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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Chromosome** | **Location** | **Base change** | **Amino Acid change** | **Functional evidence** | **Comment** | **Reference** |
| MASP2 | 1 | Exon 3 | A359G | D120G | Yes | Lowers MASP-2 concentration and the MASP-2 and may lead to inability to activate complement | Stengaard-Pedersen et al1, Thiel et al2 |
| NCF2 | 1 | Exon 13 | C1167A | H389Q | Yes | Significantly decreased binding to Vav1 resulting in reduced reactive oxygen species production | Dhillon et al3, Armstrong et al4 |
| NCF2 | 1 | Exon 15 | C1360T | P454S | Yes | Results in loss of exonic splicing enhancer leading to reduced oxidative burst response | Dhillon et al3, Denson et al5 |
| NCF2 | 1 | Exon 14 | A1256T | N419I | Yes | Significantly decreased binding to p40phox resulting in reduced reactive oxygen species production | Dhillon et al3, Denson et al5 |
| NCF2 | 1 | Exon 14 | G1184A | R395Q | Possible | R395W and R394Q have functional evidence showing reduced reactive oxygen species production | Armstrong et al4, Sancho-Shimizo et al6 |
| STAT1 | 2 | Exon 10 | G796A | V266I | Conflicting | Initially reported to have higher GAS oligonucleotide binding in response to IFN- γ but a subsequent report could not replicate this | Uzel et al7, Depner et al8 |
| NCF1 | 7 | Exon 4 | G269A | R90H | Yes | Located in PI(3,4)P2-binding domain of p47phox resulting in reduced reactive oxygen species production | Dhillon et al3, Zhao et al9 |
| NCF1 | 7 | Exon 4 | G247A | G83R | Yes | Reduced oxidative burst response to formyl peptide | Denson et al5 |
| DCLRE1C | 10 | Exon 6 | G457A | G153R | No |  |  |
| DCLRE1C | 10 | Exon 7 | C512G | P171R | Yes | Decrease in Artemis endonuclease activity in vitro (3x). Cells with variant demonstrated a DSB repair defect in G2 phase | Woodbine et al10 |
| TRIM22 | 11 | Exon 8 | G962A | R321K | Yes | Reduced or absent TRIM22-NOD2 binding with reduced downstream TNFα, IL-6, and ISG15 expression | Li et al11 |
| TRIM22 | 11 | Exon 8 | C1324T | R442C | Yes | Reduced or absent TRIM22-NOD2 binding with reduced downstream TNFα, IL-6, and ISG15 expression | Li et al11 |
| TRIM22 | 11 | Exon 4 | C731T | S244L | Yes | Reduced or absent TRIM22-NOD2 binding with reduced downstream TNFα, IL-6, and ISG15 expression | Li et al11 |
| TRIM22 | 11 | Exon 8 | C1450T | P484S | No | Reported to alter protein structure in silico, no functional evidence | Kelly et al12 |
| IL10RA | 11 | Exon 7 | C1259T | S420L | No |  |  |
| NOD2 | 16 | Exon 4 | C2104T | R702W | Yes | Reduced levels of NF-κB activation, greatly reduced response to lipopolysaccharide and peptidoglycan stimulation | Bonen et al13, Parkhouse et al14 |
| NOD2 | 16 | Exon 9 | G2863A | V955I | Conflicting | Trend towards reduced IL10 production with T cell responses, seen with enhanced CD4+ T cell proliferation. But no impact on NF-κB signalling | Hedegaard 15 |
| NOD2 | 16 | Exon 4 | C2264T | A755V | Yes | Reduced levels of NF-κB activity by 25-54% | Parkhouse et al14 |
| NOD2 | 16 | Exon 4 | A1055G | H352R | No |  |  |
| NOD2 | 16 | Exon 5 | G2470A | D824N | No |  |  |
| NOD2 | 16 | Exon 6 | A2555G | N852S | No | Located in the 4th LRR domain, widely reported association but no absolute functional evidence | Parkhouse et al14, Tukel et al16 |
| NOD2 | 16 | Exon 8 | G2722C | G908R | Yes | Reduced levels of NF-κB activation, greatly reduced response to lipopolysaccharide and peptidoglycan stimulation | Bonen et al13 |
| NOD2 | 16 | Exon 11 | 3017dupC | A1007fs | Yes | Reduced levels of NF-κB activation, no response to lipopolysaccharide and peptidoglycan stimulation | Bonen et al13 |
| NOD2 | 16 | Exon 4 | C2230T | R744W | No |  |  |
| NOD2 | 16 | Exon 4 | G2123A | R708H | No |  |  |
| NOD2 | 16 | Exon 10 | A2888G | E963G | Novel |  |  |
| BTK | X | Exon 7 | A720C | E240D | No |  |  |
| CD40LG | X | Exon 5 | G655A | G219R | Yes | Deleterious in vivo alongside XIAP mutations. In vitro functional evidence of reduced binding of CD40LG to CD40 leading possible affects on CD40L-mediated interactions | Rigaud et al17, Barnhart et al18 |
| WAS | X | Exon 4 | G391A | E131K | Yes | Absent WAS protein expression through Western blot analysis | Stewart et al19, Jin et al20 |
| WAS | X | Exon 11 | C1378T | P460S | Yes | Slowed cell growth, increased actin polymerisation impacting on cellular proliferation | Zheng et al21 |
| DKC1 | X | UTR5 | 142C>G |  | Yes | Disruption of Sp1 transcription factor binding site leading to reduced promotor activity | Salowsky et al22, Knight et al23 |
| FOXP3 | X | Exon 6 | C543T | S181S | No |  |  |
| SH2D1A | X | Exon 1 | C48T | G16G | No |  |  |
| XIAP | X | Exon 7 | A1408T | T470S | Conflicting | Too common to be disease causing, may contribute to disease in conjunction with additional variants | Uhlig et al24 |
| CYBB | X | Exon 9 | G1090C | G364R | Possible | Constituent of gp91phox adjacent to the FAD-binding domain. Widely reported but lacks functional validation to demonstrate reduced reactive oxygen species production | Dhillon et al3, Dennison et al5, O’Neill et al25 |
| POLA1 | X | Exon 32 | G3604C | D1202H | No |  |  |

Supplementary table 2- Summary of functional evidence for significant monogenic IBD variants identified within the Wessex cohort. Variants are coded as confirmed functional impact (red), possible or conflicting impact (yellow), no reported functional validation (blue) or novel variants (green).

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