TO THE EDITOR

Palmoplantar pustulosis (PPP) is a severe pustular eruption that affects the palms and/or soles, with detrimental effects on quality of life. The disease is notoriously difficult to treat because its immune and genetic determinants remain poorly defined (Twelves et al., 2019). Although mutations of the IL36RN and myeloperoxidase MPO genes have been convincingly associated with generalized pustular psoriasis, they are rarely found in patients with PPP (Haskamp et al., 2020; Twelves et al., 2019; Vergnano et al., 2020). Further candidate genes therefore need to be examined.

CARD14 encodes a keratinocyte scaffold protein that mediates NF-kB signaling downstream of TRAF2 and TRAF6. Activating CARD14 mutations have been documented in a variety of inflammatory skin disorders, including familial psoriasis, erythrodermic psoriasis, generalized pustular psoriasis, pityriasis rubra pilaris, and CARD14-associated papulosquamous eruption (Berki et al., 2015; Fuchs-Telem et al., 2012; Jordan et al., 2012b; Nieto-Beníto et al., 2020; Signa et al., 2019). More recently, loss-of-function CARD14 alleles have been observed in a small number of patients with severe atopic dermatitis, further extending the spectrum of CARD14-associated diseases (Peled et al., 2019).

In this study, we investigated the possibility that CARD14 variants might also be associated with PPP. We examined 236 unrelated cases of European descent, recruited through United Kingdom dermatology departments participating in the APRICOT clinical trial (approved by the London Dulwich Research Ethics Committee; reference 16/LO/0436 [Cro et al., 2021]) or its sister research study PLUM (approved by the London Bridge Research Ethics Committee; reference 16/LO/2190) (Supplementary Table S1). PPP was diagnosed by dermatologists in line with the consensus criteria set by the European Rare And Severe Psoriasis Expert Network (Navarini et al., 2017). The study was undertaken in accordance with the declaration of Helsinki, and all participants granted their written informed consent.

CARD14 variants were identified by querying whole-exome sequence profiles generated on an Illumina HiSeq2000 instrument (n = 212) or by Sanger sequencing the gene coding region and exon/intron junctions (n = 24). Rare changes (minor allele frequency < 1%) were assessed using three independent algorithms (see Supplementary Materials and Methods), and those that were classified as damaging by at least two predictors were considered potentially pathogenic. This approach identified eight deleterious variants, affecting 12 unrelated individuals (Table 1). Meanwhile, an analysis of 62,222 controls (non-Finnish European dataset) sequenced by the gnomAD consortium identified 1,123 rare alleles that met the same pathogenicity criteria. Fisher’s exact test showed that the CARD14 mutational burden was significantly different in the two groups (2.5 vs. 0.9%; $P = 1.5 \times 10^{-3}$; OR = 2.9, 95% confidence interval = 1.5–5.1), showing an association between rare CARD14 alleles and PPP. Importantly, the frequency of rare and synonymous CARD14 changes was comparable in cases and controls ($P > 0.05$), showing that there were no systematic differences between our patient population and the external control dataset.

We next examined low-frequency CARD14 variants, identifying multiple occurrences of a known p.Arg682Trp substitution (Jordan et al., 2012a) (Table 1). This change was also more common in cases than in controls (2.7 vs. 1.6%; $P = 0.044$; OR = 1.7; 95% confidence interval = 1.0–3.0).

Although our dataset was not powered for subgroup analysis, we found that CARD14 mutations were not restricted to a particular demographic (i.e., females or smokers) and were detectable regardless of plaque psoriasis affection status (Supplementary Table S2). Of note, this argues against the suggestion that PPP presenting with concurrent psoriasis might have a distinct genetic etiology (Murakami and Terui, 2020).

To better understand the significance of our association findings, we compared the location of the rare damaging changes detected in PPP cases with that of known CARD14 mutations. We first carried out a systematic literature review, which identified 61 CARD14 genetic studies (Supplementary Figure S1), reporting a total of 65 rare variants. We then assessed the deleterious potential of each change on the basis of their predicted pathogenicity, recurrence, and segregation (see Supplementary Materials and Methods). This identified 18 variants that were likely to be deleterious (Supplementary Table S3). Strikingly, all damaging missense alleles clustered to two specific gene regions (Supplementary Figure S2).

The gain-of-function mutations described in familial psoriasis, generalized pustular psoriasis, pityriasis rubra pilaris, and CARD14-associated papulosquamous eruption mapped between amino acids 117 and 197, affecting the
CARD14 coiled–coil and the preceding linker region. Conversely, the recurrent loss-of-function allele documented in atopic dermatitis lies within the PDZ domain (residue 593).

Interestingly, the damaging missense changes detected in PPP cases were found in both mutation hot spots. Three variants (p.Arg182Cys, p.Glu197Lys, and p.Ser384Phe) localized to the coiled–coil and three to the PDZ domain (p.Thr591Met, p.Arg597Trp, p.Arg610Cys), with one substitution mapping to the C-terminal linker region (p.Val774Ile) (Supplementary Figure S2).

These data suggest that PPP is associated with both gain- and loss-of-function CARD14 alleles. To further investigate this possibility, we overexpressed mutagenized cDNA constructs harboring representative coiled–coil (p.Arg182Cys, p.Ser384Phe) and PDZ (p.Thr591Met) variants. We found that the p.Arg182Cys and p.Ser384Phe alleles led to the formation of insoluble CARD14 aggregates (Figure 1a) because these promote constitutive NF-κB activation (Berki et al., 2015), the two variants are very likely to have gain-of-function properties. Conversely, we observed that the p.Thr591Met substitution was associated with reduced protein accumulation (Figure 1b), indicating a loss-of-function effect.

Interestingly, the notion that variants with opposing effects can result in the same clinical phenotype is supported by the characterization of CARD14 alleles associated with plaque psoriasis. This identified both gain- and loss-of-function changes, suggesting that

### Table 1. Rare- and Low-Frequency CARD14 Variants Detected in PPP Cases

| Rs Number       | Amino Acid Substitution | Minor Allele Frequency | Pathogenicity Predictions | Occurrences |
|-----------------|-------------------------|------------------------|---------------------------|-------------|
|                 |                         |                        | CADD Score2 | PROVEAN | MutationTaster | Spliceman Consensus |                  |
| rs143747620     | p.Lys78Asn              | 0.0004                 | 25.0         | Neutral | Polymorphism | —                  | Benign           | 1            |
|                 | p.Ile86Met              |                        | 17.7         | Neutral | Polymorphism | —                  | Benign           | 1            |
| rs372403419     | p.Arg182Cys             | 0.00009                 | 22.7         | Neutral | Disease causing | —                  | Deleterious      | 1            |
| rs200790561     | p.Glu197Lys             | 0.0007                  | 27.3         | Deleterious | Disease causing | —                  | Deleterious      | 1            |
| rs375882704     | p.Ala367Thr             | 0.00009                 | 24.0         | Neutral | Polymorphism | —                  | Benign           | 1            |
| rs150536049     | p.Ser378Arg             | 0.002                   | 14.8         | Deleterious | Polymorphism | —                  | Benign           | 1            |
| rs780034490     | p.Ser384Phe             | 0.000009                | 23.3         | Deleterious | Polymorphism | —                  | Deleterious      | 2            |
| rs200102454     | p.Thr591Met             | 0.00008                 | 24.3         | Neutral | Disease causing | —                  | Deleterious      | 1            |
| rs73429414      | p.Arg597Trp             | 0.000007                | 25.8         | Neutral | Disease causing | —                  | Deleterious      | 1            |
| rs371910172     | p.Arg610Cys             | 0.00003                 | 24.7         | Neutral | Disease causing | —                  | Deleterious      | 1            |
| rs13883596      | p.Val774Ile             | 0.0001                  | 16.9         | Neutral | Disease causing | —                  | Deleterious      | 1            |
| rs2289541       | p.Arg883His             | 0.0002                  | 8.1          | Neutral | Polymorphism | —                  | Benign           | 1            |
| rs146678380     | c.2569+4T>C             | 0.003                   | 3.0          | —       | Disease causing | Deleterious       | Deleterious      | 4            |
| rs61751629      | p.Glu422Lys             | 0.033                   | 14.8         | Neutral | Polymorphism | —                  | Benign           | 25           |
| rs117918077     | p.Arg882Trp             | 0.016                   | 35.0         | Deleterious | Disease causing | —                  | Deleterious      | 13           |

Low-frequency variants are reported in the two bottom rows. We reported the p.Arg182Cys and p.Thr591Met deleterious alleles in a previous study (Twelves et al., 2019).

Abbreviations: CADD, Combined Annotation Dependent Depletion; PPP, palmoplantar pustulosis.

1Frequency among non-Finnish Europeans, gnomAD 2.1.1.

2Variants with CADD scores > 15 are considered deleterious.

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**Figure 1.** Western blot analysis of FLAG–CARD14 constructs cotransfected into HEK293 cells, alongside control FLAG–TPP1 plasmids. (a) Increased formation of insoluble aggregates is observed for p.Asp176His (positive control), p.Arg182Cys, and p.Ser384Phe proteins; (b) whereas reduced accumulation of mutant p.Thr591Met proteins is apparent in the soluble fraction. Representative images are shown on the left, with average densitometry readings (n ≥ 5 independent experiments) plotted on the right. *P < 0.05, **P < 10⁻², and ***P < 10⁻³ with (a) ANOVA with Dunnett’s posthoc test or (b) t-test. HEK293, human embryonic kidney 293; WT, wild-type.
CARD14 activity levels need to be finely balanced to maintain skin immune homeostasis (Jordan et al., 2012a). Importantly, this implies that CARD14 might be a problematic therapeutic target.

Although CARD14 has been previously investigated in PPP, earlier studies were mostly restricted to the proximal coiled-coil domain and had in retrospect limited the potential to detect disease alleles (Berki et al., 2015; Mössner et al., 2017). Of note, evidence gathered in other inflammatory conditions (e.g., pityriasis rubra pilaris and CARD14-associated papulosquamous eruption) indicates that IL-12p40 blockade (ustekinumab) may be effective in individuals with CARD14 mutations (Eytan et al., 2014; Nieto-Benito et al., 2020; Signa et al., 2019). In this context, our work suggests that whole-gene mutational screens could identify patients with CARD14 disease alleles who may benefit from personalized ustekinumab treatment.

Data availability statement
All the patient allele frequency data are reported in the text and table of this manuscript. Control allele frequency data were retrieved from the gnomAD database.

CONFLICT OF INTEREST
FC has received research funding from Boehringer-Ingelheim. JNB has received research grants and/or consultancy fees from Boehringer-Ingelheim and Anaply Bios. CTW, PB, and SV are Boehringer-Ingelheim employees. HLC has received honoraria for participating in advisory boards or sponsorship to attend conferences from AbbVie, Almirall, Janssen, Leo Pharma, Lilly, Novartis, Sanoﬁ, and UCB. RBW has received research grants and/or consultancy fees from AbbVie, Almirall, Amgen, Arena, Astellas, Avilion, Biogen, Boehringer Ingelheim, Bristol Myers Squibb, Celgene, DICE, GSK, Janssen, Lilly, Leo, Medac, Novartis, Pfizer, Sanoﬁ, Sun Pharma, UCB, and UNION therapeutics. SW has received non-inancial support (sponsorship to attend conferences from AbbVie, Almirall, Janssen, Novartis, Sanoﬁ, and UCB). ADB reports honoraria for consultancy, research, and lecturing from Boehringer Ingelheim and Novartis. AEP has acted as an investigator, speaker, or advisor or received educational grants from Pfizer, AbbVie, Leo, Sanoﬁ, Galderna, Lilly, Novartis, Janssen, Amgen, Celgene, Almirall, La Roche Posay, UCB, Bris tol Myers Squibb, and Boehringer-Ingelheim. NJR reports ongoing Novartis Grant (Signature) income to Newcastle University. NJR reports consultancy/invited lectures for Boehringer Ingelheim (2022), Janssen Cilag (2022), and AbbVie (2021); and the European Society for Dermatological Research 2019 Celgene Sponsored Symposium, with income to Newcastle University (no personal income). CHS is a consultant to Novartis. CTW has received research funding from Biotherm, Sanofi, and the Medical Research Council, and NIHR partnership (grant Efficacy and Mechanism Evaluation 13/50/17). This work was supported by the European Academy of Dermatology and Venereology (grant PPRC-2018-25) and the Psoriasis Association (grants BSTOP505, ST1/17, and ST3/20). NBO was funded by an NIHR pre-doctoral fellowship (NIHR30047), and SH was funded by an Isaac Schaper Research Trust award. CEMG is funded in part by the NIHR Manchester Biomedical Research Centre and is an NIHR Emeritus Senior Investigator. NJR is an NIHR Senior Investigator. RBW is supported by the Manchester NIHR Biomedical Research Centre.

AUTHOR CONTRIBUTIONS
Conceptualization: FC; Formal Analysis: AN, AHC, CBD, SH, NBO; Funding Acquisition: FC, CHS, PB, SV; Resources: ADB, HLC, CEMG, RP, AEP; NJR, MS, SW, RBW, AW, INB, CHS; Investigation: AN, NKH; Supervision: FC; Writing – Original Draft Preparation: FC

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2022.07.031.

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SUPPLEMENTARY NOTES
Membership of the APRICOT and PLUM study team
The members of the APRICOT and PLUM include the following: Thamir Abraham (Peterborough City Hospital, Peterborough, United Kingdom), Muhmad Ali (Worthing Hospital, Worthing, United Kingdom), Suzannah August (Poole Hospital NHS Foundation Trust, Poole, United Kingdom), David Baudry (Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom), Gabrielle Becher (NHS Greater Glasgow and Clyde, Glasgow, United Kingdom), Anthony Bewley (Whipps Cross Hospital, London, United Kingdom), Victoria Cornelius (Imperial College London, London, United Kingdom), Giles Dunnill (University Hospitals Bristol NHS Foundation Trust, Bristol, United Kingdom), Adam Ferguson (Royal Derby Hospital, Derby, United Kingdom), Sharizan Ghaffar (University Hospitals of North Midlands, Derby, United Kingdom), John Ingram (University Hospital of Wales, Cardiff, United Kingdom), Svetlana Kavakleiva (Royal Lancaster Infirmary, Lancaster, United Kingdom), Susan Kelly (The Royal Shrewsbury Hospital, United Kingdom), John Lachmann (Royal Free Hospital, London, United Kingdom), Effie Ladoyanni (Rutgers Hall Hospital, Dudley, United Kingdom), Helen McAteer (The Psoriasis Association, Northampton, United Kingdom), John McKenna (Leicester Royal Infirmary, Leicester, United Kingdom), Freya Meynell (Guy’s and St Thomas’ NHS Foundation Trust, London, United Kingdom), Prakash Patel (Guy’s and St Thomas’ NHS Foundation Trust), Trixie Patel (Guy’s and St Thomas’ NHS Foundation Trust), Alan Pusparajah (Guy’s and St Thomas’ NHS Foundation Trust), Catriona Sinclair (Mid and South Essex NHS Foundation Trust, Southend-on-Sea, United Kingdom), Rachel Wachsmuth (Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom), Rosemary Wilson (Guy’s and St Thomas’ NHS Foundation Trust).

SUPPLEMENTARY MATERIALS AND METHODS
Whole-exome sequencing and Sanger sequencing
A total of 95 affected individuals were whole-exome sequenced as part of a previous study (Vergnano et al., 2020). The same reagents and computational pipeline were then used to generate variant profiles for a further 117 patients. Briefly, libraries were prepared with Agilent SureSelect Human All Exome kit and run on an Illumina HiSeq instrument (Illumina Inc, San Diego, CA). Reads were aligned to the hg19 genome using Novalo (Novocraft Technologies, Petaling Jaya, Malaysia), and variants were called with SAMtools (Li et al., 2009) and annotated with ANNOVAR (Wang et al., 2010). A total of 24 additional palmoplantar pustulosis cases were screened by Sanger sequencing using the primers in Supplementary Table S4.

Pathogenicity predictions
The rare CARD14 alleles detected in palmoplantar pustulosis cases and gnomAD controls were analyzed using the same approach. Briefly, the impact of missense variants was assessed with Combined Annotation Dependent Depletion (CADD) (Rentzsch et al., 2019), MutationTaster (Schwarz et al., 2010), and PROVEAN (Choi and Chan, 2015). Given that the latter program can only be used for the analysis of coding changes, splicing variants were assessed using CADD, MutationTaster, and Spliceman (Lim and Fairbrother, 2012). Missense and splice site variants that were classified as damaging by at least two predictors were considered potentially pathogenic. Frameshift and nonsense variants were automatically considered potentially pathogenic.

Systematic literature review
A systematic literature review was performed by interrogating the PubMed database with the terms (CARD14 or CARMA2) and (variants or mutations or GWAS or genome-wide linkage). The cut-off date was October 31, 2021. Duplicate articles, conference abstracts, reviews, irrelevant studies, and papers that were not written in English were removed. The rare variants (minor allele frequency < 1%) described in the remaining studies were classified as pathogenic if they were predicted to be damaging by at least two algorithms and met one of the following criteria: described in at least two case reports, segregating with inflammatory skin disease in pedigrees, inherited de novo.

In vitro mutagenesis
Mutant constructs were generated using the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA), and primers were designed using Agilent’s QuikChange Primer Design tool (https://www.agilent.com/store/primerDesignProgram.jsp) (Supplementary Table S5). All constructs were validated by sequencing the entire CARD14 coding region, pCMV promoter, bovine growth hormone polyadenylation site, and the FLAG sequence.

Cell culture
Human embryonic kidney 293 cells were cultured in DMEM supplemented with 2 mM L-Glutamine, 50 U/ml of penicillin, and 50 μg/ml of streptomycin (all from Life Technologies, Carlsbad, CA) and 10% fetal calf serum (LabTech, Heathfield, United Kingdom). Lipofectamine 2000 (Life Technologies) was used to cotransfect cells with the FLAG–CARD14 constructs and FLG–TPP1 (kindly provided by Tracey Mitchell, King's College London, London, United Kingdom). Pellets were harvested after 48 hours.

Western blotting
Cell pellets were incubated with non-denaturing lysis buffer (50 mM Tris-hydrogen chloride [pH 7.4], 50 mM sodium chloride, 10% glycerol, 5 mM EDTA, 1% NP-40) and then centrifuged. The supernatant containing the soluble protein fraction was frozen, and the pellet with the insoluble proteins was resuspended in denaturing cell extraction buffer (Thermo Fisher Scientific, Waltham, MA). The two fractions were analyzed by western blotting using a mouse anti-FLAG antibody (Sigma-Aldrich, St. Louis, MO) at 1:3,000 dilution. Autoradiography films were analyzed with ImageJ (National Institutes of Health, Bethesda, MD).
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(Schneider et al., 2012) to measure FLAG–CARD14/FLAG–TPP1 ratios.

**Statistics**
Counts of rare and damaging CARD14 alleles were compared in cases vs. controls using Fisher’s exact test. Densitometry results were analyzed with a t-test or one-way ANOVA followed by Dunnett’s post-test, as appropriate. P < 0.05 was deemed statistically significant.

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Supplementary Figure S1. Flowchart illustrating the steps taken during the literature review.

Supplementary Figure S2. Distribution of CARD14 missense alleles associated with inflammatory skin disease. The diagram shows the localization of the CARD14 mutations validated through the systematic literature review and the rare variants associated with PPP. The D176H allele is shown in brackets because its frequency exceeds 1% in Asian populations. GUK, guanylate kinase; PPP, palmoplantar pustulosis.
## Supplementary Table S1. Patient Demographics

| Demographic                        | Observations                      |
|-----------------------------------|-----------------------------------|
| Sex (%)                           | 169 females (79.2%); 43 males (20.8%) |
| Median age of onset (IQR)         | 46 (36–55)                        |
| Median PPPASI (IQR)               | 9.6 (3.5–16.2)                    |
| Concurrent plaque psoriasis, n (%)| 67 (31.6%)                        |
| Current/former smokers, n (%)     | 181 (76.4%)                       |

Abbreviations: IQR, interquartile range; PPASI, palmoplantar pustulosis area and severity index.

## Supplementary Table S2. Characteristics of the 12 Individuals Harboring CARD14 Mutations

| Demographic                        | Observations                      |
|-----------------------------------|-----------------------------------|
| Sex (%)                           | 11 females (91.6%); 1 male (8.4%)  |
| Median age of onset (IQR)         | 48 (35.5–56.5)                    |
| Median PPPASI (IQR)               | 10.3 (2.2–18)                     |
| Concurrent plaque psoriasis, n (%)| 4 (33.3%)                         |
| Current/former smokers, n (%)     | 7 (58.3%)                         |

Abbreviations: IQR, interquartile range; PPASI, palmoplantar pustulosis area and severity index.

## Supplementary Table S3. Damaging CARD14 Alleles Associated with Inflammatory Skin Disease

| Change   | Reference(s)                                                                 |
|----------|-----------------------------------------------------------------------------|
| p.Gly117Ser | Ammar et al., 2016; Craiglow et al., 2018; Eskin-Schwartz et al., 2016;   |
|          | Jordan et al., 2012; Mössner et al., 2017; Takeichi et al., 2017b         |
| c.349+1G>A   | Fuchs-Telem et al., 2012; Takeichi et al., 2017a                            |
| c.349+5G>A   | Jordan et al., 2012                                                        |
| p.Met119Arg  | Craiglow et al., 2018; Lwin et al., 2018                                   |
| p.Met119Thr  | Craiglow et al., 2018; Frare et al., 2021                                  |
| p.Leu124Pro  | Craiglow et al., 2018; Eytan et al., 2014; Spoerri et al., 2018           |
| p.Cys127Ser  | Craiglow et al., 2018; Takeichi et al., 2017b                             |
| p.Gln136Leu  | Takeichi et al., 2017b                                                     |
| p.Glu138Lys  | Has et al., 2016                                                           |
| p.Glu138Ala  | Jordan et al., 2012                                                        |
| p.Glu138del  | Fuchs-Telem et al., 2012                                                   |
| p.Leu149Arg  | Signa et al., 2019                                                         |
| p.Cys153Ser  | Chiramel et al., 2020                                                      |
| p.Leu156Pro  | Fuchs-Telem et al., 2012                                                   |
| p.Asp176His  | Berki et al., 2015; Mössner et al., 2017; Sugira et al., 2014; Takeichi et al., 2017b |
| p.Glu197Lys  | Ammar et al., 2016                                                         |
| p.Ile593Thr  | Peled et al., 2019                                                         |
| p.Ser602Leu  | Ammar et al., 2016                                                         |
### Supplementary Table S4.  *CARD14* Sequencing Primers

| Target | Primer Name               | Sequence (5′–3′) | Annealing (°C) |
|--------|---------------------------|------------------|----------------|
| Exon 4 | CARD14_Exon4F             | ATGGCCACTGGAATGCTTC | 63             |
|        | CARD14_Exon4R             | CAGGAGGAGAGAGACCCC |               |
| Exon 5 | CARD14_Exon5F             | ACGGACGGAGAGAGAGAAA | 64             |
|        | CARD14_Exon5R             | AAGGGGGAGAGAGCATTAC |               |
| Exon 6 | CARD14_Exon6F             | TGCTCACTCTGCTCACCTAC | 66             |
|        | CARD14_Exon6R             | AAGGAGGAGAGAGATGGA |               |
| Exon 7 | CARD14_Exon7F             | TCTTCTCTGAGGCGGAGAAG | 63             |
|        | CARD14_Exon7R             | AAGAAGGAGAGAGACCATC |               |
| Exon 8 | CARD14_Exon8F             | AAGACTGAGCTCGCTAGGCTG | 63             |
|        | CARD14_Exon8R             | AATTAGTGAGCTCGCAGGG |               |
| Exon 9 | CARD14_Exon9F             | AGAAGCTTCTTCCTCCTCC | 66             |
|        | CARD14_Exon9R             | GTGAGAGAGAGAGAGAGAG |               |
| Exon 10| CARD14_Exon10F            | CACTGACATGTAAGACAGA | 63             |
|        | CARD14_Exon10R            | TCGGTCACTAGTGAAGAC |               |
| Exon 11| CARD14_Exon11F            | CGGAAGGAGAGAGAGAGAGAG | 63             |
|        | CARD14_Exon11R            | CGTACGACGCTACTTCTCC |               |
| Exons 12 + 13 | CARD14_Exon12_13F | TGCTTCTCTTCTTCTCCTC | 66             |
|        | CARD14_Exon12_13R | TATCTTCTCTTCTCCTCCTCCT |               |
| Exons 14 + 15 | CARD14_Exon14_15F | AGATCTGAGGAGGAGGCCCTT | 66             |
|        | CARD14_Exon14_15R | TGAAGTCTGACTCTGCTCCCTCCT |               |
| Exon 16 + 17 | CARD14_Exon16_17F | TCGAAGGAGCTGTCTTCTC | 63             |
|        | CARD14_Exon16_17R | CGTACGACGCTACTTCTCC |               |
| Exon 18 | CARD14_Exon18F            | AAAGCTTCTGAGGAGACTGCG | 63             |
|        | CARD14_Exon18R            | TTTGAAAGGGGTGAGGAGAGAG |               |
| Exon 19 | CARD14_Exon19F            | ACACACACACTTCTTCTCGT | 63             |
|        | CARD14_Exon19R            | CCCAGCCCATGATCTTCTCCT |               |
| Exon 20 + 21 | CARD14_Exon20_21F | TGAATTCTTCTGCTCCTGCTCCTG | 64             |
|        | CARD14_Exon20_21R | GATGAGGTGTGACCTACTCAGCT |               |
| Exon 22 | CARD14_Exon22F            | AAATCTACTACCTAGCTTCTC | 63             |
|        | CARD14_Exon22R            | TCCAGGAAGGGGAGGAGGAGG |               |
| Exon 23 | CARD14_Exon23F            | TGCTCAGCTACTACCTAGCT | 63             |
|        | CARD14_Exon23R            | GCCTCAGTGAGGAGAGAGAGAG |               |

### Supplementary Table S5.  Mutagenesis Primers

| Variant  | Primers (5′–3′) |
|----------|-----------------|
| p.Arg182Cys  | CACAGCCGCATGAAAGTGAGGTTAGGCAC |
|           | GTGCCGCaACACTTCACTCAGTCGAGGAGG | 64 |
| p.Ser384Phe | CTGCCGAGAGGGTCCTTCTCCACACAGG |
|           | CCTGAGGAGAGGACTTCCCTCCCGAG | |
| p.Thr591Met | GAAGATGCCCCATAGGTTCCTGCGAGGAAG |
|           | TATGAGGAGAGGACTTCCCTCCCGAG | |

The p.Asp176His mutagenesis primers were described by Berki et al. (2015).