CORRESPONDENCE

Mutant *CARD10* in a family with progressive immunodeficiency and autoimmunity

Dan-hui Yang[1,2,3], Ting Guo[1,2,3], Zhuang-zhuang Yuan[4], Cheng Lei[1,2,3], Shui-zi Ding[1,2,3], Yi-feng Yang[5], Zhi-ping Tan[5] and Hong Luo[1,2,3]

Cellular & Molecular Immunology (2020) 17:782–784; https://doi.org/10.1038/s41423-020-0423-x

Autoimmunity and immunodeficiency were previously considered to be mutually exclusive conditions. However, an increased understanding of the complex immune regulatory systems and signaling mechanisms, coupled with the application of genetic analysis, has demonstrated the complex relationships between the two kinds of diseases.[1] In recent years, several mild forms of primary immunodeficiencies have been discovered, presenting with opportunistic infections overlapping autoimmunity and/or allergy late in life.[1]

Caspase recruitment domain (CARD)-containing proteins, CARD9, CARD10 (CARMA3), CARD11 (CARMA1), and CARD14 (CARMA2), are members of the membrane-associated guanylate kinase family. These proteins function as molecular scaffolds by forming an intracellular complex with B-cell lymphoma protein 10 (BCL10) and mucosa-associated lymphoid tissue lymphoma translocation gene 1 (MALT1), which are critical for the activation of several signaling pathways, such as the nuclear factor binding near the κ light chain gene in B cells (NF-κB), c-Jun N-terminal kinase, and mammalian target of rapamycin pathways, during adaptive immunity.[2,3] In addition, dominant or recessive mutations in the *CARD9*, *CARD11*, and *CARD14* genes have been identified as genetic causes of immunodeficiencies in the presence/absence of autoimmune diseases and tumors in patients and families.[4–6]

As a ubiquitously expressed protein, CARD10 activates the NF-κB pathway following G-protein-coupled receptor- and epidermal growth factor receptor-induced signaling.[2] De Diego et al. hypothesized that *CARD10* mutations could be associated with inflammatory colitis in humans.[7] However, the consequences of *CARD10* mutations in humans have remained elusive.

In this study, we reported two siblings of a consanguineous family (Fig. 1a) affected by immunodeficiency with autoimmunity. The two patients suffered from recurrent infections, asthma, autoimmune anemia, and Crohn's disease (CD) (Fig. 1b, c). We used exome sequencing to determine the causative genes in the patients. A novel homozygous missense mutation (c.1258C>T; p.R420C) in exon 7 of *CARD10* (Fig. 1d), predicted to be a disease-causing variant by means of different prediction methods, was identified. This variant (R420C) is located in the coiled-coil domain of CARD10, a key and highly conserved domain (Fig. 1e), and might affect the hydrophobicity and stability of the alpha helix (Fig. 1f). Sanger sequencing analysis further verified the mutation (Fig. 1g). Reconstitution studies demonstrated decreased expression of *CARD10* mRNA and CARD10 protein in the patient with the R420C mutation (Fig. S1).

Our study suggests that the R420C mutation is associated with recurrent infections, CD, allergic diseases, and other disorders in patients. We found that both affected siblings suffered from asthma, while their blood eosinophils were low. This phenomenon is consistent with the features seen in *Card10*-deficient mice. In the *Card10*$^{-/-}$ mouse asthma model, airway eosinophils are decreased but airway hyperresponsiveness is not decreased compared with the respective levels in WT mice.[8] In the affected family member compared with the sibling, we observed that immune cells and cytokine production were decreased (Tables S1 and S2), while autoimmune antibodies and chemokine levels were increased (Fig. 1h and Table S1), implying that *CARD10* mutations might lead to immunodeficiency and autoimmunity as a causative genetic factor. Given the high expression of CARD10 in epithelial cells of the gastrointestinal tract and the involvement of the CARD10-BCL10-MALT1 complex in TLR4 signaling, CARD10 has been suggested to be associated with inflammation and colitis.[9,10] Similarly, inflammatory bowel disease was hypothesized to be linked with *CARD10* defects in humans.[7] *CARD10* gene expression was significantly lower in CD patients than in ulcerative colitis patients and healthy controls.[11] Consistently, the pathological examination of the gastrointestinal biopsy sample of the proband's sister revealed early signs of CD (Fig. 1c).

Monocytes are typical leukocytes in the innate immune response that can sense pathogen-related molecules and cytokines. Human monocytes are classified into three categories: classical ($CD14^{high}CD16^{-}$), intermediate ($CD14^{high}CD16^{+}$), and nonclassical ($CD14^{+}CD16^{high}$); intermediate monocytes play an important role in promoting inflammation, while nonclassical monocytes are associated with antiviral responses. In our study, flow cytometry analysis showed that the numbers of $CD14^{high}CD16^{+}$, $CD14^{+}CD16^{high}$, and monocyte-derived $HLADR^{+}CD11c^{+}CD16^{+}$ cells were all reduced in the patient compared with the respective numbers in the sibling. In mice, dendritic cell maturation and Ag presentation have been shown to be impaired in *Card10*-deficient airway epithelial cells.[8] In the affected patient, a cytokine assay showed that IL-8, GROα, MCP-1, MIP-1α, SDF1α, and other chemokine family members were significantly elevated, which was consistent with the increased

[1]Department of Respiratory Medicine, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China; [2]Research Unit of Respiratory Disease, Central South University, Changsha, Hunan 410011, China; [3]Hunan Diagnosis and Treatment Center of Respiratory Disease, Changsha, Hunan 410011, China; [4]School of Life Sciences, Central South University, Changsha, Hunan 410013, China and [5]Clinical Center for Gene Diagnosis and Therapy, Department of Cardiovascular Surgery, The Second Xiangya Hospital of Central South University, Changsha, Hunan 410011, China
Correspondence: Zhi-ping Tan (zhipingtan@csu.edu.cn) or Hong Luo (luohonghuxi@csu.edu.cn)
These authors contributed equally: Dan-hui Yang, Ting Guo

Received: 15 March 2020 Accepted: 17 March 2020
Published online: 1 April 2020

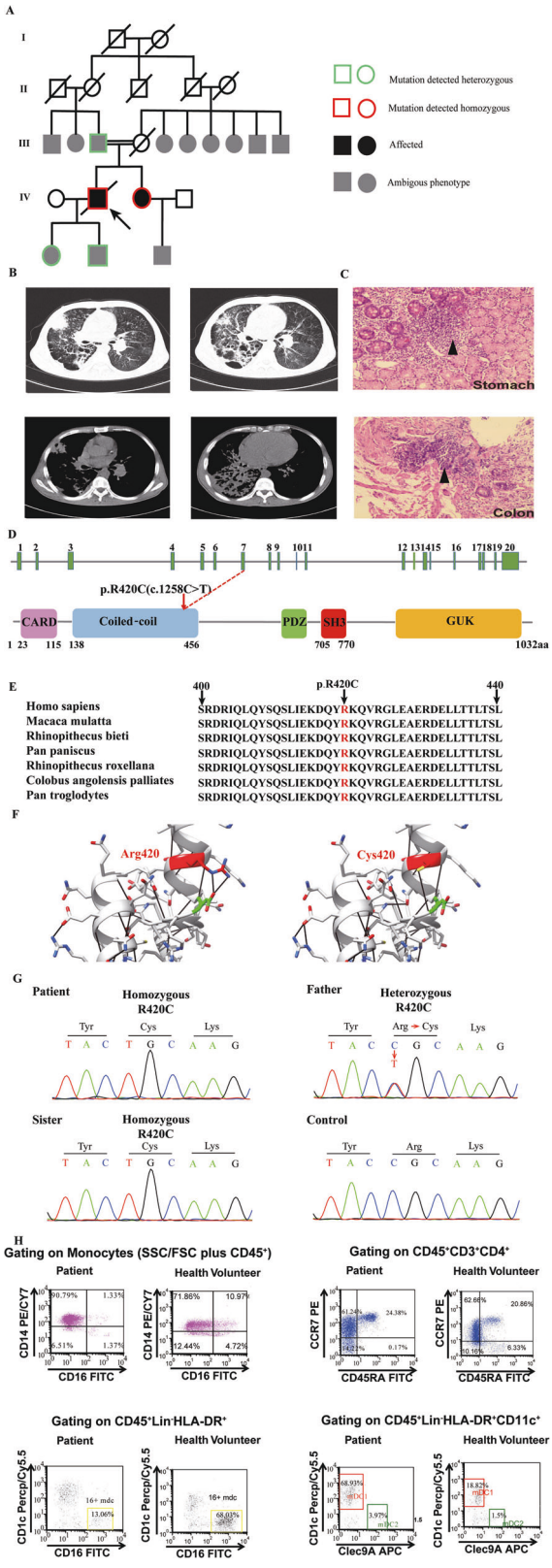

Fig. 1 Genetic analysis, mutation characterization, clinical images, and pathological and immunophenotypic findings in the patients. **a** Pedigree of the *CARD10*-deficient family. The pedigree shows five generations of the family. Roman numerals refer to generations. Circles refer to female subjects. Squares refer to male subjects. Solid symbols refer to affected subjects. Crossed-out symbols refer to deceased subjects. The arrow indicates the proband. **b** High-resolution computed tomography of the proband at the age of 42 revealed bronchiectasis with infection, lung abscess, and pulmonary bulla. **c** H&E micrograph (×100 magnification) of the gastrointestinal mucosal biopsy sample of the proband's sister at the age of 42 showing local proliferative gastritis and colitis. **d** Exon structure of the *CARD10* gene. Domain structure of the CARD10 protein; aa amino acid number. **e** Sequence alignment of mammalian CARD10 proteins. R420, marked by the red box, is located in a highly conserved amino acid region (from UniProt). **f** The CARD10 protein tertiary structure was predicted by Swiss-model software based on the crystal structure of a coiled-coil segment of Pyrococcus yayanosii Smc (5xg2.1.pdb). The amino acid substitution from arginine to cysteine at position 420 likely affects intramolecular hydrogen bond formation, which is predicted to affect the hydrophobicity and stability of the alpha helix. **g** Sanger sequencing analysis of the *CARD10* gene in the patients, their family members, and the controls. **h** Analysis of CD14 and CD16 expression on monocytes, CD45RA and CCR7 expression on CD3+CD4+ cells, CD1C and CD16 expression on DCs and CD1C and Clec9A expression on CD11c+DCs

expression of chemokines in autoimmune diseases.[12] Other inflammatory factors, such as IL6, TNFα, IFNα, IL-1α, TNF-β, IL-21, IL-22, IL-23, and IL-27, generally remain normal or are decreased, which may reflect either a reduction in the quantity or functional deficiency of immune cells, such as monocytes or DC cells after mutation of *CARD10*. The airway epithelial cells of *Card10*-deficient mice also present a decrease in type 2 cytokine levels.[8] The two siblings showed varying degrees of immunodeficiency, for which the underlying mechanism remains to be clarified.

Our findings further imply that environmental factors might play an important role in the diversity of phenotypes.[13] In this consanguineous family, the two siblings with the same homozygous disease-causing mutations manifested different clinical phenotypes. The elder brother, who lived an unhealthy lifestyle and was exposed to metal dust, suffered immunodeficiency and autoimmune disease much earlier than his sister. The younger sister, who lived a healthy lifestyle, had an apparently milder clinical phenotype, presenting with unexplained small-cell hypochromic anemia, gastrointestinal discomfort, and seasonal urticaria. Similar to other known genes that cause PIDs, *CARD10* mutations were proposed to lead to the development of rare diseases that predominantly present with autoimmune or allergic symptoms, and the diseases appear to progress slowly for 20–30 years.[1] Such genetic defects also predispose individuals to recurrent infections and can cause primary immunodeficiencies.[1] The symptoms in our patients were consistent with these predictions.

In conclusion, by applying genetics and immunology approaches, we identified a *CARD10* mutation as the possible cause of a novel form of autosomal recessive genetic disease characterized by primary immunodeficiency accompanied by autoimmune disease. Our study also links the *CARD10* mutation with CD. Therefore, genetic mutations affecting *CARD10* and *CARD10*-related genes could be collectively considered when attempting to understand these types of diseases.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

ACKNOWLEDGEMENTS

We thank all the patients and their family and the healthy volunteers for participating in the present study. We thank Long Ma, Wei-nian Shou, and Wen-jun Zhang for critical reading of the paper. This study was supported by the National Natural

Science Foundation of China (81770002 to HL, 81470445 to Z-p.T., 81900002 to T.G., and 81570288 to Y-f.Y.), the Planned Science and Technology Project of Hunan Province, China (2015JC3032 to Y-f.Y.), the Science and Technology Program of Changsha, China (kq1901120 to H.L.), and the National Key Clinical Specialty Construction Projects of China.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: H.L. and Z-p.T. Performed the experiments: D-h.Y., T.G., and Z-z.Y., Collected samples: D-h.Y., T.G., and S-z.D. Analyzed the data: Z-p.T., H.L., D-h.Y., T.G., and Y-f.Y. Wrote the text of the main paper: D-h.Y., Z-p.T., and H. L. Prepared the figures: D-h.Y. and L.C. All authors reviewed and revised the paper.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41423-020-0423-x) contains supplementary material.

Competing interests: The authors declare no competing interests.

Ethical approval: The present study was approved by the Ethics Committee of the Second Xiangya Hospital, Central South University (Changsha, China).

Informed consent: Written informed consent was obtained from the patient and his parents for publication.

REFERENCES

1. Schmidt, R. E., Grimbacher, B. & Witte, T. Autoimmunity and primary immunodeficiency: two sides of the same coin? *Nat. Rev. Rheumatol.* **14**, 7–18 (2017).
2. Blonska, M. & Lin, X. NF-kappaB signaling pathways regulated by CARMA family of scaffold proteins. *Cell Res.* **21**, 55–70 (2011).
3. Zhang, Q., Lenardo, M. J. & Baltimore, D. 30 Years of NF-kappaB: a blossoming of relevance to human pathobiology. *Cell* **168**, 37–57 (2017).
4. Lanternier, F. et al. Deep dermatophytosis and inherited CARD9 deficiency. *N. Engl. J. Med.* **369**, 1704–1714 (2013).
5. Greil, J. et al. Whole-exome sequencing links caspase recruitment domain 11 (CARD11) inactivation to severe combined immunodeficiency. *J. Allergy Clin. Immunol.* **131**, 1376–1383.e1373 (2013).
6. Jordan, C. T. et al. Rare and common variants in CARD14, encoding an epidermal regulator of NF-kappaB, in psoriasis. *Am. J. Hum. Genet.* **90**, 796–808 (2012).
7. Perez de Diego, R. et al. Genetic errors of the human caspase recruitment domain-B-cell lymphoma 10-mucosa-associated lymphoid tissue lymphoma-translocation gene 1 (CBM) complex: molecular, immunologic, and clinical heterogeneity. *J. Allergy Clin. Immunol.* **136**, 1139–1149 (2015).
8. Causton, B. et al. CARMA3 is critical for the initiation of allergic airway inflammation. *J. Immunol.* **195**, 683–694 (2015).
9. Bhattacharyya, S. et al. Carrageenan-induced colonic inflammation is reduced in Bcl10 null mice and increased in IL-10-deficient mice. *Mediat. Inflamm.* **2013**, 397642 (2013).
10. Bhattacharyya, S. et al. Toll-like receptor 4 mediates induction of the Bcl10-NFkappaB-interleukin-8 inflammatory pathway by carrageenan in human intestinal epithelial cells. *J. Biol. Chem.* **283**, 10550–10558 (2008).
11. Yamamoto-Furusho, J. K. et al. Caspase recruitment domain (CARD) family (CARD9, CARD10, CARD11, CARD14 and CARD15) are increased during active inflammation in patients with inflammatory bowel disease. *J. Inflamm.* **15**, 13 (2018).
12. Chen, K. et al. Chemokines in homeostasis and diseases. *Cell. Mol. Immunol.* **15**, 324–334 (2018).
13. Picard, C. et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for primary immunodeficiency 2015. *J. Clin. Immunol.* **35**, 696–726 (2015).