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Autoimmunity and immunodeficiency associated with monoallelic LIG4 mutations via haploinsufficiency

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Title:**Autoimmunity and immunodeficiency associated with monoallelic *LIG4* mutations *via* haploinsufficiency**

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61

62 **Conflict of Interest Disclosure**

63 The authors declare no competing financial interests.

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67 **Abstract**

68 **Background:** Biallelic mutations in *LIG4* encoding DNA-ligase 4 cause a rare immunodeficiency syndrome
69 manifesting as infant-onset life-threatening and/or opportunistic infections, skeletal malformations,
70 radiosensitivity and neoplasia. *LIG4* is pivotal during DNA repair and during V(D)J recombination as it performs
71 the final DNA-break sealing step.

72 **Objective:** We explored whether monoallelic *LIG4* missense mutations may underlie immunodeficiency and
73 autoimmunity with autosomal dominant inheritance.

74 **Methods:** Extensive flow-cytometric immune-phenotyping was performed. Rare variants of immune system
75 genes were analyzed by whole exome sequencing. DNA repair functionality and T cell-intrinsic DNA damage
76 tolerance was tested with an ensemble of *in vitro* and *in silico* tools. Antigen-receptor diversity and autoimmune
77 features were characterized by high-throughput sequencing and autoantibody arrays. Reconstitution of wild-
78 type vs. mutant *LIG4* were performed in *LIG4* knock-out Jurkat T cells and DNA damage tolerance was
79 subsequently assessed.

80 **Results:** A novel heterozygous *LIG4* loss-of-function mutation (p.R580Q), associated with a dominantly inherited
81 familial immune-dysregulation consisting of autoimmune cytopenias, and in the index patient with
82 lymphoproliferation, agammaglobulinemia and adaptive immune cell infiltration into nonlymphoid organs.
83 Immunophenotyping revealed reduced naïve CD4⁺ T cells and low TCR-V α 7.2⁺ T cells, while T/B-cell receptor
84 repertoires showed only mild alterations. Cohort screening identified two other non-related patients with the
85 monoallelic *LIG4* mutation p.A842D recapitulating clinical and immune-phenotypic dysregulations observed in
86 the index family and displaying T cell-intrinsic DNA damage intolerance. Reconstitution experiments and
87 molecular dynamics simulations categorize both missense mutations as loss-of-function and haploinsufficient.

88 **Conclusion:** We provide evidence that certain monoallelic *LIG4* mutations may cause human immune
89 dysregulation *via* haploinsufficiency.

90

91 **Clinical implications**

92 *LIG4* haploinsufficiency should be considered in patients with immune dysregulation of unidentified cause, as
93 it may have prognostic as well as therapeutic consequences.

94

95 **Capsule Summary**

96 This is the first description of *LIG4* haploinsufficiency-associated combined immunodeficiency in humans.

97

98 **Key words**

99 DNA ligase 4 – DNA damage - autoimmunity – haploinsufficiency – autosomal dominant – inborn errors of
100 immunity – immunodeficiency – primary immunodeficiency

101

102 **Abbreviations**

103 AIHA (autoimmune hemolytic anemia), AIRR-seq (adaptive immune receptor repertoire-sequencing),
104 autoinflamm. (autoinflammation), BCR (B cell receptor), BE (Binding energy), cDNA (copy deoxyribonucleic
105 acid), CDR3 (complementarity-determining region 3), CID (combined immunodeficiency), comp. het.
106 (compound heterozygous), CTV (CellTrace™ violet), DSB (DNA double-strand breaks), HD (healthy donors),
107 homo. (homozygous), IGH (immunoglobulin heavy chain), IGHA (immunoglobulin heavy constant alpha), IGHG
108 (immunoglobulin heavy constant gamma), IgL (immunoglobulin light constant), IR (ionizing radiation), ITP
109 (immune thrombocytopenia), LAG3 (lymphocyte-activation gene-3), *LIG4* (DNA ligase 4), MD (molecular
110 dynamics), mRNA (messenger ribonucleic acid), NHEJ (nonhomologous end-joining), OBD (Oligonucleotide/
111 oligosaccharide-fold domain), OH (hydroxyl), PBMC (peripheral blood mononucleated cells), PAD (primary
112 antibody deficiency), PCR (polymerase chain reaction), PD-1 (programmed cell death-1), PID (primary

113 immunodeficiency), SCID (severe combined immunodeficiency), SHM (somatic hypermutations), TCR (T cell
114 receptor), TCRA (T cell receptor α -chain), TCRB (T cell receptor β -chain), WES (whole exome-sequencing), WT
115 (wild-type).

116

117 Introduction

118 The three mammalian DNA ligases (LIG1, LIG3, LIG4) are pivotal for genomic recombination, replication and
119 repair⁽¹⁾. LIG4 is essential for resolving DNA double-strand breaks (DSB) - the most noxious DNA lesions⁽²⁾. DSB
120 mending engages the ubiquitous non-homologous end-joining (NHEJ) repair pathway, which utilizes LIG4 for
121 the last step of DNA re-ligation⁽²⁾.

122 NHEJ is preferentially used after genotoxic assaults like ionizing radiation (IR) as well as physiologically
123 during V(D)J recombination, a crucial step in the T and B cell receptor generation (TCR respectively BCR)⁽³⁾. V(D)J
124 recombination is mandatory for the development of adaptive immunity, as the variability and consequently,
125 the antigen recognition is ensured by the semi-stochastic recombination of the variable (*V*), diversity (*D*) and
126 joining (*J*) gene segments encoding the variable domains of both T and B cell receptors⁽³⁾. A well-regulated DNA-
127 damage response is therefore imperative for immune homeostasis and to guarantee immunocompetence and
128 immune tolerance.

129 Although the first LIG4 deficient patient was characterized 33 years ago, only 120 patients with either
130 homozygous or compound heterozygous mutated *LIG4* have been published to date (reviewed in **Table I**). LIG4
131 haploinsufficiency caused by monoallelic *LIG4* mutations has not been reported in human patients, whereas
132 murine data suggests that a single functional *LIG4* allele may not be sufficient to protect from malignancy and
133 may reduce survival⁽⁴⁻⁶⁾. Here we identified two novel monoallelic *LIG4* missense variants associated with
134 impaired tolerance to DNA damage in primary T cells and combined immunodeficiency, in four patients from
135 three non-related families.

136

137 Methods

138 *Ethics approval and human subjects*

139 Following informed consent, the patients and family members were included into a prospective cohort that was
140 approved by the Ethics committee of the Northwestern and central Switzerland (EKNZ 2015-187), complying
141 with all national and international ethical regulations. Blood samples from healthy donors were obtained after
142 informed consent from the Blood Donor Center, University Hospital Basel.

143

144 *Genetic analysis*

145 Genomic DNA was isolated from cultured T-cell blasts or peripheral blood mononuclear cells (PBMCs) using the
146 QIAamp DNA Blood Mini Kit (Qiagen). Whole exome sequencing was performed as described earlier^(7, 8).

147 The *LIG4* variant was confirmed by Sanger sequencing of PCR amplification products of cDNA derived from
148 PBMCs. After running the amplicon on an 1.5% agarose gel, DNA was extracted with QIAquick Gel Extraction Kit
149 (Qiagen). The purified PCR products were then bidirectionally sequenced by Microsynth (Switzerland).

150

151 *Cell isolation and immunophenotyping*

152 Patient- and healthy control-derived PBMCs were isolated from whole blood, *via* Ficoll density gradient
153 separation using LymphoprepTM (density 1.077g/mL, Axonlab).

154 Cells were stained in PBS containing 2.5% human AB serum, NaH₃ 0.01%, HEPES 25mM, Fc block (BioLegend #
155 426101) for 30min at 4°C. Chemokine receptor staining was performed at 37°C for 20min. All primary/
156 secondary antibody conjugates are listed in supplemental methods. Cell viability was assessed using Live/Dead

157 Fixable NIR (# L34975, Invitrogen™, ThermoFisher Scientific). Data analysis was performed using FlowJo
158 software (Version 10.5.2, TreeStar, USA).

159
160 Additional methods are reported in the **supplementary material** section.

161 162 **Results**

163 Dominantly inherited immune-dysregulation

164 P1, presented at the age of two years with autoimmune hemolytic anemia (AIHA) and immune thrombo-
165 cytopenia (ITP) (**Fig 1, A**). During the disease course, P1 developed lymphoproliferation (splenomegaly and
166 lymphadenopathy) and multiple infections including opportunistic pathogens (**Fig 1, A**). At the age of eleven
167 years, P1 developed biopsy-proven interstitial nephritis with polyclonal T and B cell infiltrations (**Fig 1, B**). At the
168 transition into the adult immunology service, being under immune suppression with mycophenolate,
169 agammaglobulinemia was noted. Immunoglobulin replacement therapy was started at this time. Despite
170 normalized serum IgG levels, P1 developed life-threatening non-infectious pneumonitis, again characterized by
171 polyclonal lymphocyte infiltration (**Fig 1, C - E**). Lastly, sterile granulomatous parotitis was diagnosed (**Fig 1, F**).
172 Her father and two paternal uncles experienced several adult-onset ITP episodes that responded to systemic
173 steroids.

174 A detailed immunological evaluation was performed in P1 and her father (P2). The father had mildly
175 reduced lymphocytes ($1.02 \times 10^9/L$) and thrombocytes ($114 \times 10^9/L$), in the absence of immune modulating
176 treatment (**Table E1**). Analysis of PBMCs revealed a reduced frequency of naïve $CD27^+CD45RO^-$ T cells in both
177 patients (**Fig 1, G and H**). T cell proliferation upon mitogen stimulation was enhanced (**Fig 1, I**). Peripheral blood-
178 derived $CD4^+$ T regulatory cells (T_{reg} , $CD25^{hi}CD127^{low}$) were reduced in frequency in both P1 and her father
179 compared to healthy donors (HDs) (**Fig E1, A**). Those T_{reg} displayed an activated and proinflammatory phenotype
180 (**Fig E1, B**). $CD4^+$ T cells also displayed a phenotype skewed towards T_{H1} (**Fig E1, C**). Autoreactivity of B cells was
181 investigated by probing the father's serum immunoglobulins against different self-antigens on a protein
182 microarray and compared with gender-matched controls. Four of the tested IgG autoantibody specificities were
183 found to be elevated in the serum of the father (**Fig E1, D and F**), including augmented IgG directed against
184 genomic DNA (**Fig E1, E**). Endogenous IgG of P1 could not be tested due to the agammaglobulinemia and the
185 immunoglobulin substitution. Low T cells bearing the $TCR V\alpha 7.2^+$ were noted in both (**Fig 1, J**), similarly to what
186 was found in some other patients diagnosed with CID in our cohort (**Fig 1, K**).

187 Since low $TCR V\alpha 7.2^+$ T cells have been reported as a hallmark observed in patients with V(D)J
188 recombination defects^(9, 10), we performed TCR and BCR high throughput sequencing.

189 190 Preserved TCR/BCR repertoires

191 The most common TCR loci were sequenced, using DNA derived from peripheral blood T cells from P1 and her
192 parents. The distribution of the most variable region of the TCR, the complementarity-determining region 3
193 (CDR3) lengths in the T cell receptor α -chain (*TCRA*, **Fig 2, A**) and β -chain (*TCRB*) sequences (**Fig E2, A**) were
194 comparable in P1 and her parents. To account for the entire repertoire diversity and clonality, the Shannon's
195 (*H*) entropy⁽¹¹⁾ and Simpson's clonality⁽¹²⁾ indices were computed and found to be normal (**Fig 2, B and C**,
196 respectively).

197 We focused on the individual *TCRA V* gene segment usage, as this locus can adopt a directional
198 multistage recombination, which is halted only upon positive thymocyte selection⁽¹³⁾. We found only the V-gene
199 segment *27-01-03* to be significantly overrepresented in the two patients compared to healthy donors (HD) (**Fig**
200 **2, D**).

201 To investigate the pairing of *TCRA V* with *J* gene segments, heatmaps were computed. The pairing was
 202 overall maintained, in total (**Fig E2, B**) as well as in unique *TCRA* sequences (**Fig 2, E**), including distal gene
 203 segment pairing (**Fig 2, E, Fig E2, B**).

204 The autoimmune disposition in P1 and her father could reflect differences in B cell subsets and/or BCR
 205 repertoire, thus peripheral blood B cells were immunophenotyped and RNA-derived immunoglobulin heavy
 206 chain (*IGH*) repertoires were sequenced using isotype-resolved barcode based adaptive immune receptor
 207 repertoire-sequencing (AIRR-seq) technology.

208 P1 displayed an inverted BCR light chain (κ vs. λ) expression on B cells compared to HDs (**Fig 2, F**). Both
 209 patients had an increased percentage of CD21^{low} B cells (**Table E1**). The vast majority of P1's B cells included
 210 unmutated naïve and memory IgM/IgD (*MD*) transcripts (**Fig E2, C**). Further, the constant region segment
 211 utilization was investigated (**Fig 2, G**). In P1 IgG (*IGHG*) and IgA (*IGHA*) transcripts were barely detectable (**Fig**
 212 **E2, C**). Both patients displayed a tendency for a reduced *IGHG2* subclass frequency (**Fig 2, H**). In addition, P1's B
 213 cells transcripts showed a skewing towards the utilization of the *IGHG3* subclass (**Fig 2, H**).

214 P1's *MD* memory B cells had an increased usage of the *V_{H4}* gene family at the expense of *V_{H3}* (**Fig 2, I**).
 215 In both patients the memory *MD* B cell transcripts harbored less abundantly the *J_{H4}* gene segment (**Fig 2, J**).

216 Affinity maturation was analyzed *via* the quantification of somatic hypermutations (SHM) detected in
 217 memory B cell transcripts, being below the normal range for P1 and marginally low in the paternal memory *MD*
 218 compartment (**Fig 2, K**). An increased ratio of replacement mutations (R) compared to silent mutations (S) (R/S
 219 ratio) in the CDRs may point at antigen selection^(14, 15). P1's *IGHG* and memory *MD* B cell transcripts showed a
 220 decreased R/S ratio compared to HDs (**Fig 2, L**), while in the father's B cells, the R/S ratio was only marginally
 221 low in *MD* memory B cells (**Fig 2, L**).

222 Novel heterozygous *LIG4* missense variant

223 We next investigated PBMC-derived DNA of P1, her parents and the clinically healthy brother using whole-
 224 exome sequencing (WES), followed by custom-designed PID gene panel filtering. In both diseased individuals
 225 we detected a c.G1739A heterozygous missense variant in *LIG4* (**Table EII**). Sanger sequencing confirmed
 226 heterozygosity. The healthy mother and brother did both not carry the *LIG4* variant (**Fig 3, A**). The c.G1739A
 227 variant causes replacement of an arginine at position 580 by a glutamine (p.R580Q). The Arg580 is highly
 228 conserved across various vertebrates (**Fig 3, B**) and locates within the oligonucleotide/oligosaccharide-binding
 229 domain (OBD), crucial for complete *LIG4* encirclement of the DNA during NHEJ⁽¹⁶⁾ (**Fig 3, C**). The variant is
 230 predicted to have functional impact on the *LIG4* protein (CADD-PHRED score 33⁽¹⁷⁾, PolyPhen-2⁽¹⁸⁾ score 1 and
 231 SIFT⁽¹⁹⁾ 21 score 0) (**Table EII**). This *LIG4* variant has so far not been described in the literature (**Table I**). *LIG4*
 232 mRNA was somewhat low in the father when compared to HDs but was normal in P1 (**Fig 3, D**). Immunoblots
 233 from T cell blast derived protein revealed conserved *LIG4* protein levels in P1 (**Fig 3, E**).

234 In addition, a novel homozygous missense variant in *FAS* (c.G383A, p.R128K, **Table EII**) was detected in
 235 the father. Both children, P1 and her healthy brother, were heterozygous carriers for this *FAS* variant. Based on
 236 unobtrusive *FAS*-related serum biomarkers, normal *FAS*-related apoptosis studies in T cell blasts of P1 and the
 237 fact that the healthy brother carried the same heterozygous *FAS* variant, we excluded the rare *FAS* variant to
 238 drive the disease in P1 and her father (**Fig E3, A-E**). In keeping, structure analysis predicted the extracellular
 239 R128K *FAS* mutation to be functionally conservative (**Fig E3, E**)

240 The R580Q variant reduces DSB ligation and DNA binding

241 The clinical phenotype of the *LIG4* variant carriers pointed to a protein loss of function associated with the
 242 R580Q variant. We performed substrate ligation assays comparing the enzymatic activity of recombinant wild-
 243

246 type (WT) vs. mutant (R580Q) LIG4 protein (**Fig 4, A**). As substrate, a 42 base pairs nicked oligonucleotide duplex
 247 (42mer) with attached fluorescent dye was used (**Fig 4, B**). Applying increasing substrate concentration (**Fig 4,**
 248 **C**) and reaction duration (**Fig 4, D**) we observed reduced amounts of ligated products in the R580Q LIG4
 249 presence as compared to WT.

250 Reduced biochemical ligation activity of the mutant R580Q LIG4 prompted us to study the LIG4-DNA
 251 interaction at the structural level. We performed molecular dynamics simulations, an approach allowing to
 252 efficiently interpret the effect of mutations on protein function^(8, 20, 21). The simulations focused on the catalytic
 253 domain of LIG4 in closed conformation with a nicked adenylated-DNA substrate (PDB 6BKG). Twelve
 254 independent unbiased trajectories of > 500ns, six for the WT and six for the R580Q mutant were computed.
 255 The Arg580 interacts with the broken 5' AMP-carrying DNA strand, with its guanidium moiety at a salt bridge
 256 distance from two phosphate groups (**Fig 4, E**) likely stabilizing the protein-DNA complex. Using the Molecular
 257 Mechanics Poisson-Boltzmann Surface Area (MMPBSA) approach⁽²²⁻²⁴⁾, we calculated the free binding energy
 258 between the WT vs. R580Q LIG4 to the DNA. We found that the binding energy was lower in the case of the
 259 R584Q ligand (**Fig 4, F and G, Fig E4, A and B**). The weakened R580Q LIG4-DNA binding could not be
 260 compensated by any of the 632 neighboring residues (**Fig E4, B**). Thus, the residue 580 accounted alone for the
 261 largest binding energy (BE) reduction.

262 Next, we focused the conformational analysis on the interactions of the residue with the DNA backbone
 263 and on their torsion angles. The dihedral χ_1 angle indicates the orientation of the sidechain with respect to the
 264 protein mainchain. The WT Arg580 experienced negligible oscillations in all trajectories, while the mutant
 265 Gln580 displayed greater dihedral χ_1 angle fluctuations including a bimodal χ_1 angle orientation (**Fig 4, H and**
 266 **I**). This suggested that Gln580 was still sampling new conformations after 500ns. The fluctuations of Gln580
 267 affected the secondary structure, causing a strong increase of the backbone torsion angles ϕ and ψ dynamics
 268 (**Fig E4, C – F**). Quantification of either the salt bridges and hydrogen bonds formed between WT Arg580
 269 respectively mutant Gln580 and the DNA (**Fig 4, J-L**), disclosed a higher abundance of salt-bridges being formed
 270 for the WT (**Fig 4, M, Fig E4, G**), significantly outnumbering the weaker hydrogen bonds for the mutant R580Q
 271 with the DNA (**Fig 4, M, Fig E4, H, video E1**).

272 Several mutations affecting the LIG4 catalytic domain have been reported. We wondered whether any of
 273 the previously reported mutations (**Table I**) would be related to DNA binding, similarly to the one characterized
 274 here. The location of all human missense mutations affecting the LIG4 catalytic domain was compared to those
 275 of the trajectories in which the distance between enzyme and DNA was $\leq 3\text{\AA}$. Three residues other than the
 276 Arg580 were identified: p.278, p.447 and p.449 (**Fig 4, N**). The positions p.278 and p.449 are well-described
 277 ATP-binding residues and a biochemical characterization for the p.447 mutation was not found in the literature.
 278 Consequently, the here described mutation at p.580 is to our knowledge the first with experimental evidence
 279 for reduced LIG4-DNA binding.

280

281 Dysregulated DSB repair response in heterozygous *LIG4* mutated primary T cells

282 To experimentally address LIG4 functionality in the context of a heterozygous missense variant, we
 283 characterized the DSB response in T cells of the patients *in vitro*.

284 After two days of *in vitro* culture, we observed spontaneously increased phosphorylation of two
 285 important DNA damage associated proteins H2Ax (γ H2Ax) and 53BP1 (p53BP1)^(25, 26) in T cells of both *LIG4*
 286 variant carriers (**Fig 5, A and B**). Next, we measured nuclear γ H2Ax kinetics after DSB induction *via* ionizing
 287 radiation (IR). Memory CD45RO⁺CD4⁺ T cells of both patients displayed higher γ H2Ax⁺ levels beyond 48 hours
 288 after IR compared to cells from HDs (**Fig 5, C**). The father's memory CD45RO⁺CD4⁺ T cells showed a trend and
 289 P1's memory CD4⁺ T cells a distinctly augmented proportion H2Ax phosphorylation after *in vitro* treatment of
 290 PBMCs with the DSB inducing drug Bleomycin sulfate⁽²⁷⁾ (**Fig 5, D**). This was paralleled by reduced cell viability

291 after *in vitro* Bleomycin sulfate exposure in naïve (CD45R0⁻) and memory (CD45R0⁺) CD4⁺ T cells of both patients
292 as compared to cells of HDs (Fig 5, E).

293 T cell proliferation capacity after IR plus mitogen stimulation, was studied by labelling peripheral blood-
294 derived T cells with CellTrace™ violet (CTV). Proliferation was quantified by assessing the CTV dye dilution. With
295 rising IR-doses, we observed a trend for a decreased relative proliferation index in both CD4⁺ and CD8⁺ T cells
296 of the two *LIG4* variant carriers compared to healthy T cells (Fig 5, F and G).

297

298 The monoallelic *LIG4* mutation p.A842D recapitulates impaired T-cell intrinsic DNA damage response and is
299 linked with combined immunodeficiency

300 In our cohort of patients with immunodeficiency/immune-dysregulation, we identified two additional unrelated
301 patients (P3 and P4), carrying an another functionally so far unstudied monoallelic *LIG4* mutation encoding
302 p.A842D (Fig 6, A and Table E2). Rare variants in other IEL-related genes filtered by WES in P3 and P4 were listed
303 as benign or variant of unknown significance (VUS) on gnomAD/ClinVar and did not align with reported clinical
304 features or zygosity reported by the international union of immunologic societies (IUIS)⁽²⁸⁾. Both were adult
305 patients with hypogammaglobulinemia, both sharing reduced naïve CD4⁺ T cells with the *LIG4* p.R580Q
306 mutation carriers of the index family (Table E1).

307 The alanine at position 842 is being conserved across species (Fig 6, B) within the BRCT2 domain of *LIG4*
308 interacting with its cofactor *XRCC4* (Fig 6, C). The distance of the proximal *XRCC4* residues (Gln159, Glu163 and
309 Val166) and *LIG4* is exceeding 8Å in a reported 2.4 Å resolution model centered around the *LIG4* BRCT segment-
310 *XRCC4* interaction (PDB 3II6) implying an indirect influence of the A842D substitution on molecular
311 interaction⁽²⁹⁾. We conducted 500 ns long independent unbiased MD trajectories, four of the WT and four of
312 the A842D variant. The analyses focused on residues located within a range of 15Å of the Cα atom of residue
313 842 (Fig 6, C and Fig E5). Results delineated potential alteration of a network of salt bridges involving multiple
314 residues of *XRCC4* and *BRCT*. A domino-effect of the A842D mutation was predicted to skew four pairs of acidic
315 and basic residues located in *BRCT2* and *XRCC4* (Fig E5). These changes are predicted to shift binding along the
316 *XRCC4* helices (see legend of Fig E5 for detailed description). The effect of the A842D mutation was conceptually
317 analogous to a *XRCC4* R161Q mutation causing reduced DNA repair⁽³⁰⁾.

318 We next re-addressed immune cell-intrinsic consequences of both R580Q and A842D mutations in
319 heterozygous state in primary T cells. Bleomycin treatment of PBMCs derived from A842D-mutated P3 and P4
320 resulted in significantly elevated CD3⁺ T cell death equivalent to re-analyzed R580Q-mutated P1 (Fig 6, D and
321 E). TCR Vα7.2⁺ frequencies in T cells (Table E1 and Fig 6, F) were low similar to P1 (Fig 6, G). When Vα7.2⁺ TCR
322 frequencies and T cell bleomycin induced cell death rates were correlated, two-dimensional plotting resulted
323 in a distinct segregation of *LIG4*-mutated patients P1, P3 and P4 with healthy control and also with unrelated
324 immune disease patients (Fig 6, H). When the slope of (% bleomycin-induced cell death)/(% Vα7.2⁺) was
325 computed for each individual, this T-cell functional index distinctly differentiated *LIG4*-mutated patients from
326 all other individuals examined (Fig 6, H). Subset-level analysis of bleomycin-induced cell death in CD4⁺ T cells
327 showed for naïve CD4⁺ T cells a notable acceleration (Fig E6, A). This was in keeping with the low *ex vivo*
328 frequencies of this subset as naïve CD4⁺ T-cell frequencies were lower and central memory CD4⁺ T-cell
329 frequencies reciprocally higher in patients P1-P4 compared with examined healthy and disease controls (Fig E6,
330 B-D).

331 In summary, accelerated DNA damage-induced T-cell death is a common feature in the currently
332 identified heterozygous *LIG4* R580Q and A842D monoallelic mutated patients.

333

334 *LIG4* R580Q and A842D mutations are functionally haploinsufficient

335 We next addressed the T cell-intrinsic consequences of the LIG4 R580Q and A842D mutations by reconstituting
336 LIG4 in a newly generated *LIG4*-knock-out (*LIG4*-KO) Jurkat T-cell line. Using the CRISPR-Cas9 system we
337 generated Jurkat T cells carrying a frameshift mutation in the *LIG4* gene resulting in LIG4 loss of expression as
338 confirmed by western blot and flow cytometry (**Fig 7, A**). Bleomycin treatment of *LIG4*-KO Jurkat T cells
339 resulted in augmented apoptosis in a dose- and time-dependent manner as compared with LIG4 competent
340 cells (**Fig 7, B and C**), functionally verifying that tolerance towards DNA damage is LIG4 dependent.
341 We next designed a transient transfection/overexpression-based LIG4 reconstitution in the *LIG4*-KO Jurkat T
342 cells (**Fig 7, D**, top left). A combined usage of a cationic polymer with magnetofection reproducibly attained
343 reporter protein/LIG4 protein-positive populations (**Fig 7, D**, left bottom). This occurred with a low basal
344 cytotoxicity enabling quantitative analysis upon *in vitro* DNA damage induced by bleomycin. Wild type (WT)
345 LIG4-expressing Jurkat T cells typically demonstrated a rescue from cell death which was not observed in R580Q
346 and A842D LIG4 reconstituted cells (**Fig 7, D and E**). There was certain inter-assay variability in these complex
347 reconstitution experiments whereas genotype differences (WT vs. MUT) were consistent. Thus, both LIG4
348 mutant proteins are loss of function in this reconstitution system.

349 A mixed reconstitution of WT and R580Q or A842D LIG4 did not significantly alter T cell apoptosis
350 compared to reconstitution with WT alone (**Fig 7, F**), even when using a 3:1 ratio in favor of the mutant LIG4.
351 These results rule out a dominant negative function of the R580Q and the A842D LIG4 variants.

352 In summary, the LIG4 R580Q and A842D mutations are loss of function causing LIG4 haploinsufficiency
353 upon DNA damage when present in heterozygous state.

354

355 Discussion

356 The clinical phenotype of human LIG4 deficiency is broad, ranging from asymptomatic carriers to death *in utero*
357 (**Table I**). To our knowledge, all LIG4 deficient patients described so far carried homozygous or compound
358 heterozygous *LIG4* mutations. However, Rucci et al. described reduced survival in mice carrying a heterozygous
359 *Lig4* missense mutation⁽⁶⁾. The immune-phenotype and clinical status of parents or siblings of published LIG4
360 deficient patients has not been studied systematically yet, albeit collective experience suggests immune-
361 competence in those monoallelic LIG4^{mut} carriers.

362 All four patients with monoallelic novel *LIG4* mutations characterized here had hypogamma-
363 globulinemia, low naïve CD4⁺ T cells, low V α 7.2 TCR segment usage and displayed augmented T cell intrinsic
364 cell death upon bleomycin exposure. T cell intrinsic hypersensitivity to experimental DNA damage in the four
365 heterozygous *LIG4* mutation carriers analyzed here is a key characteristic in LIG4 deficiency⁽³¹⁾.

366 The diversified TCR repertoire in both heterozygous *LIG4* mutation carriers analyzed is in keeping with TCR
367 repertoire analysis of published patients with compound heterozygous *LIG4* mutations⁽³²⁻³⁵⁾. These similarities
368 between published biallelic and the here presented monoallelic *LIG4* mutation carriers might be explained by
369 by the degree of functional hypomorphism⁽³¹⁾. This has however not been studied so far. Besides the role for
370 LIG4 in thymic T cell development, resting peripheral T cells have been found to be particularly sensitive to DNA
371 damage⁽³⁶⁾, possibly contributing to the observed low naïve T cell frequencies in heterozygous *LIG4* mutation
372 carriers.

373 We have documented immunodeficiency, lymphoproliferation and autoimmunity in the patients
374 analyzed here, including unique complications not yet documented in association with *LIG4* deficiency.
375 However, the full clinical spectrum associated with LIG4 haploinsufficiency is predicted to widen as more
376 patients are identified^(37,38). We can currently not conclude on the clinical penetrance of LIG4 haploinsufficiency.
377 Penetrance and also clinical phenotypes are known to be modified by environmental influence (e.g. immune-
378 suppressive treatment or recurrent x-ray based imaging in P1), epigenetics and also rare germline variants in
379 other immune-system genes⁽³⁹⁾.

380 Our newly established transfection platform to test functionality of identified rare *LIG4* variants, in
381 combination with molecular dynamic simulations, may guide definitive molecular diagnosis in possible *LIG4*
382 haploinsufficiency.

383 In summary, this is to our knowledge the first report of *LIG4* haploinsufficiency associated with
384 monoallelic *LIG4* mutations, driving human immune-dysregulatory disease that may segregate as an autosomal
385 dominant trait. In patients with immune-dysregulation of unknown cause, we encourage to consider *LIG4*
386 haploinsufficiency as it may have specific prognostic and therapeutic consequences.

387
388

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Figure legends

FIG. 1| Multiple autoimmune manifestations and reduction of naïve T cells in the peripheral blood of P1 and her father. A) Clinical manifestations in the index patient P1, thrombocyte counts, hemoglobin levels, grey background depicts reference range. Ears nose throat ENT, varicella-zoster virus VZV. **B)** P1's kidney biopsy during interstitial nephritis. Immunohistochemistry staining with anti-CD20 and anti-CD4. **C)** Pulmonary tissue gated computer tomography scan of P1 during the pneumonitis episode and **D)** after steroid treatment. **E)** Lung biopsy specimens during the pneumonitis episode and stained with anti-CD20 and anti-CD3. **F)** Cranial magnetic resonance imaging, showing parotid gland swelling (white arrowheads). **G)** Peripheral blood T cell subsets with naïve ($CD27^+CD45RO^-$), effector memory (EM, $CD27^-CD45RO^+$) and central memory (CM, $CD27^+CD45RO^+$) and **H)** quantification. **I)** CellTrace™ violet (CTV) dilution after 5 days of *in vitro* stimulation. **J)** Enumeration of T cells bearing the TCR $V\alpha 7.2$ segment by flow-cytometry. The number indicates the frequency within the $CD3^+$ T cell population. **K)** Comparison of the TCR $V\alpha 7.2^+$ T cell frequency in P1 and her father with patients affected by combined immunodeficiency (CID), primary antibody deficiency (PAD), autoinflammation (Autoinflamm.) or to healthy donors (HD). (K) non-parametric Kruskal-Wallis test with Dunn's correction ** $p < 0.01$.

FIG. 2| Preserved B and T cell receptor repertoires. A) High throughput sequencing of the T cell receptor loci. CDR3 length distribution. **B)** Shannon's (H) entropy index, grey shadow for HD values⁽⁴⁰⁾. **C)** Simpson clonality index. **D)** Individual V gene segment usage. **E)** Heatmaps displaying VJ gene pairing, box indicates most distal gene pairing. **F)** Surface expression of the BCR light chains. **G)** IGH locus cartoon for the constant region (adapted from⁽⁴¹⁾). IGH high-throughput RNA sequencing for the determination of B cell maturation status and constant region gene usage. **H)** IgA and IgG subclass utilization. Box-plot indicates age-matched HDs values. **I)** V family and **J)** J gene segment usage. Box-plot indicates values of age-matched HDs. **K)** Average of somatic hypermutations (SHM). The black line indicates the model fitting the SHM increase by age, gray lines indicate the 95% confidence interval. **L)** Antigen selection was quantified by the computation of the mean replacement/silent (R/S) ratio. The black line indicates the model fitting, the R/S increase by age, gray lines indicate the 95% confidence interval. (D) differential expression analysis empirical Bayes method. (F) Mann-Whitney test with post-hoc correction, the HDs SD was added to the value of P1.

629 **FIG. 3 | Novel missense variant within the catalytic core of DNA ligase 4. A)** Sanger sequencing of c.A1739G
 630 in bulk T cell-derived DNA, the resulting amino acid change at p.R580Q is indicated. **B)** Multiple LIG4 protein
 631 sequence alignment, p.580 position is highlighted. **C)** Molecular representation in ribbons of the human LIG4
 632 catalytic core bound to a DNA duplex. The WT Arg580 is shown as stick (arrow). The corresponding β sheet 18
 633 is indicated. The mutated amino acid resides in the catalytic oligonucleotide/oligosaccharide-fold domain
 634 (OBD, blue). Numbers indicate the amino acid position in NP_001091738. BRCT1: BRCA1 C terminus; BRCT2:
 635 BRCA2 C terminus; DBD DNA binding domain in green; NTD nucleotidyltransferase in orange. **D)** Qualitative
 636 polymerase chain reaction (qPCR) was used to measure *LIG4* mRNA levels in PBMCs of the two patients and
 637 healthy controls including the mother. The relative quantity (RQ) was normalized to multiple housekeeping
 638 genes and to the mean of the HDs. **E)** The LIG4 protein levels were quantified by separating PHA T cell blast
 639 cell lysates by SDS-PAGE electrophoresis and probed with rabbit-anti LIG4. Right side normalization of LIG4
 640 protein levels to β -actin levels. (d) non-parametric Mann-Whitney rank test, ns not significant.

641

642

643 **FIG. 4 | LIG4 R580Q reduces DNA-ligation activity and weakens DNA-binding. A)** Normalization of
 644 recombinant WT or R580Q LIG4 proteins. **B)** 42mer nicked DNA-duplex. Multiple turnover-ligations for WT vs.
 645 R580Q LIG4 with **C)** increasing unadenylated 42mer concentrations and **d)** time. Product separation on a TBE-
 646 Urea polyacrylamide gel. **E)** Molecular OBD representation, the Arg580 represented as stick (arrows: nearby
 647 DNA-backbone phosphorous atoms). **F)** Computed LIG4 binding energy (BE) between the WT vs. R580Q LIG4
 648 and adenylated-DNA complex. Twelve independent trajectories, each >500ns. **G)** Residues with BE difference
 649 >20 kJ/mol between WT and R580Q. **H)** Dihedral φ_1 angle time series and **I)** distribution focused on residue
 650 580. **J)** WT LIG4 and **(K)** R580Q LIG4 (stick) with the adenylated nicked-DNA as ball and stick. 3rd and 4th
 651 phosphate group of DNA-backbone (arrows). **L)** Minimal distance between the residue sidechain and DNA-
 652 backbone phosphate groups. The phosphate group-numbering is indicated. **M)** Temporal fraction, during
 653 which residue 580 sidechain and the DNA-backbone phosphate were < 4 Å. **N)** Bottom: Identification of likely
 654 DNA-interacting residues (distance to DNA < 3 Å). Middle: Human *LIG4* missense mutations (Table I). Top:
 655 Missense mutations with potential DNA binding. Mann-Whitney testing (F) with multiple comparison
 656 correction (L), (G) 2wayANOVA with Šídàk correction.

657

658 **FIG.5 | Augmented DNA-damage susceptibility *in vitro*.** T cells derived from PBMCs were cultured for two
 659 days without stimulation. The phosphorylation of H2Ax (γ H2Ax) and 53BP1 (p53BP1) were assessed by flow
 660 cytometry. **A)** Quantification (mean of triplicates) and **(B)** representative flow cytometric plots of the
 661 γ H2Ax⁺p53BP1⁺ population in bulk CD3⁺ T cells. **C)** Kinetics of γ H2Ax in CD45R0⁺CD4⁺ helper T cells after 10Gy
 662 irradiation (IR). **D)** Analysis of the nuclear γ H2Ax⁺ fraction in memory CD45R0⁺ CD4⁺ T cells after *in vitro*

663 treatment of PBMCs with Bleomycin sulfate for 24 hours at indicated concentrations. **E)** Cell death after 24
 664 hours *in vitro* Bleomycin sulfate exposure of CD4⁺ T cells (naïve CD45R0⁻ and memory CD45R0⁺). **F)** T cell
 665 proliferation after IR. T cells were labelled with CellTrace™ violet (CTV), followed by IR and stimulation for five
 666 days *in vitro* with anti-CD3/anti-CD28 (aCD3/aCD28). Gray shaded population indicates the maternal non-
 667 stimulated condition of T cells. **G)** The relative proliferation index was computed for CD4⁺ and CD8⁺ T cells
 668 after different IR intensities, stimulation of cells as in (F). (A) Kruskal-Wallis test, (C/D/E/G) 2wayANOVA with
 669 Šídàk correction. Single points represent mean values of duplicates or triplicates for the patients.

670

671

672 **FIG. 6 | A novel LIG4 A842D mutation substantiates linkage of monoallelic LIG4 mutations with DNA damage-**
 673 **induced T-cell death and immunodeficiency. A)** Sanger sequencing chromatogram of heterozygous LIG4
 674 A842D mutation in P3 and P4. **B)** Cross-species alignment of A842-proximal LIG4 residues. **C)** LIG4-XRCC4
 675 molecular complex highlighting residue 846-proximal area of BRCT2. Structural domains shown in black
 676 (BRCT1/BRCT2), blue (XRCC4-A) and red (XRCC4-B). Simulation snapshots in boxes for WT (top) and A842D
 677 (bottom) LIG4. Salt bridges shown as dashed lines when distances were mostly below 5Å during simulation.
 678 **D)** Dead cell stain-positive frequencies (mean ± SD) in T cells following 24-hour bleomycin exposure in blood-
 679 donors (n = 15, black), disease-controls (green) and patients P1 (R580Q), P3 and P4 (A842D). **E)** Post-hoc
 680 comparisons of one-way ANOVA for bleomycin-treated groups. Representative data shown as mean of pooled
 681 triplicate/quadruplicate (P1), duplicate/triplicate (P3) or triplicate/quadruplicate (P4). **F)** Flow-cytometric
 682 plots of TCRVα7.2⁺ T cells. **G)** TCR Vα7.2⁺ T cell frequencies of healthy controls (gray), disease-controls (green)
 683 and in LIG4-mutated patients (pink). **H)** Two-dimensional plot of *ex vivo* TCRVα7.2⁺ versus *in vitro* 24-hour 50
 684 μM bleomycin-induced T-cell death. An empirical slope of 2 is appended. **I)** One-way ANOVA of T cell-
 685 functionality slope defined as (24-hour bleomycin-induced dead frequencies)/(TCRVα7.2-positive
 686 frequencies).

687

688 **FIG 7 | LIG4 R580Q and A842D loss-of-function mutants manifest haploinsufficiency upon reconstitution.**

689 **A)** Verification of CRISPR-Cas9-mediated LIG4 knockout in Jurkats (Top). LIG4-expression impairment was
 690 verified by intracellular staining (bottom left) and western blotting (bottom right). **B)** Flow-cytometric plots of
 691 WT (left) versus LIG4-knocked out (LIG4-KO) (right) Jurkat T-cells exposed to bleomycin (12 hours). **C)** Dose-
 692 (12h) and time- (50μM) dependent frequencies of Annexin V-positive apoptotic cell frequencies following
 693 bleomycin exposure. Performed in triplicate (0μM, 10μM) or quadruplicate (50μM) and compared by unpaired
 694 t-tests. **D)** LIG4 functional reconstitution schematic via transient overexpression in LIG4-KO Jurkat T-cells. Cells
 695 were magnetofected via cationic polymers with a dual-promoter, LIG4/mCherry co-expressing vector
 696 (representative flow plot: bottom), then exposed to bleomycin and evaluated for Annexin V positivity in

697 mCherry(/LIG4)-positive/negative populations. A representative calculation is shown. **E)** Comparison of post-
698 bleomycin survival rates in mCherry+ cells normalized against intra-well mCherry- fractions upon WT versus
699 mutant *LIG4* transfection. Representative of two independent experiments performed in quadruplicate.
700 Compared by unpaired t-tests. **F)** Comparison of post-bleomycin incubation survival rates in mCherry+ cells
701 upon WT and mutant LIG4 co-transfection at indicated ratios. Post-hoc comparisons of one-way ANOVA are
702 shown. Pooled data of two independent experiments performed in triplicate/quadruplicate/control are shown
703 (mean \pm SEM).

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Table I | Clinical and genetic features of published patients with confirmed *LIG4* mutation. Patients are ordered according to the 5' position of the first mutated allele. cDNA sequence refers to NM_001098268.

c.C8T + c.C26T	c.2736+3delC	p.A3V + p.T9I	NA	Comp. het.	Additional polymorphisms in <i>ATM, NOD2, NLRP3</i>	none	(42)	R_001
c.C32G	c.T1236T	p.A11G	p.N412K	Comp. het.		none	(43)	R_002
c.C32G	c.T1236T	p.A11G	p.N412K	Comp. het.	(brother with p.A11G/ c.C32G, p.N412K/ c.T1236T)	none	(43)	R_003
c.C32G	c.T1236T	p.A11G	p.N412K	Comp. het.	(brother with p.A11G/ c.C32G, p.N412K/ c.T1236T)	none	(43)	R_004
c.T57G	c.1904delA	p.L19W	p.K635fs*10X	Comp. het.		none	(44)	R_005
c.597_600delTCAG	c.597_600delTCAG	p.Q200Kfs*201	p.Q200Kfs*201	Homo.		none	(45)	R_006
c.597_600delTCAG	c.597_600delTCAG	p.Q200Kfs*201	p.Q200Kfs*201	Homo.		none	(45)	R_007
c.597_600delTCAG	c.597_600delTCAG	p.Q200Kfs*201	p.Q200Kfs*201	Homo.		none	(45)	R_008
c.613delT	c.1904delA	p.S205Lfs*29X	p.K635fs*10X	Comp. het.		generalised erythema and dry cracked skin	(46, 47)	R_009
c.613delT	c.C845A	p.S205Lfs*29X	p.H282L	Comp. het.	balanced translocation t(1;19)(q21;p13))	hepatomegaly, skin scaly, dry, pale, hair was dry, brittle and scarce	(48)	R_010
c.613delT	c.C845A	p.S205Lfs*29X	p.H282L	Comp. het.	balanced translocation t(1;19)(q21;p13))	NA	(48)	R_011
c.613delT	c.C2440T	p.S205Lfs*29X	p.R814X	Comp. het.		none	(46, 49, 50)	R_012
c.A745G	c.1270_1274delAAAGA	p.M249V	p.K424Rfs*20X	Comp. het.		none	(51)	R_013
c.A745G	c.1271_1275delAAAGA	p.M249V	p.K424Rfs*20X	Comp. het.		jaundice, sclerosing cholangitis, hepatosplenomegaly	(43)	R_014
c.A745G	c.1271_1275delAAAGA	p.M249V	p.K424Rfs*20X	Comp. het.		jaundice, sclerosing cholangitis, hepatosplenomegaly	(43)	R_015
c.G827A	c.233_236delAGAG	p.G276D	p.R78Wfs*15X	Comp. het.		disseminated erythematous maculopapules after Rubella vaccine, hepatosplenomegaly.	(52)	R_016
c.G833A	c.G833A	p.R278H	p.R278H	Homo.		NA	(53)	R_017
c.G833A	c.G833A	p.R278H	p.R278H	Homo.		hypopigmentation, bronchiectasis	(44)	R_018
c.G833A	c.G833A	p.R278H	p.R278H	Homo.		hypopigmentation	(44)	R_019
c.G833A	c.G833A	p.R278H	p.R278H	Homo.	for all 3 mutations + p.A3V + p.T9I/ c.C8T + c.C26T	none	(54-57)	R_020
c.G833A	c.G833A	p.R278H	p.R278H	Homo.		none	(54, 58, 59)	R_021
c.G833A	c.1271_1275delAAAGA	p.R278H	p.K424fs*20X	Comp. het.		NA	(60)	R_022
c.G833A	c.1271_1275delAAAGA	p.R278H	p.K424fs*20X	Comp. het.		NA	(53)	R_023

c.G833A	c.1271_1275del	p.R278H	p.K424Rfs*21X	Comp. het.		NA	(10)	R_024
c.G833T	c.G833T	p.R278L	p.R278L	Homo.		none	(35, 61)	R_025
c.G833T	c.G833T	p.R278L	p.R278L	Homo.		none	(35)	R_026
c.G833T	c.935delC	p.R278L	p.P313Hfs*19	Homo.		AIHA	(35, 61)	R_027
c.G833T	c.1142_1143delCT	p.R278L	p.L382Efs*4	Comp. het.	c.C26T/ p.T9I	AIHA	(35, 61)	R_028
c.G833T	c.1144_1145delCT	p.R278L	p.L382Efs*5	Comp. het.		gastrointestinal ulcers	(62)	R_029
c.G833T	c.1271_1275delAAAGA	p.R278L	p.K424Rfs*20X	Comp. het.		vitiligo	(62)	R_030
c.G833T	c.1271_1275delAAAGA	p.R278L	p.K424Rfs*20X	Comp. het.		erythroderma	(62)	R_031
c.G833T	c.1271_1275delAAAGA	p.R278L	p.K424Rfs*20X	Comp. het.		eczema, generalized lymphadenopathy	(62)	R_032
c.G833T	c.1271_1275delAAAGA	p.R278L	p.K424Rfs*20X	Comp. het.		none	(62)	R_033
c.G833T	c.1271_1275delAAAGA	p.R278L	p.K424Rfs*20X	Comp. het.		none	(62)	R_034
c.G833T	c.1271_1275delAAAGA	p.R278L	p.K424Rfs*20X	Comp. het.		none	(35, 61)	R_035
c.G833T	c.1271_1275delAAAGA	p.R278L	p.K424Rfs*20X	Comp. het.		colitis	(35, 61)	R_036
c.G833T	c.1271_1275delAAAGA	p.R278L	p.K424Rfs*20X	Comp. het.		AIHA, purpura	(35)	R_037
c.G833T	c.1271_1275delAAAGA	p.R278L	p.K424Rfs*20X	Comp. het.		none	(35)	R_038
c.G833T	c.1271_1275delAAAGA	p.R278L	p.K424Rfs*20X	Comp. het.		AIHA	(35)	R_039
c.G833T	c.1271_1275delAAAGA	p.R278L	p.K424Rfs*20X	Comp. het.		anti-human globulin test, anti-thrombocytes antibodies, anti-HLA antibodies	(63)	R_040
c.G833T	c.1277_1278delAA	p.R278L	p.E426Gfs*19	Comp. het.		none	(62)	R_041
c.G833T	c.G2113T	p.R278L	p.E705X	Comp. het.		none	(35, 61)	R_042
c.G833T	c.2134_2135delTA	p.R278L	p.I712Afs*5	Comp. het.		AIHA	(35, 61)	R_043
c.G833T	c.C2710T	p.R278L	p.Q904X	Comp. het.	p.S12T/ c.T34A	none	(35)	R_044
c.G833T	loss exon2 (189-4043)	p.R278L	none	Comp. het.		none	(35)	R_045
c.G833C	NA	p.R278P	p.E582Dfs	Comp. het.		none	(64)	R_046
c.A840G	c.1271_1275delAAAGA	p.Q280R	p.K424Rfs*20X	Comp. het.	no AV3, T9I	none	(34, 57)	R_047
c.A840G	c.1271_1275delAAAGA	p.Q280R	p.K424Rfs*20X	Comp. het.	no AV3, T9I	none	(34, 57)	R_048
c.A845T	c.1544_1548delAAAGA	p.H282L	p.K424Rfs*19X	Comp. het.		veno-occlusiv disease	(33, 57)	R_049
c.A845T	c.1544_1548delAAAGA	p.H282L	p.K424Rfs*19X	Comp. het.		autoimmune cytopenia	(33, 57)	R_050
c.C845T	c.1746_1750delAAGAT	p.H282L	p.R581fsX	Comp. het.	c.C26T/ p.T9I	Omenn syndrome (scaly erythroderma), hepatosplenomegaly, lymphadenopathy	(57, 65)	R_051
c.C847G	c.C847G	p.K283E	p.K283E	Homo.		NA	(66)	R_052

c.A847A	c.1271_1275delAAAGA	p.K283E	p.K424Rfs*20X	Comp. het.		NA	(67)	R_053
c.A847A	c.1271_1275delAAAGA	p.K283E	p.K424Rfs*20X	Comp. het.		none	(67)	R_054
c.A875G	c.1307_1311del	p.Q229R	p.K436Rfs*20	Comp. het.		NA	(10)	R_055
c.G907A	c.1904delA	p.P231T	p.A562fs21X	Comp. het.		None	(68)	R_056
c.T980G	c.2585_5886del	p.I327S	p.H826Rfs*6	Comp. het.		AIHA	(35)	R_057
c.G1102T	c.G1102T	p.D368Y	p.D368Y	Homo.		Eczema	(69)	R_058
c.A1103T	c.G1341T	p.D368V	p.W447C	Comp. het.		bronchiectasis, villous atrophy, liver lesions, granulomatous dermatitis (after Rubella vaccination, nodular, superficial and deep dermal lymphohistiocytic infiltrate with scattered lymphohistiocytic cells)	(70)	R_059
c.G1237T	c.G1341	p.E413*	p.W447C	Comp. het.		epithelioid cell granuloma (absence of infection)	(57, 71)	R_060
c.1245_1250dupGATGC	c.C2440T	p.L418Mfs*3	p.R814X	Comp. het.		none	(47)	R_061
c.1271_1274delAAAG	c.C2440T	p.K424Rfs*20X	p.R814X	Comp. het.		NA	(10)	R_062
c.1271_1275delAAAGA	c.C2440T	p.K424Rfs*20X	p.R814X	Comp. het.		psoriasis	(47)	R_063
c.1271_1275delAAAGA	c.C2440T	p.K424Rfs*20X	p.R814X	Comp. het.		none	(47)	R_064
c.1271_1275delAAAGA	c.C2440T	p.K424Rfs*20X	p.R814X	Comp. het.		none	(47)	R_065
c.1271_1275delAAAGA	c.C2440T	p.K424Rfs*20X	p.R814X	Comp. het.		hypopigmentation	(47)	R_066
c.1271_1275delAAAGA	c.C2440T	p.K424Rfs*20X	p.R814X	Comp. het.		none	(47)	R_067
c.1271_1275delAAAGA	c.C2440T	p.K424Rfs*20X	p.R814X	Comp. het.		none	(47)	R_068
c.1271_1275delAAAGA	c.C2440T	p.K424Rfs*20X	p.R814X	Comp. het.		none	(67)	R_069
c.1271_1275delAAAGA	c.C2440T	p.K424Rfs*20X	p.R814X	Comp. het.		cutaneous abnormalities	(66)	R_070
c.A1296T	c.C1672T	p.K432N	p.Q558X	Comp. het.		none	(35)	R_071
c.1297_1299delCAA	c.1297-1299delCAA	p.Q433del	p.Q433del	Homo.		none	(57, 72)	R_072
c.T1312c	c.T1312c	p.Y438H	p.Y438H	Homo.	LRIG2 mutations (homo)	nail dystrophy, sparse and thin hair	(73)	R_073
c.A1345C	c.C2440T	p.K449Q	p.R814X	Comp. het.		none	(32)	R_074
c.A1345C	c.C2440T	p.K449Q	p.R814X	Comp. het.		none	(32)	R_075
c.A1345C	c.C2440T	p.K449Q	p.R814X	Comp. het.		none	(32)	R_076
c.A1345C	c.C2440T	p.K449Q	p.R814X	Comp. het.		NA	(53)	R_077
c.G1406A	c.C2440T	p.G469E	p.R814X	Comp. het.		psoriasiform erythrodermic squamous skin patches	(55, 56, 74)	R_078
c.G1406A	c.C2440T	p.G469E	p.R814X	Comp. het.		none	(75)	R_079

c.1512_1513delTC	c.C2440T	p.R505Cfs*12X	p.R814X	Comp. het.		none	(47)	R_080
c.1751_1755delTAAGA	c.C2440T	p.I584Rfs*2X	p.R814X	Comp. het.		none	(76)	R_081
c.1762delAAG	c.1762delAAG	p.K588del	p.K588del	Homo.		none	(77)	R_082
c.1762delAAG	c.1762delAAG	p.K588del	p.K588del	Homo.		none	(77)	R_083
c.C1738T	c.C2440T	p.R580X	p.R814X	Comp. het.		hypothyroidism, hypogonadism, diabetes, chronic cutaneous affection, photosensitivity, telangiectasia	(55)	R_084
c.C1738T	c.C2440T	p.R580X	p.R814X	Comp. het.		hypothyroidism, amenorrhea, photosensitivity, psoriasis	(55)	R_085
c.1904delA	c.C2440T	p.K635fs*10X	p.R814X	Comp. het.		NA	(66)	R_086
c.1904delA	c.C2440T	p.K635fs*10X	p.R814X	Comp. het.		NA	(66)	R_087
c.C2094T	c.C2440T	p.Y698X	p.R814X	Comp. het.		None	(47)	R_088
c.C2094T	c.C2440T	p.Y698X	p.R814X	Comp. het.	Xp22.31p22.32 duplication	none	(78)	R_089
c.2386_2389dupATTG	c.C2440T	p.A797Dfs*3	p.R814X	Comp. het.		cutis marmorata	(47)	R_090
c.C2440T	c.C2440T	p.R814X	p.R814X	Homo.		hypogonadism, asthma, lymphadenopathy, hepatomegaly.	(79)	R_091
c.G2612A	c.G2612A	p.R871H	p.R871H	Homo.		recurrent meningitis (sterile), recurrent genital/oral ulcers, anterior uveitis, intermittent attacks of non-erosive arthritis.	(80)	R_092
NA	NA	NA	NA	NA	AML: 48, XX, +2, der(5)t(5;17)(q11;q11), -7, +8, +11, -17, +20/46, XX	none	(81)	R_093
NA	NA	NA	NA	NA		none	(57)	R_094
NA	NA	NA	NA	NA		autoimmunity, Omenn phenotype	(57)	R_095
NA	NA	NA	NA	NA		none	(57)	R_096
NA	NA	NA	NA	NA		none	(57)	R_097
NA	NA	NA	NA	NA		none	(57)	R_098
NA	NA	NA	NA	NA		none	(57)	R_099
NA	NA	NA	NA	NA		none	(57)	R_100
NA	NA	NA	NA	NA		none	(57)	R_101
NA	NA	NA	NA	NA		none	(57)	R_102
NA	NA	NA	NA	NA		none	(57)	R_103

NA	NA	NA	NA	NA		none	(57)	R_104
NA	NA	NA	NA	NA		none	(57)	R_105
NA	NA	NA	NA	NA		none	(57)	R_106
NA	NA	NA	NA	NA		autoimmunity	(57)	R_107
NA	NA	NA	NA	NA		none	(57)	R_108
NA	NA	NA	NA	NA		none	(57)	R_109
NA	NA	NA	NA	NA		none	(57)	R_110
NA	NA	NA	NA	NA		none	(57)	R_111
NA	NA	NA	NA	NA		none	(57)	R_112
NA	NA	NA	NA	NA		none	(57)	R_113
NA	NA	NA	NA	NA		none	(57)	R_114
NA	NA	NA	NA	NA		none	(57)	R_115
NA	NA	NA	NA	NA		none	(57)	R_116
NA	NA	NA	NA	NA		none	(57)	R_117
NA	NA	NA	NA	NA		NA	(57)	R_118
NA	NA	NA	NA	NA		NA	(57)	R_119
NA	NA	NA	NA	NA		NA	(57, 82)	R_120

Journal Pre-proof











