

A novel, heterozygous three base-pair deletion in CARD11 results in B cell expansion with NF- κ B and T cell anergy disease

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1 **A novel, heterozygous three base-pair deletion in CARD11 results in B cell expansion with NF- κ B**
2 **and T cell anergy disease.**

3

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19

20 **Abstract**

21

22 Germline gain-of-function mutations in *CARD11* lead to the primary immunodeficiency, B cell
23 expansion with NF- κ B and T cell anergy (BENTA). Herein, we report the case of a girl, presenting at
24 2 years of age with lymphocytosis and splenomegaly in whom a novel, in-frame, three base pair
25 deletion in *CARD11* was identified resulting in the deletion of a single lysine residue (K215del) from
26 the coiled-coil domain. *In vitro* functional assays demonstrated that this variant leads to a subtle
27 increase in baseline NF- κ B signaling and impaired proliferative responses following T cell receptor
28 and mitogenic stimulation. Previously reported immunological defects associated with BENTA
29 appear mild in our patient who is now 6 years of age; a B cell lymphocytosis and susceptibility to
30 upper respiratory tract infections persist, however, she has broad, sustained responses to protein-
31 polysaccharide conjugate vaccines and displays normal proliferative responses to *ex vivo* T-cell
32 stimulation.

33

34 **Case Presentation**

35

36 A 22 month old girl was referred for assessment of a persistently elevated lymphocyte count
37 identified during the investigation of coryzal symptoms and intermittent diarrhoea. Her symptoms
38 had persisted for over 2 months and culminated in a short hospital admission where she was found
39 to have low-grade pyrexia of 37.8°C, splenomegaly and cervical lymphadenopathy. Total white cell
40 count was elevated at 31.5×10^9 cells/L (neutrophils 3.1×10^9 /L, lymphocytes 26.6×10^9 /L,
41 monocytes 1.6×10^9 /L) but C-reactive protein was < 2 mg/L; blood and stool cultures were
42 negative. Symptoms improved following an empirical three day course of azithromycin, however,
43 lymphadenopathy, lymphocytosis and splenomegaly persisted (10.6 cm on ultrasound, upper limit
44 of normal for age: 7 cm).

45

46 Past medical history was unremarkable; she had been delivered at term, had no neonatal issues
47 and was growing on the 75th centile for weight. Mild asthma had been treated with a salbutamol
48 inhaler. There was no family history of immunodeficiency or lymphoproliferative disease, however,
49 a maternal great aunt had died of systemic lupus erythematosus. Her parents were not
50 consanguineous.

51

52 A summary of immunological investigations is provided in **Table 1**. The CD19⁺ B cell population was
53 expanded at presentation (7.19×10^9 cells/L, normal range $0.6-3.1 \times 10^9$ cells/L), and although it
54 has declined with age, it has consistently accounted for over 50% of the total lymphocyte
55 population (**Figure 1a and 1b**). Longitudinal B cell immunophenotyping (**Figure 1c and Table 2**) has
56 demonstrated expanded populations of naïve B cells with reduced populations of unswitched and
57 switched memory B cells compared to established, age-adjusted reference ranges (1). The B cell
58 population was polyclonal (**Figure 1d**) and a lymph node excision biopsy, performed at 33 months
59 to exclude lymphoproliferative disease in the context of persistent B cell lymphocytosis, was
60 normal.

61

62 In light of the B cell lymphocytosis, genetic investigations were undertaken to search for putative
63 gain-of-function mutations in Caspase Recruitment Domain family member 11 (*CARD11*) leading to

64 the B cell expansion with NF- κ B and T cell anergy (BENTA) phenotype. Sanger sequencing revealed
65 a heterozygous, in-frame three base pair deletion that causes the deletion of a single lysine residue
66 at position 215 within the coiled-coil domain of CARD11 (NG_027759.1: c.645-647del; p.Lys215del)
67 (**Figure 2b**). This variant was not identified in the patient's mother; evaluation of the patient's
68 father was not possible (**Figure 2a**). *In silico* analysis of the variant was undertaken: the variant has
69 not been previously reported in the ClinVar, ExAC, gnomAD or dbSNP databases confirming it was
70 novel. The Mutation Taster score was 1.00, predicting a high probability that the variant is disease
71 causing. PROVEAN analysis (2), an approach that infers the functional consequences of amino acid
72 substitutions and indels, predicted the variant to be deleterious with a score of -14.046.
73 Phylogenetic analysis shows the K215 residue of CARD11 is highly conserved across taxa from
74 human to zebrafish (**Figure 2c**). Other known variants causing the BENTA phenotype are shown in
75 **Figure 2d**. Interestingly, the K215del variant has been reported in the Catalogue of Somatic
76 Mutations in Cancer (COSMIC) (3) as a haematological malignancy-associated somatic mutation in
77 two individuals: one with primary CNS lymphoma (4) and one with chronic lymphocytic leukaemia
78 with Richter's transformation (5).

79

80 The functional consequences of the K215del *CARD11* variant on NF- κ B signaling were assessed by
81 transfection of *CARD11*-deficient JPM50.6 cells with mutant and wild-type *CARD11* constructs.
82 Compared to wild-type *CARD11*, the K215del variant showed increased baseline NF- κ B signaling,
83 but mildly reduced NF- κ B activation following stimulation with anti-CD3/CD28, and substantially
84 reduced activation following stimulation with PMA and ionomycin (**Figure 3a and 3b**). In contrast,
85 the BENTA-associated mutation E134G, increased NF- κ B activity following anti-CD3/CD28 ligation.
86 All *CARD11* proteins were equally expressed (data not shown). To investigate the effect of the
87 variant in the heterozygous state, K215del *CARD11* was co-transfected in a 1:1 ratio with wild type
88 *CARD11*. Slightly increased baseline NF- κ B signaling was again observed, although it did not reach
89 statistical significance. Moreover, modest attenuation of NF- κ B signaling in response to both
90 mitogenic and TCR stimulation were again observed. These findings are largely consistent with
91 reported abnormalities in BENTA patients, providing evidence of the functional relevance of this
92 variant in our patient.

93

94 The patient, now 6 years old, has been followed up for over 46 months since her original
95 presentation. She continues to suffer from frequent episodes of upper respiratory tract infections

96 and otitis media that respond well to empirical antibiotic therapy; infection frequency was not
97 reduced by prophylactic antibiotic therapy. There has been a modest reduction in the magnitude of
98 her lymphocytosis over time (**Figure 1a**) and malignant lymphoproliferation and autoimmunity
99 have not emerged. She remains Epstein-Barr virus naïve and continues under close surveillance for
100 future complications of BENTA.

101 Discussion

102

103 CARD11 is a scaffold protein, uniquely expressed in the haemopoetic lineage, responsible for
104 coordinating signaling events downstream of the B and T cell receptor. It is essential for the
105 activation of the canonical NF- κ B signaling cascade: following the activation of lymphocyte surface
106 antigen receptors and proximal signaling events, the linker region of CARD11 is phosphorylated by
107 PKC- θ or PKC- β in T cells and B cells, respectively, inducing a conformational change in CARD11 that
108 releases autoinhibition. The subsequent recruitment of BCL10 and MALT1 forms the CBM
109 signalosome complex, and MALT1 recruits TRAF6 to initiate canonical NF- κ B signaling (6). CARD11
110 can also activate the JNK pathway (7) and independently regulates glutamine flux and mTORC1
111 signaling with consequent effects on Th1/Th17 cell differentiation (8).

112

113 Somatically acquired, oncogenic *CARD11* mutations were first identified in tumour samples from
114 individuals with the activated-B-cell subtype of diffuse large B cell lymphoma. The mutations
115 clustered in the CARD11 coiled-coil domain and conferred constitutive activating signals upon the
116 NF- κ B pathway (9). CARD11 was subsequently shown to be a critical checkpoint regulating B cell
117 fate following B cell receptor engagement; lymphoma-associated *CARD11* mutations can drive
118 antigen-dependent B cell expansion, providing evidence of synergy between autoreactive B cell
119 receptors and somatic activating mutations within downstream signaling molecules in
120 lymphomagenesis and autoimmunity (10).

121

122 The primary immunodeficiency BENTA arises due to germline gain-of-function mutations in
123 *CARD11*. Similar to somatically acquired oncogenic mutations, BENTA-associated mutations tend to
124 cluster in the "LATCH" and coiled-coil domains of CARD11 and constitutively activate NF- κ B (11).
125 This leads to a peripheral, polyclonal B cell lymphocytosis, splenomegaly and lymphadenopathy
126 that develops within the first year of life, with the potential to transform into malignant
127 lymphoproliferation (12). The observed B cell lymphocytosis in BENTA is driven by a combination of
128 increased bone marrow output leading to increased transitional B-lymphocytes
129 (CD10⁺CD24^{hi}CD38^{hi}) and enhanced survival in peripheral lymphoid tissue leading to expansion of
130 the naïve B cell compartment. Functionally, naïve B cells from BENTA patients proliferate normally
131 to mitogenic stimuli and show enhanced viability *in vitro*, which contributes to their persistence
132 (12, 13). Despite this, the efficient generation of short-lived plasmablasts and long-lived plasma

133 cells can be impaired in BENTA due to poor induction of key differentiation factors including BLIMP-
134 1 and XBP-1 (14). Consequently, recurrent sinopulmonary bacterial infections and upper
135 respiratory tract infections are common in BENTA, as is a failure to respond to polysaccharide
136 vaccination and, in some patients, protein-conjugate vaccination (12, 15). Our patient's burden of
137 infectious disease remains modest, restricted to the upper respiratory tract, and she has mounted
138 robust and sustained responses to all vaccines administered routinely as part of the UK vaccine
139 schedule including the 13-valent pneumococcal conjugate vaccine. Autoimmunity may also
140 complicate BENTA less frequently; haemolytic anaemias (16) have been noted, but these have not
141 occurred in our patient.

142

143 Constitutive increased CARD11 activity downstream of the T cell receptor (TCR) does not lead to a
144 numerical expansion of T cells, but induces a degree of hyporesponsiveness that can be rescued
145 using very strong stimuli (12). Accordingly, our patient displays normal *ex vivo* proliferative
146 responses to bead-conjugated anti-CD2, anti-CD3 and anti-CD28 stimulation. The T cell defect in
147 BENTA is believed to underlie a susceptibility to complications of EBV infection. Low level chronic
148 EBV viraemia emerges in over 80% of BENTA patients with serological evidence of EBV exposure
149 and is hypothesized to arise secondary to poor TCR induced IL-2 secretion, consequent NK cell
150 dysfunction and an expanded pool of naïve B cells that can maintain lytic infection (13). There may
151 be a wider susceptibility to viral infections in some patients: molluscum and BK virus infection have
152 also been reported (12). To date, our patient remains EBV naïve.

153

154 Herein, we report the case of a 6 year-old girl with a BENTA phenotype driven by the deletion of
155 the lysine 215 residue from the coiled-coil domain of CARD11. We confirm this variant leads to
156 both modest enhancement of baseline NF- κ B signaling and attenuation of T cell responses to
157 mitogenic and TCR stimuli, consistent with a milder phenotype. The relationship between *CARD11*
158 genotypes, the structural biology of mutant CARD11 within the CBM signalosome complex, the
159 potency of the gain-of-function effect and final immunological and clinical phenotype requires
160 further exploration. For example, contrasting clinical outcomes have been reported for patients
161 possessing the C49Y variant ranging from mild disease to fatal haemophagocytic
162 lymphohistiocytosis (16, 17). A distinct mutation in the coiled-coil (p.His234_Lys238delinsLeu)
163 produced a "mixed" clinical BENTA phenotype with atopic disease features, consistent with both

164 gain-of-function and dominant negative signaling effects (18). Longitudinal studies of patients with
165 *CARD11* mutations are necessary to fully inform our understanding of this rare disease.

166

167

168 **Conflict of Interest Statement**

169 The authors declare that the research was conducted in the absence of any commercial or financial
170 relationships that could be construed as a potential conflict of interest

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177 **References**

- 178 1. Piątosza B, Wolska-Kuśnierz B, Pac M, Siewiera K, Gałkowska E, Bernatowska E. B cell subsets in
179 healthy children: Reference values for evaluation of B cell maturation process in peripheral blood.
180 Cytom Part B - Clin Cytom. 2010;78(6 B):372–81.
- 181 2. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the functional effect of amino acid
182 substitutions and indels. PLoS One. 2012;7(10):e46688.
- 183 3. Bamford S, Dawson E, Forbes S, Clements J, Pettett R, Dogan A, Flanagan A, Teague J, Futreal
184 PA, Stratton MR, Wooster R. The COSMIC (Catalogue of Somatic Mutations in Cancer) database
185 and website. Br J Cancer. 2004 Jul 19;91(2):355-8.
- 186 4. Montesinos-Rongen M1, Schmitz R, Brunn A, Gesk S, Richter J, Hong K, Wiestler OD, Siebert R,
187 Küppers R, Deckert M. Mutations of CARD11 but not TNFAIP3 may activate the NF-kappaB
188 pathway in primary CNS lymphoma. Acta Neuropathol. 2010 Oct;120(4):529-35.
- 189 5. Fabbri G, Khiabani H, Holmes AB, Wang J, Messina M, Mullighan CG, Pasqualucci L, Rabadan
190 R, Dalla-Favera R. Genetic lesions associated with chronic lymphocytic leukemia transformation to
191 Richter syndrome. J Exp Med. 2013 Oct 21;210(11):2273-88.
- 192 6. Turvey SE, Durandy A, Fischer A, Fung SY, Geha RS, Gewies A, et al. The CARD11-BCL10-MALT1
193 (CBM) signalosome complex: Stepping into the limelight of human primary immunodeficiency. J
194 Allergy Clin Immunol. 2014;134(2):276–84.
- 195 7. Hara H, Wada T, Bakal C, Koziaradzki I, Suzuki S, Suzuki N, Nghiem M, Griffiths EK, Krawczyk C,
196 Bauer B, D'Acquisto F, Ghosh S, Yeh WC, Baier G, Rottapel R, Penninger JM. The MAGUK family
197 protein CARD11 is essential for lymphocyte activation. Immunity. 2003 Jun;18(6):763-75.
- 198 8. Nakaya M, Xiao Y, Zhou X, Chang JH, Chang M, Cheng X, et al. Inflammatory T cell responses rely
199 on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation.
200 Immunity. 2014;40(5):692–705
- 201 9. Lenz G, Davis RE, Ngo VN, Lam L, George TC, Wright GW, Dave SS, Zhao H, Xu W, Rosenwald A,
202 Ott G, Muller-Hermelink HK, Gascoyne RD, Connors JM, Rimsza LM, Campo E, Jaffe ES, Delabie J,
203 Smeland EB, Fisher RI, Chan WC, Staudt LM. Oncogenic CARD11 mutations in human diffuse large
204 B cell lymphoma. Science. 2008 Mar 21;319(5870):1676-9.
- 205 10. Jeelall YS1, Wang JQ, Law HD, Domaschensz H, Fung HK, Kallies A, Nutt SL, Goodnow CC,
206 Horikawa K. Human lymphoma mutations reveal CARD11 as the switch between self-antigen-
207 induced B cell death or proliferation and autoantibody production. J Exp Med. 2012 Oct
208 22;209(11):1907-17.
- 209 11. Chan W, Schaffer TB, Pomerantz JL. A quantitative signaling screen identifies CARD11
210 mutations in the CARD and LATCH domains that induce Bcl10 ubiquitination and human
211 lymphoma cell survival. Mol Cell Biol. 2013 Jan;33(2):429-43
- 212 12. Snow AL, Xiao W, Stinson JR, Lu W, Chaigne-Delalande B, Zheng L, et al. Congenital B cell
213 lymphocytosis explained by novel germline *CARD11* mutations. J Exp Med. 2012;209(12):2247–61.
- 214 13. Arjunaraja S, Angelus P, Su HC, Snow AL. Impaired control of Epstein-Barr virus infection in B-
215 cell expansion with NF-κB and T-cell anergy disease. Front Immunol. 2018 Feb 8;9:198.

- 216 14. Arjunaraja S, Nosé BD, Sukumar G, Lott NM, Dalgard CL, Snow AL. Intrinsic plasma cell
217 differentiation defects in B cell expansion with NF- κ B and T cell anergy patient B cells. *Front*
218 *Immunol.* 2017 Aug 2;8:913.
- 219 15. Brohl AS, Stinson JR, Su HC, Badgett T, Jennings CD, Sukumar G, et al. Germline CARD11
220 Mutation in a Patient with Severe Congenital B Cell Lymphocytosis. *J Clin Immunol.* 2015;35(1):32–
221 46.
- 222 16. Gupta M, Aluri J, Desai M, Lokeshwar M, Taur P, Lenardo M, et al. Clinical, immunological, and
223 molecular findings in four cases of B cell expansion with NF- κ B and T cell anergy disease for the
224 first time from India. *Front Immunol.* 2018 Jun 14;9:1049
- 225 17. Buchbinder D, Stinson JR, Nugent DJ, Heurtier L, Suarez F, Sukumar G, et al. Mild B-cell
226 lymphocytosis in patients with a CARD11 C49Y mutation. *Journal of Allergy and Clinical*
227 *Immunology.* 2015. p. 819–821.e1
- 228 18. Desjardins M, Arjunaraja S, Stinson JR, Dorjbal B, Sundaresan J, Niemela J, et al. A Unique
229 Heterozygous CARD11 Mutation Combines Pathogenic Features of Both Gain- and Loss-of-
230 Function Patients in a Four-Generation Family. *Front Immunol.* 2018 Dec 12;9:2944.
- 231

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Figure legends

Figure 1 - Immunophenotyping of peripheral blood lymphocytes: (A) Longitudinal analysis of the total peripheral blood lymphocyte count, total B cell count and (B) relative size of the CD19⁺ and CD3⁺ lymphocyte populations from 26 months of age to present. (C) Flow cytometric analysis of PBMC performed at 69 months of age demonstrating an expanded population of naïve B lymphocytes (CD19⁺, IgD⁺, CD27⁻), reduced populations of unswitched (CD19⁺, IgD⁺, CD27⁺) and switched (CD19⁺, IgD⁻, CD27⁺) memory B cells and increased transitional B cells (CD19⁺, IgM^{hi}, CD38^{hi}) compared to established, age-adjusted reference ranges (1). (D) Flow cytometric analysis performed at 26 months of age demonstrating polyclonal B cell kappa/lambda usage and normal CD4/CD8 populations

Figure 2 – Pedigree and sequencing of c.645-647del mutation in CARD11: (A) Pedigree of kindred. (B) Sanger sequencing chromatograms demonstrating c.645-647del in-frame 3 bp deletion in proband. NG_027759.1 is the reference sequence. (C) Protein alignment of CARD11 K215 demonstrating conservation across taxa. The conserved lysine residue is highlighted in red. Numbers represent the position of the lysine residue relative to N-terminus in each species. (D) Schematic representation of CARD11 protein showing locations of known CARD11 mutations resulting in BENTA (blue) and the deletion of lysine 215 found in the proband (red).

Figure 3 – *In vitro* functional validation of the K215del variant: CARD11-deficient Jurkat T cells containing an NF-κB-driven GFP reporter (JPM50.6) were transfected with mutant or wild-type CARD11 (A and B) or mutant and wild-type CARD11 in a 1:1 ratio (C and D). Transfected cells were stimulated with anti-CD3/anti-CD28 or phorbol 12-myristate 13-acetate (PMA) and ionomycin. The E134G variant is a known BENTA-causing variant and serves as a positive control. Representative histograms of NF-κB reporter activity (A and C; numbers denote % GFP⁺ cells) and GFP mean fluorescence intensity (B and D) are shown (n = 3). Statistical significance was determined using a

one-way ANOVA comparing each variant to empty vector/empty vector plus wild-type for a given treatment condition (*denotes $p < 0.05$ after adjustment for multiple comparisons with Dunnett's test).