal CHD, non-fatal myocardial infarction, or fatal or non-fatal stroke) on trial for those who carried the *ABCA1* R219K variant as compared to those who did not. The overall adjusted hazard ratio on trial for carriers versus non-carriers was 1.22 (95%CI 1.06–1.40, $p = 0.006$). Examining two treatment arms, it was seen that for carriers in the placebo group the HR was 1.08 (0.89–1.30, $p = 0.447$), while for carriers in the pravastatin group it was 1.41 (1.15–1.73, $p = 0.001$).

Table 2
Adjusted baseline lipid levels (mean ± SD) by gender and genotype.

ABCA1 SNP rs2230806	R219K			p[a]
	GG	GA	AA	
All (*n*)	2834	2167	413	
TC (mmol/L)	5.69 ± 0.91	5.68 ± 0.89	5.75 ± 0.93	0.463
LDL-C (mmol/L)	3.81 ± 0.80	3.79 ± 0.80	3.80 ± 0.80	0.655
HDL-C (mmol/L)	1.27 ± 0.34	1.28 ± 0.35	1.30 ± 0.34	0.024
TG (mmol/L)	1.56 ± 0.72	1.53 ± 0.69	1.52 ± 0.64	0.140
apoA-I (g/L)	1.32 ± 0.24	1.33 ± 0.25	1.35 ± 0.23	0.066
apoB (g/L)	1.15 ± 0.23	1.15 ± 0.23	1.15 ± 0.22	0.222
Men (n)	1395	1040	184	
TC (mmol/L)	5.36 ± 0.80	5.36 ± 0.80	5.31 ± 0.71	0.819
LDL-C (mmol/L)	3.59 ± 0.72	3.58 ± 0.73	3.54 ± 0.62	0.820
HDL-C (mmol/L)	1.17 ± 0.31	1.18 ± 0.33	1.21 ± 0.30	0.133
TG (mmol/L)	1.50 ± 0.74	1.50 ± 0.74	1.44 ± 0.59	0.568
apoA-I (g/L)	1.24 ± 0.21	1.25 ± 0.24	1.26 ± 0.22	0.166
apoB (g/L)	1.11 ± 0.22	1.10 ± 0.21	1.09 ± 0.19	0.227
Women (*n*)	1439	1127	229	
TC (mmol/L)	6.00 ± 0.89	5.97 ± 0.88	6.11 ± 0.93	0.110
LDL-C (mmol/L)	4.01 ± 0.81	3.99 ± 0.81	4.07 ± 0.82	0.281
HDL-C (mmol/L)	1.36 ± 0.35	1.38 ± 0.35	1.40 ± 0.34	0.091
TG (mmol/L)	1.61 ± 0.69	1.55 ± 0.64	1.60 ± 0.68	0.127
apoA-I (g/L)	1.39 ± 0.25	1.40 ± 0.24	1.42 ± 0.21	0.221
apoB (g/L)	1.20 ± 0.23	1.18 ± 0.22	1.20 ± 0.23	0.577

[a] p values using the three genotypes, men and women combined; adjusted for gender, body mass index, age, alcohol, smoking, diabetes, apoE phenotype, and country. As for Bonferroni correction of multiple testing, *p*-value threshold divided by independent tests would be 0.05/12 = 0.004.

Table 3
A. Percent LDL-C response to pravastatin by genotype. B. Percent LDL-C response to placebo by genotype.

Gene	Genotype	Adjusted mean percent LDL-C reduction[a]					
		n	6 months	p^b	*n*	12 months	p^b
A							
ABCA1							
All	GG	1346	−33.06 ± 14.16	0.374	1318	−32.04 ± 15.70	0.767
	GA	1010	−33.26 ± 14.31		972	−31.72 ± 15.65	
	AA	199	−33.84 ± 12.72		198	−31.77 ± 15.46	
Men	GG	673	−32.37 ± 13.82	0.604	662	−30.99 ± 15.79	0.519
	GA	459	−32.73 ± 13.32		443	−30.69 ± 15.01	
	AA	107	−32.57 ± 12.78		104	−29.54 ± 16.46	
Women	GG	673	−33.75 ± 14.46	0.502	656	−33.10 ± 15.56	0.797
	GA	551	−33.69 ± 15.09		529	−32.59 ± 16.13	
	AA	92	−35.32 ± 12.55		94	−34.24 ± 13.95	
B							
ABCA1							
All	GG	1334	−1.70 ± 11.89	0.257	1297	−1.11 ± 13.22	0.952
	GA	1044	−1.42 ± 11.24		1029	−1.31 ± 13.55	
	AA	193	−0.85 ± 0.95		190	−0.78 ± 0.12	
Men	GG	652	−1.82 ± 11.43	0.107	632	−0.69 ± 12.77	0.881
	GA	526	−1.25 ± 12.26		525	−1.66 ± 13.94	
	AA	66	0.69 ± 8.32		68	1.34 ± 10.14	
Women	GG	682	−1.58 ± 12.32	0.957	665	−1.51 ± 13.63	0.830
	GA	518	−1.60 ± 10.11		504	−0.95 ± 13.14	
	AA	127	−1.65 ± 10.05		122	−1.34 ± 13.37	

[a] Values are provided as mean ± S.D.
[b] *p* values for data combining men and women, adjusted for gender, body mass index, age, alcohol, smoking, diabetes, apoE phenotype, baseline HDL-C levels, and country.

An interaction between this genetic variation and treatment benefit was very close to statistical significance ($p = 0.058$), suggesting that the R219K variant is a novel genetic marker for pravastatin treatment benefit in the elderly. Similar findings for this variant were observed in both men and women (Table 4), but an interaction between this genetic variant and treatment benefit (primary endpoint) in both gender did not reach statistical significance, $p = 0.206$ in men and 0.148 in women.

4. Discussion

We examined the association of a prevalent genetic polymorphism, R219K, at the *ABCA1* gene locus with baseline lipids and vascular disease, pravastatin induced LDL-C lowering response, and cardiovascular endpoints on trial in PROSPER, in which participants who had been randomized to pravastatin 40 mg/day or placebo and were followed for a mean of 3.2 years [8]. We found that the presence of this variant was associated with modestly, but significantly greater levels of baseline HDL-C. More importantly we noted that, in this trial of pravastatin in elderly subjects, those on active treatment carrying variant allele had a significantly greater cardiovascular risk (close to that in the placebo group) than those on pravastatin without the variant.

Amino acid 219 is located in the long extracellular loop of *ABCA1*, a region of the transporter known to contain various glycosylation sites [23,24]. The substitution of an arginine by a lysine at this residue probably alters the conformation of the extracellular domain of the *ABCA1* protein, and enhances its interaction with apoA-I, thereby increasing the efficiency of phospholipid and cholesterol transfer from the plasma membrane to HDL particles [25–27]. These increases could therefore elevate baseline HDL-C levels. To date, the association between *ABCA1* R219K variant and increased HDL-C level remains controversial.

To our knowledge, only one small study examined potential effects of the R219K variant on statin induced lipid responses [28]. These investigators reported similar LDL-C reductions among the three *ABCA1* genotypic groups after 12 weeks of treatment with pravastatin. Our study in a much larger population with a longer treatment period supports these prior observations and indicates that this polymorphism does not plays a significant role as a determinant of LDL-C lowering response to pravastatin treatment.

Our study did not provide direct evidence for the mechanism by which the R219K variant affect the statin effects in cardiovascular event reduction. Wild-type homozygotes for this variant showed significantly lower HDL-C levels at baseline. If this could be recognized as a simple representation of high-risk population, our observation might be the case that greater benefit from pravastatin treatment could be expected in the higher risk group. Another possible mechanism is that R219K variant plays a novel role in lipid metabolism. Supporting this is a recent study suggesting that the R219K variant not only affects apoA-I and HDL metabolism, but may also significant influence postprandial lipid metabolism [29]. Further *in vitro* and *in vivo* study would clearly be required to establish the significance of this genetic variation as determinant for statin response. In comparison with previous studies, the present results are concordant with some and discordant with others to investigate *ABCA1* polymorphism with respect to both cardiovascular diseases and statin response. As for R219K variant, a meta-analysis including 5388 participants reported opposite genotype effects, K-variant as a protective allele, in Chinese population [30]. However, authors of this report found significantly more frequent prevalence of K-variant allele in Chinese population as compared to Caucasians, and recommended the interpretation with great caution. Another meta-analysis including 42 studies for R219K variant reported a potential protective role of K-allele, but again suggested limited interpretation of results because of significant heterogeneity among enrolled studies [31]. As for statin response, only one study examined potential influence of *ABCA1* polymorphism [32]. This pharmacogenetic study enrolled 1686 patients with familial hypercholesterolemia without history of coronary heart disease and analyzed statin-*ABCA1* C69T, not R219K, polymorphism interaction by comparing treated and untreated patients. They found that TT genotype was associated with increased disease risk in untreated patients, but that the risk was no longer significantly different between genotypes, at least partially explained by a higher rise in HDL-C levels (despite similar pretreatment levels) during statin treatment in TT individuals. Effects of this polymorphism on *ABCA1* transcription were proposed, but

Table 4
Analysis of incidence of new events on trial by *ABCA1* genotype.

Gene	Primary endpoints		p^d	CHD death or non-fatal MI		p^d	Cardiovascular events		p^d
Genotype	New case/total subjects (%)	HR[c]		New case/total subjects (%)	HR[c]		New case/total subjects (%)	HR[c]	
ABCA1									
Unadjusted									
GG	386/2834 (13.6)	1	0.005	277/2834 (9.7)	1	0.002	439/2834 (15.5)	1	0.029
GA + AA	420/2580 (16.3)	1.22 (1.06–1.40)		321/2580 (12.4)	1.30 (1.10–1.52)		256/2580 (17.7)	1.16 (1.02–1.32)	
Adjusted[a]									
GG	386/2834 (13.6)	1	0.006	277/2834 (9.7)	1	0.001	439/2834 (15.5)	1	0.021
GA + AA	420/2580 (16.3)	1.22 (1.06–1.40)		321/2580 (12.4)	1.30 (1.11–1.53)		456/2580 (17.7)	1.17 (1.02–1.33)	
On placebo									
GG	216/1412 (15.3)	1	0.447	157/1412 (11.1)	1	0.192	241/1412 (17.1)	1	0.404
GA + AA	219/1320 (16.6)	1.08 (0.89–1.30)		170/1320 (12.5)	1.16 (0.93–1.44)		242/1320 (18.3)	1.08 (0.90–1.29)	
On pravastatin[b]									
GG	170/1422 (12.0)	1	0.001	120/1422 (8.4)	1	0.001	198/1422 (13.9)	1	0.013
GA + AA	201/1260 (16.0)	1.41 (1.15–1.73)		151/1260 (12.9)	1.51 (1.18–1.92)		214/1260 (17.0)	1.28 (1.06–1.56)	
Men on placebo									
GG	127/685 (18.5)	1	0.568	97/685 (14.2)	1	0.279	142/685 (20.7)	1	0.396
GA + AA	128/634 (20.2)	1.08 (0.84–1.38)		106/634 (16.7)	1.17 (0.88–1.54)		146/634 (23.0)	1.11 (0.88–1.40)	
Men on pravastatin									
GG	97/710 (13.7)	1	0.026	72/710 (10.1)	1	0.029	116/710 (16.3)	1	0.087
GA + AA	107/590 (18.1)	1.37 (1.04–1.80)		83/590 (14.1)	1.42 (1.04–1.95)		117/590 (19.8)	1.25 (0.97–1.62)	
Women on placebo									
GG	89/727 (12.2)	1	0.661	60/727 (8.3)	1	0.496	99/727 (13.6)	1	0.806
GA + AA	91/686 (13.3)	1.07 (0.80–1.43)		64/686 (9.3)	1.13 (0.79–1.61)		96/686 (14.0)	1.04 (0.78–1.38)	
Women on pravastatin									
GG	73/712 (10.3)	1	0.011	48/712 (6.7)	1	0.008	82/712 (11.5)	1	0.046
GA + AA	94/670 (14.0)	1.50 (1.10–2.05)		68/670 (10.1)	1.67 (1.14–2.44)		97/670 (14.5)	1.36 (1.01–1.83)	

[a] *p* values for men and women combined; adjusted for gender, body mass index, age, alcohol, smoking, diabetes, hypertension, apoE phenotype, baseline HDL-C levels, randomized treatment, and country. No significant differences were noted when men and women were separated.
[b] *p* values for men and women combined; adjusted for gender, body mass index, age, alcohol, smoking, diabetes, hypertension, apoE phenotype, baseline HDL-C levels, and country.
[c] Hazards ratio (95% confidence interval).
[d] As for Bonferroni correction of multiple testing, *p*-value threshold divided by independent tests would be 0.05/4 = 0.0125.

further studies appear to be required to confirm this consideration. In addition, difference in study populations, familial hypercholesterolemia without coronary disease (mean age 39 years) in this study and elderly (75 years) in our study could not allow us to consider these two studies equally.

In the original analysis in PROSPER, subgroup analysis demonstrated that the benefit of pravastatin in the prevention for primary endpoint was predominantly in the lowest tertile of HDL-C with significant interaction [8]. Our current observations, associations with wild allele homozygosity, lower HDL-C levels, and fewer cardiovascular events on pravastatin, could provide one possible genetic explanation for this finding. This kind of issue which could only be obtained through a well-conducted large randomized study enrolling significant number of both genders and examining genotypic effects on treatment response is the most important strength of the present study. Despite that the primary weakness of the study is its narrow focus, only one polymorphism investigated and lack of providing evidence of possible explanation for observations, further *in vitro* and clinical studies are required to extend our observations to more wide range of populations.

In conclusion, *ABCA1* R219K variant might be a novel genetic determinant for pravastatin treatment response in heart disease risk reduction in the elderly.

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