CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This study was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI14C1277).

Eun Ju Kim[1,2,3,5], Dong Hun Lee[1,2,3,5], Yeon Kyung Kim[1,2,3], Hee Chul Eun[1,2,3] and Jin Ho Chung[1,2,3,4]

[1]Department of Dermatology, Seoul National University College of Medicine, Seoul, Korea; [2]Laboratory of Cutaneous Aging Research, Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea; [3]Institute of Human-Environment Interface Biology, Seoul National University, Seoul, Korea and [4]Institute on Aging, Seoul National University, Seoul, Korea
E-mail: jhchung@snu.ac.kr
[5]These authors contributed equally to this work.

REFERENCES

Berardesca E, Fluhr JW, Maibach HI (2006) *Sensitive Skin Syndrome*, 1st (edn) CRC Press: New York, 1–5

Deval E, Gasull X, Noel J *et al.* (2010) Acid-sensing ion channels (ASICs): pharmacology and implication in pain. *Pharmacol Ther* 128:549–58

Dykes AC, Fultz ME, Norton ML *et al.* (2003) Microtubule-dependent PKC-alpha localization in A7r5 smooth muscle cells. *Am J Physiol Cell Physiol* 285:C76–87

Farage MA, Maibach HI (2010) Sensitive skin: closing in on a physiological cause. *Contact Dermatitis* 62:137–49

Goldstein BJ, Scalia R (2004) Adiponectin: a novel adipokine linking adipocytes and vascular function. *J Clin Endocrinol Metab* 89: 2563–8

Hardie DG, Hawley SA, Scott JW (2006) AMP-activated protein kinase–development of the energy sensor concept. *J Physiol* 574:7–15

Holzer P (2009) Acid-sensitive ion channels and receptors. *Handb Exp Pharmacol*, 283–332

Kadowaki T, Yamauchi T, Kubota N *et al.* (2006) Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* 116:1784–92

Kim EJ, Lee DH, Kim YK *et al.* (2014) Decreased ATP synthesis and lower pH may lead to abnormal muscle contraction and skin sensitivity in human skin. *J Dermatol Sci* 76:214–21

Krause MP, Liu Y, Vu V *et al.* (2008) Adiponectin is expressed by skeletal muscle fibers and influences muscle phenotype and function. *Am J Physiol Cell Physiol* 295:C203–12

Reeh PW, Kress M (2001) Molecular physiology of proton transduction in nociceptors. *Curr Opin Pharmacol* 1:45–51

Ruderman NB, Carling D, Prentki M *et al.* (2013) AMPK, insulin resistance, and the metabolic syndrome. *J Clin Invest* 123:2764–72

Sattar AA, Sattar R (2012) Globular adiponectin activates Akt in cultured myocytes. *Biochem Biophys Res Commun* 424:753–7

Ständer S, Schneider SW, Weishaupt C *et al.* (2009) Putative neuronal mechanisms of sensitive skin. *Exp Dermatol* 18:417–23

Yamauchi T, Kamon J, Minokoshi Y *et al.* (2002) Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 8:1288–95

See related commentary on pg 2153

PLCG1 Gene Mutations Are Uncommon in Cutaneous T-Cell Lymphomas

Journal of Investigative Dermatology (2015) **135,** 2334–2337; doi:10.1038/jid.2015.161; published online 14 May 2015

TO THE EDITOR

The molecular events underlying the oncogenesis of cutaneous T-cell lymphomas (CTCLs) remain largely unknown, especially when considering primary or driver mutations. Global genomic approaches have revealed the existence of recurrent chromosomal or genetic alterations, some of which having potential diagnosis or prognosis value (Scarisbrick *et al.*, 2000; Vermeer *et al.*, 2008; van Doorn *et al.*, 2009; Laharanne *et al.*, 2010a, 2010b; Cristofoletti *et al.*, 2013). However, no specific gene mutation is currently assessed for the management of patients with CTCLs.

Vaque *et al.* (2014) recently identified somatic mutations of the Phospholipase C Gamma 1 (*PLCG1*) gene in about 20% of epidermotropic CTCLs, being notably more frequent in transformed/tumoral mycosis fungoides (T-MF; 8/30 cases) and the Sézary syndrome (SS; 1/2 cases) than in erythrodermic and folliculotropic mycosis fungoides (1/20 cases). They reported a c.1034C>T/p.S345F mutation in exon 11 in nine patients (eight T-MF and one SS) and a c.1559C>T/p.S520F mutation in exon 15 (in one erythrodermic mycosis fungoides case). Such mutations, especially the p.S345F mutation, conferred enhanced signaling capacity and transforming property. Finally, *PLCG1* mutations were suggested to be possibly associated with a higher rate of disease-related death (Vaque *et al.*, 2014).

These findings prompted us to investigate the *PLCG1* gene status in our series of CTCLs, including T-MF ($n=37$) and SS ($n=39$). We also studied other CTCL subtypes including lymphomatoid papulosis (LyP; $n=4$), cutaneous anaplastic large cell lymphomas (c-ALCL, $n=14$), and the following T cell or CTCL cell lines: MyLa, SeAX, HH, Hut78, FEPD, and 1301 (for origin see Chevret *et al.* (2014)).

All cases were retrieved from the Aquitaine database of cutaneous

Abbreviations: c-ALCL, cutaneous anaplastic large cell lymphoma; CTCL, cutaneous T-cell lymphoma; HRM, high-resolution melting; LyP, lymphomatoid papulosis; PLCG1, Phospholipase C Gamma 1; SS, Sézary syndrome; T-MF, transformed/tumoral mycosis fungoide

Accepted article preview online 24 April 2015; published online 14 May 2015

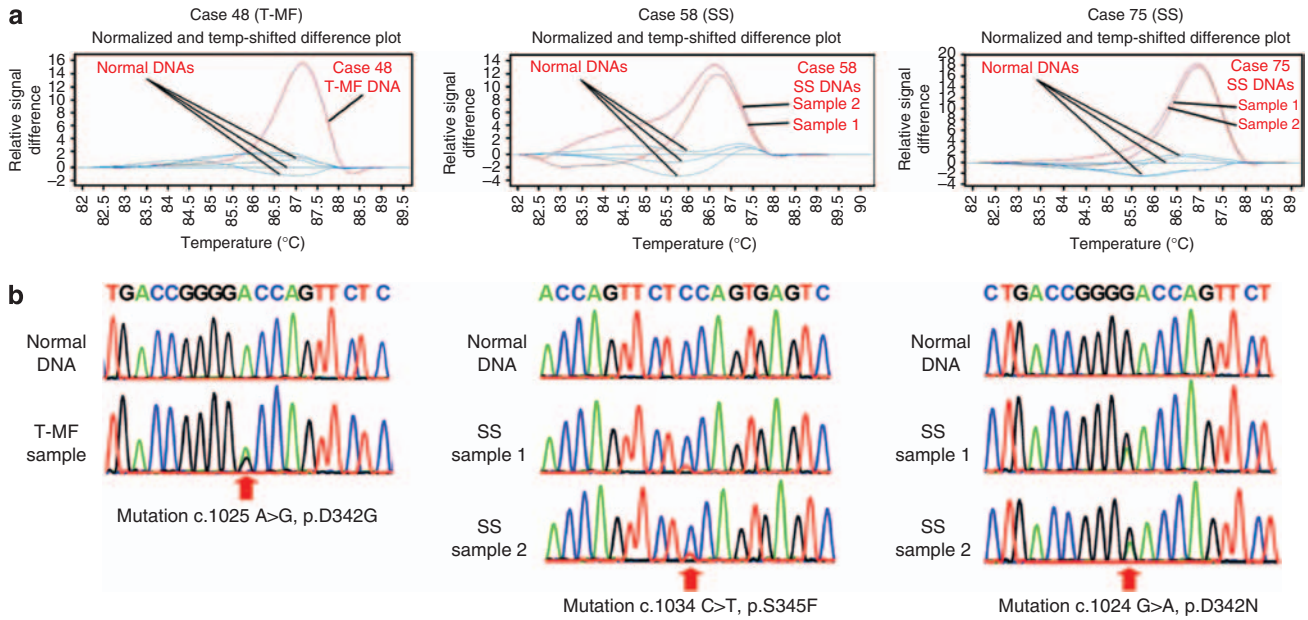

Figure 1. Identification of Phospholipase C Gamma 1 (*PLCG1*) exon 11 mutations in cutaneous T-cell lymphoma (CTCL) samples. (**a**) High-resolution melting (HRM) curves and (**b**) Sanger sequencing profiles (partial sequence, sense strand) of *PLCG1* exon 11 PCR products obtained from normal and CTCL DNA samples. The three CTCL cases (one transformed/tumoral mycosis fungoides (T-MF) and two Sézary syndrome (SS)) in which *PLCG1* exon 11 mutations were identified are illustrated.

Table 1. CTCL patient features and PLCG1 status

Diagnosis	Number of cases	Gender	Mean age ± SD (years)	Status *PLCG1* exon 11	Status *PLCG1* exon 15
cALCL	*N* = 14	F = 3 M = 7	58 ± 6	All WT	All WT
LyP	*N* = 4	F = 3 M = 1	30 ± 15	All WT	All WT
MF	*N* = 37 (MF = 5; T-MF = 32)	F = 14 M = 23	68 ± 9	1 Mutant (c.1025A > G; p.D342G) 40 WT	All WT
SS	*N* = 39	F = 17 M = 22	70 ± 6	1 Mutant (c.1034 C > T; p.S345F) 1 mutant (c.1024 G > A; p.D342N) 46 WT	All WT

Abbreviations: cALCL, cutaneous anaplastic large cell lymphomas; F, female; LyP, lymphomatoid papulosis; M, male; MF, mycosis fungoide; SS, Sézary syndrome; T-MF, transformed mycosis fungoides; WT, wild type.

lymphomas, with approval from the regional bioethics committee and informed written consent, in accordance with the Declaration of Helsinki Principles. Skin or blood* samples (*for patients with SS) were analyzed. The selected cases contained at least 40% of tumor cells, as found by histo/cytopathological or flow cytometric evaluation. DNA was extracted from frozen CTCL samples or cell lines with the DNA easy kit (Qiagen, Courtaboeuf, France). All exhibited a dominant monoclonal rearrangement of the T-cell receptor gamma (*TCRG*) gene (Beylot-Barry *et al.*, 2001), as well as chromosomal imbalances (Laharanne *et al.*, 2010b). In cases with *PLCG1* mutation, constitutional DNA was extracted from histologically normal tissue and checked for the absence of the monoclonal *TCRG* gene rearrangement. The mutational status of *PLCG1* exon 11 and 15 was determined by high-resolution melting (HRM) analysis on a LC480 device (Roche Diagnostics, Meylan, France), followed by Sanger sequencing for samples exhibiting variant profiles, as reported for *BRAF* status determination (Boursault *et al.*, 2013). The primers used were the following: 5′-GCCCATCTGACCATACC TAC-3′ and 5′-TGGACCCCACGCACAC TCA-3′ (exon 11) and 5′-CTCACAAGTC CCTCTTTGGTC-3′ and 5′-GACCTGA GCTGGTTCCTCAC-3′ (exon 15).

Only one of the 37 tested T-MF cases (2.7%) from our series harbored a mutation in exon 11 (c.1025A > G/p.D342G; case 48, Figure 1, Table 1). Two out of the 39 tested SS cases (5%) exhibited exon 11 mutations, the c.1024 G > A/p.D342N mutation (case 75) and the c.1034C > T/p.S345F mutation (case 58; Figure 1,Table 1), the latter being previously reported to be a recurrent event in CTCLs (Vaque *et al.*, 2014). All

three mutations were somatic, as not detected in the control constitutional DNA. Interestingly, the p.D342G and p.D342N mutations had not been reported either in CTCLs (Vaque *et al.*, 2014) or in other types of cancers (http://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=PLCG1#dist). According to the PROVEAN (http://provean.jcvi.org/seq_submit.php) or PolyPhen (http://genetics.bwh.harvard.edu/pph2/) software analysis, they were predicted to alter the function of the PLCG1 protein catalytic domain. In the two mutated SS cases, the same mutation was detected in blood samples at two time points of the disease, with a 5-year (case 58) and a 1-year (case 75) interval, respectively (Figure 1, Table 1). Although all cases contained at least 40% of tumor cells, the mutated allele was more prominent in samples from the T-MF case 48 and the SS case 75 than in the SS case 58 (Figure 1), suggesting a possible tumor cell heterogeneity for the *PCLG1* status in this case. In addition, none of our T-MF and SS cases exhibited *PLCG1* exon 15 mutation, and none of the tested c-ALCL and LyP cases and T-cell leukemia/CTCL cell lines showed mutations of *PLCG1* exons 11 or 15.

We thus report a low frequency of *PLCG1* mutations in our T-MF (1 of 37, 2.7%) and SS (2 of 39, 5%) cases and the fact that the c.1034C>T/p.S345F mutation is quite uncommon in CTCLs (one out of the three mutations found in the 94 CTCL patients tested). We also document the absence of *PLCG1* exon 11 and 15 mutations in the tested c-ALCL and LyP cases, as well as in the main CTCL cell lines.

For SS, differences in sample numbers can explain the discrepancy between frequencies of *PLCG1* mutations found in our study (2/39 cases) and the first one (1/2 cases; Vaque *et al.*, 2014). For T-MF, we and the previous report analyzed comparable number of samples; thus, the distinct mutation frequencies (1/37 vs. 8/30) may come from technical aspects. Indeed, in the previous study, five out of the nine cases harboring the c.1034C>T/p.S345F were identified only with a sensitive allele-specific quantitative PCR assay (Vaque *et al.*, 2014). On the other hand, our HRM/Sanger sequencing strategy identified two previously unknown PLCG1 mutations affecting the catalytic domain that would have been missed by the allele-specific mutation analysis used by our colleagues (Vaque *et al.*, 2014; Manso *et al.*, 2015).

To exclude technical bias, we checked the sensitivity of our methodology by diluting the mutated samples within normal DNA. As illustrated for sample 2 from case 75, containing 40% of Sézary cells (Figure 1 and Supplementary Figure S1 online -Pure-), dilution up to 4-fold still allowed a clear detection of variant HRM profile, as well as a mutant peak on Sanger sequencing profiles (Supplementary Figure S1A and S1B online). Higher dilutions (8-fold) yielded normal HRM profiles, and the mutation was barely detected by Sanger sequencing. Therefore, our approach is capable to detect *PLCG1* mutation in samples with 10% of tumor cells (5% of mutant allele) in accordance with our previous evaluation of this technique for *BRAF* analysis in melanomas (Boursault *et al.*, 2013).

Using allele-specific mutation analysis, the same group reported the presence of the PLCG1^{S345F} in about 13% of peripheral T-cell lymphoma, with significant association with CD30 expression and p50 nuclear expression, suggesting an increased NF-kB activity (Manso *et al.*, 2015). Such an association was not observed in our CTCL series, which also includes CD30+ T-MF cases and CD30+ cutaneous lymphoproliferative disorders with abundant tumor cell content (Fauconneau *et al.*, 2015).

If assessment of the PLCG1 mutations, notably the p.S345F mutation, is only feasible with highly sensitive methods, especially in our samples containing more than 40% of tumor cells, this would suggest that PLCG1 mutations do not represent an initiating oncogenic event but may be acquired by some aggressive subclones with possible prognosis impact on the overall survival in CTCL and PTCL (Vaque *et al.*, 2014; Manso *et al.*, 2015). Further investigations by other groups and next-generation sequencing techniques are required to establish the real prevalence of PLCG1 mutations, which appeared unusual in our series of otherwise well-characterized CTCL subtypes (Laharanne *et al.*, 2010b; Chevret *et al.*, 2014; Fauconneau *et al.*, 2015).

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by grants from the Ligue Contre le Cancer, Comité de Gironde, the Cancéropôle Grand Sud-Ouest and the Institut National du Cancer (INCA) for supporting the Aquitaine database of cutaneous lymphoma and the Tumor Bank of CHU de Bordeaux. We also thank Nathalie Carrere, and Séverine Verdon (Tumor Bank and Tumor Biology Laboratory, Centre Hospitalier Universitaire de Bordeaux, Pessac, France) for help in the preparation of control and patient DNA samples and Christine Alfaro (Department of Dermatology, Centre Hospitalier Universitaire de Bordeaux, Hôpital Haut-Lévêque, Pessac, France) for help in collecting clinicopathological data.

Charline Caumont[1,2], Audrey Gros[1,2], Cécile Boucher[2], Pierre Mélard[3], Martina Prochazkova-Carlotti[1], Elodie Laharanne[2], Anne Pham-Ledard[1,4], Béatrice Vergier[1,3], Edith Chevret[1], Marie Beylot-Barry[1,4], Jean-Philippe Merlio[1,2] and David Cappellen[1,2]

[1]EA2406, Histology and Molecular Pathology of Tumours, University of Bordeaux, Bordeaux, France; [2]Tumor Bank and Tumor Biology Laboratory, Centre Hospitalier Universitaire de Bordeaux, Hôpital Haut-Lévêque, Pessac, France; [3]Department of Pathology, Centre Hospitalier Universitaire de Bordeaux, Hôpital Haut-Lévêque, Pessac, France and [4]Department of Dermatology, Centre Hospitalier Universitaire de Bordeaux, Hôpital Haut-Lévêque, Pessac, France
E-mail: jp.merlio@u-bordeaux.fr or david.cappellen@u-bordeaux.fr

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Beylot-Barry M, Sibaud V, Thiébaut R *et al.* (2001) Evidence that an identical skin and blood T-cell clone is an independent prognostic factor in primary cutaneous T-cell lymphomas. *J Invest Dermatol* 117:920–6

Boursault L, Haddad V, Vergier B *et al.* (2013) Tumor homogeneity between primary and metastatic sites for BRAF status in metastatic melanoma determined by immunohistochemical and molecular testing. *PLoS One* 8: e70826

Chevret E, Andrique L, Prochazkova-Carlotti M *et al.* (2014) Telomerase functions beyond telomere maintenance in primary cutaneous T-cell lymphoma. *Blood* 123:1850–9

Cristofoletti C, Picchio MC, Lazzeri C *et al.* (2013) Comprehensive analysis of PTEN status in Sezary syndrome. *Blood* 122:3511–20

Fauconneau A, Pham-Ledard A, Cappellen D *et al.* (2015) Assessment of diagnostic criteria between primary cutaneous anaplastic large cell lymphoma and CD30-rich transformed mycosis fungoides. A study of 66 cases. *Br J Dermatol*10.1111/bjd.13690

Laharanne E, Chevret E, Idrissi Y *et al.* (2010a) CDKN2A-CDKN2B deletion defines an aggressive subset of cutaneous T-cell lymphoma. *Mod Pathol* 23:547–58

Laharanne E, Oumouhou N, Bonnet F *et al.* (2010b) Genome-wide analysis of cutaneous T-cell lymphomas identifies three clinically relevant classes. *J Invest Dermatol* 130: 1707–18

Manso R, Rodriguez-Pinilla SM, Gonzalez-Rincon J *et al.* (2015) Recurrent presence of the PLCG1 S345F mutation in nodal peripheral T-cell lymphomas. *Haematologica* 100:e25–7

Scarisbrick JJ, Woolford AJ, Russell-Jones R *et al.* (2000) Loss of heterozygosity on 10q and microsatellite instability in advanced stages of primary cutaneous T-cell lymphoma and possible association with homozygous deletion of PTEN. *Blood* 95:2937–42

van Doorn R, van Kester MS, Dijkman R *et al.* (2009) Oncogenomic analysis of mycosis fungoides reveals major differences with Sezary syndrome. *Blood* 113:127–36

Vaque JP, Gomez-Lopez G, Monsalvez V *et al.* (2014) PLCG1 mutations in cutaneous T-cell lymphomas. *Blood* 123:2034–43

Vermeer MH, van Doorn R, Dijkman R *et al.* (2008) Novel and highly recurrent chromosomal alterations in Sézary syndrome. *Cancer Res* 68:2689–98