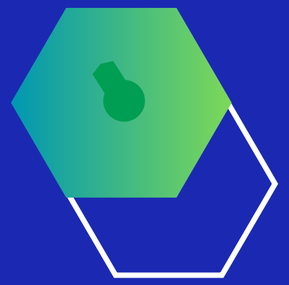
Association of *CLYD* and *NOD2* variants with susceptibility to IBD

Axel Rodriguez

The data

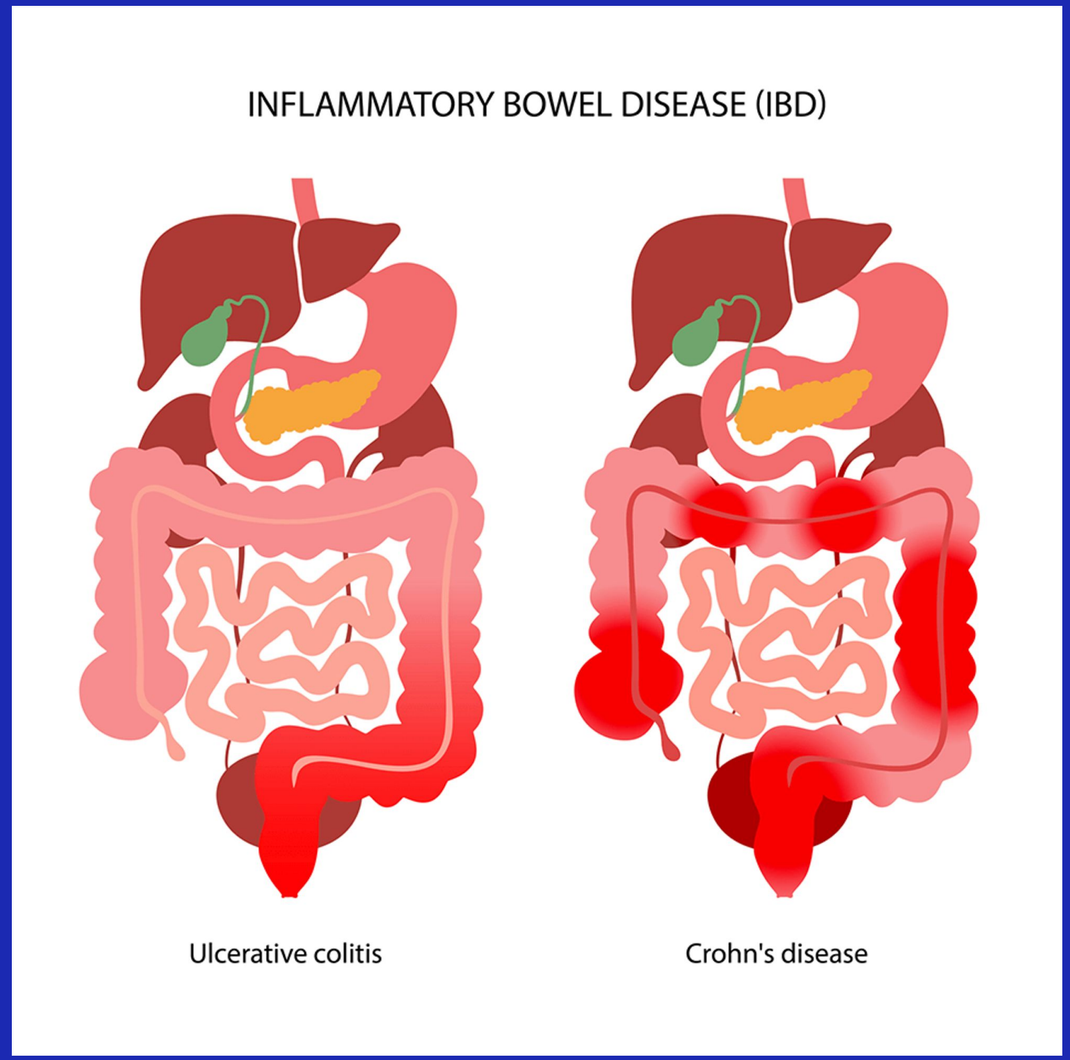
- **n = 1,401**
- Genotyping information of **500,000 SNPs**
- Measures of age, sex, high-density lipoprotein (HDL)-cholesterol (HDL-C), low-density lipoprotein (LDL)-cholesterol, triglycerides (TGs), Coronary Artery Disease (CAD) status and Inflammatory Bowel Disease (IBD) status.
- Participant identification information
- **1,281 complete observations**
- Age of range of study participants: from **22 to 87 y/o**, with a mean of **55 y/o**
- **367 IBD positive cases.**

The trait



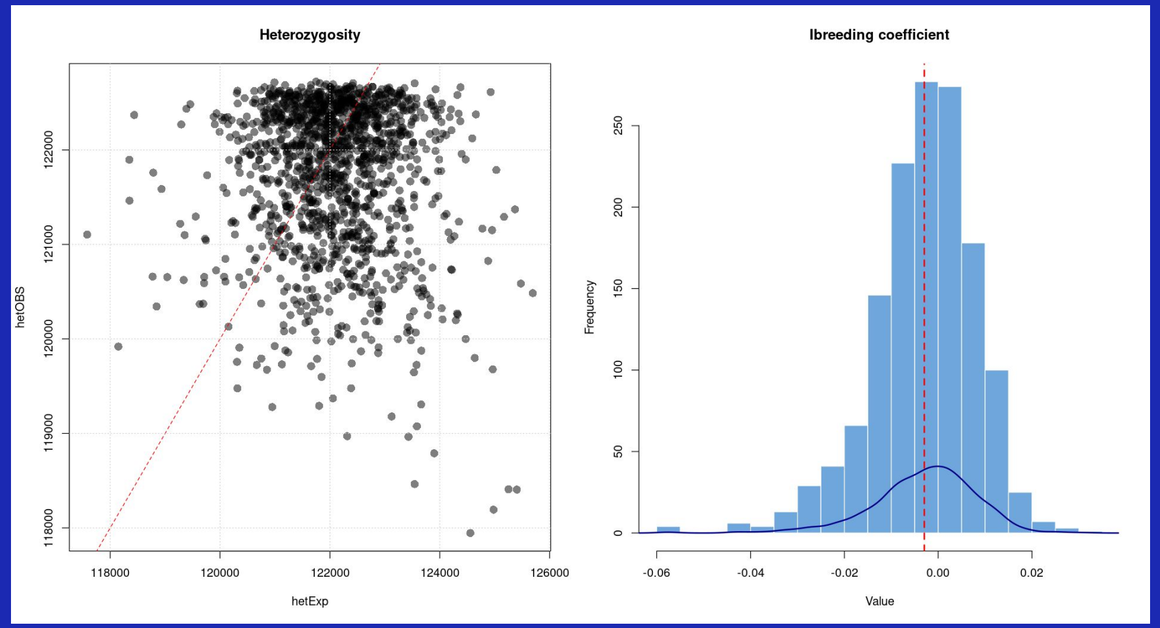
- Inflammatory Bowel Disease (IBD)
- Repetitive episodes of inflammation of the gastrointestinal tract caused by an **abnormal immune response** to gut microflora.
- Encompasses **two types of idiopathic intestinal disease** that are differentiated by their location and depth of involvement in the bowel wall.
- **Both are not curable**, and they both carry enormous morbidity. Finally, both increase the risk of colorectal cancer.
- Is estimated to affect more than 3 million people in the USA and Europe

The trait


INFLAMMATORY BOWEL DISEASE (IBD)
Ulcerative colitis
Crohn's disease

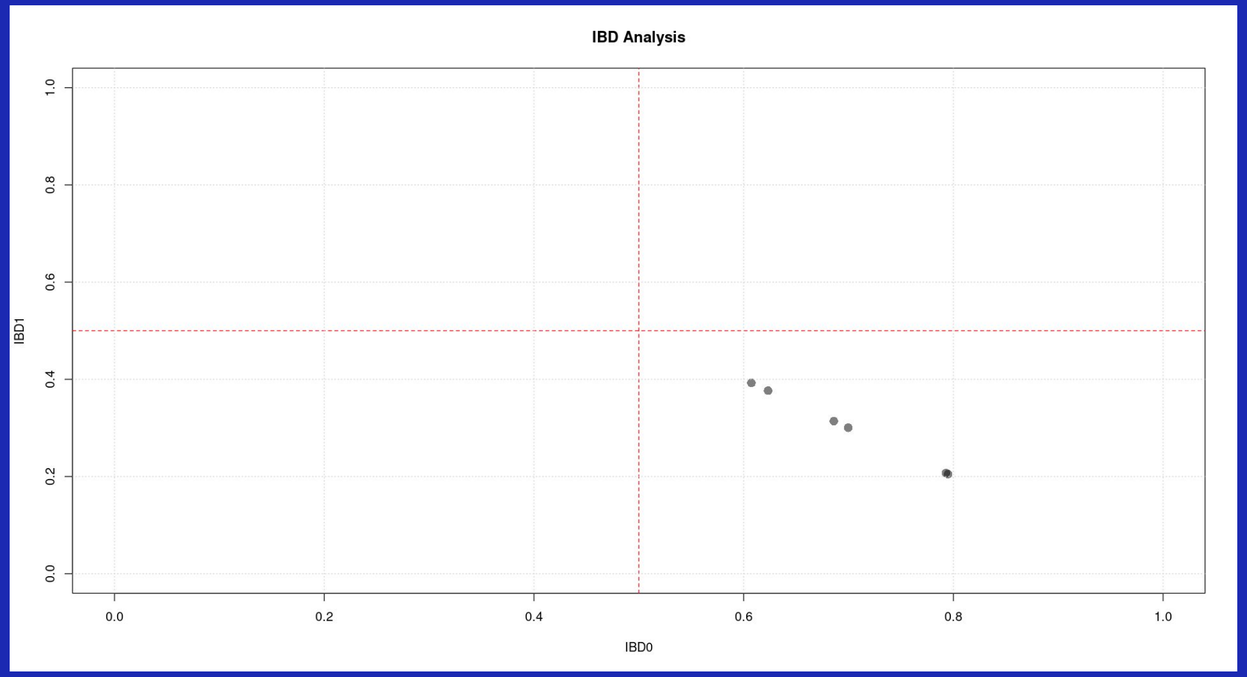
Quality control

- **54,583 SNPs** removed due to **low call rate** (CR < 95%, 445,417 remaining SNPS)
- **63,395 SNPs** removed due to low **MAF** (MAF < 1%, 382,022 remaining SNPS)
- **4 individuals** removed due to unusual **inbreeding coefficient** (1,397 remaining individuals).



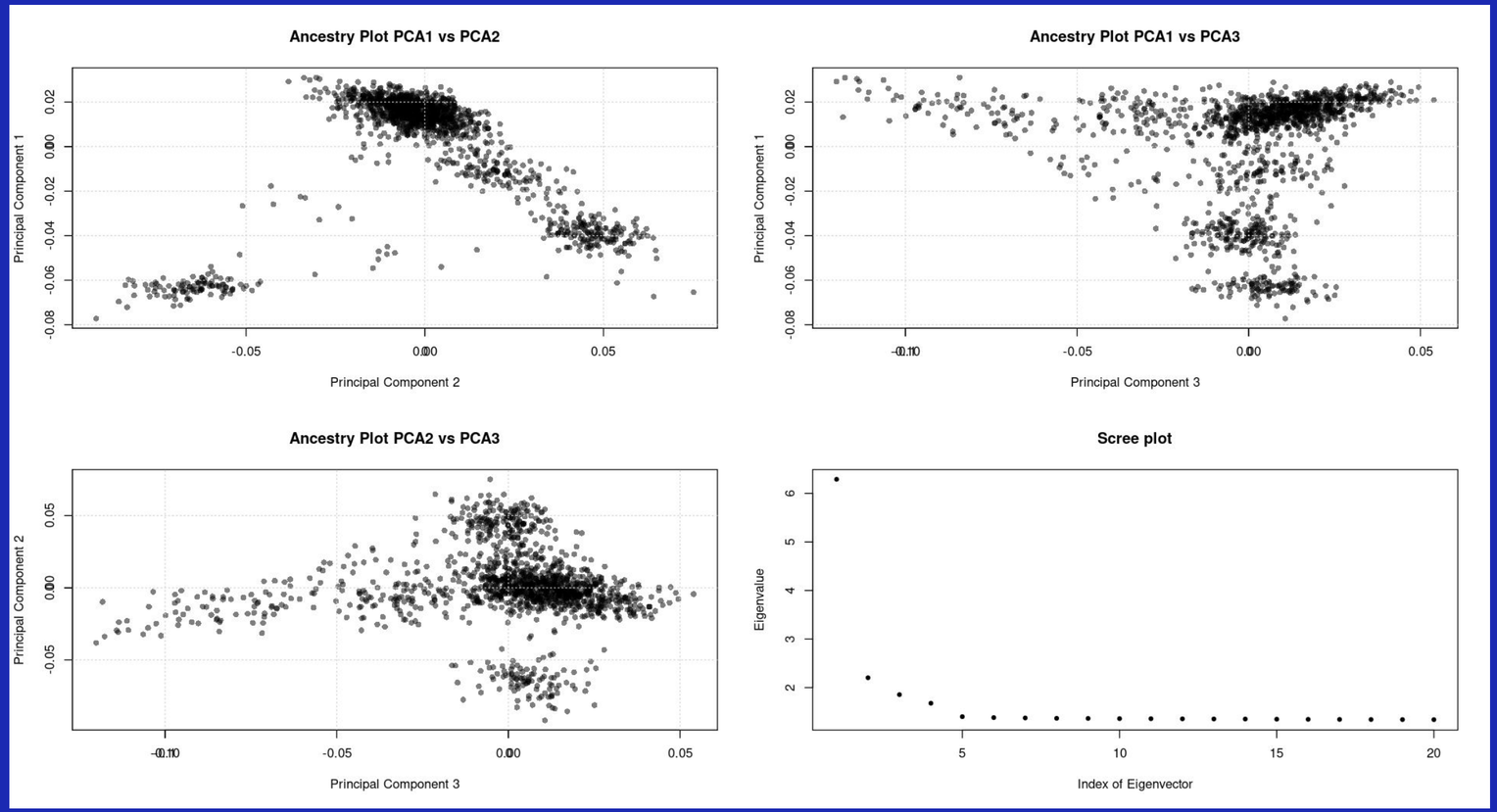
Quality control

- **1,187 SNPs** removed due to high **HWE** (380,835 remaining SNPs).
- 380,369 SNPs used in IBD analysis.
- **5 similar samples** removed due to **correlation coefficient** >= 0.05



Quality control

- **Three PCAs** retained



- **1,392 samples** & **380,835 SNPs** included in analysis

The model

$$Y = \alpha * SNP + \text{age} + \text{CAD} + \text{sex} + \text{tg} + \text{hdl} + \text{ldl} + \text{PC1} + \text{PC2} + \text{PC3}$$

```
result <- summary(glm(phenotype ~ . - FamID, family = binomial, data = cbind(phenodata, snp = genoNum[, snp.name])))
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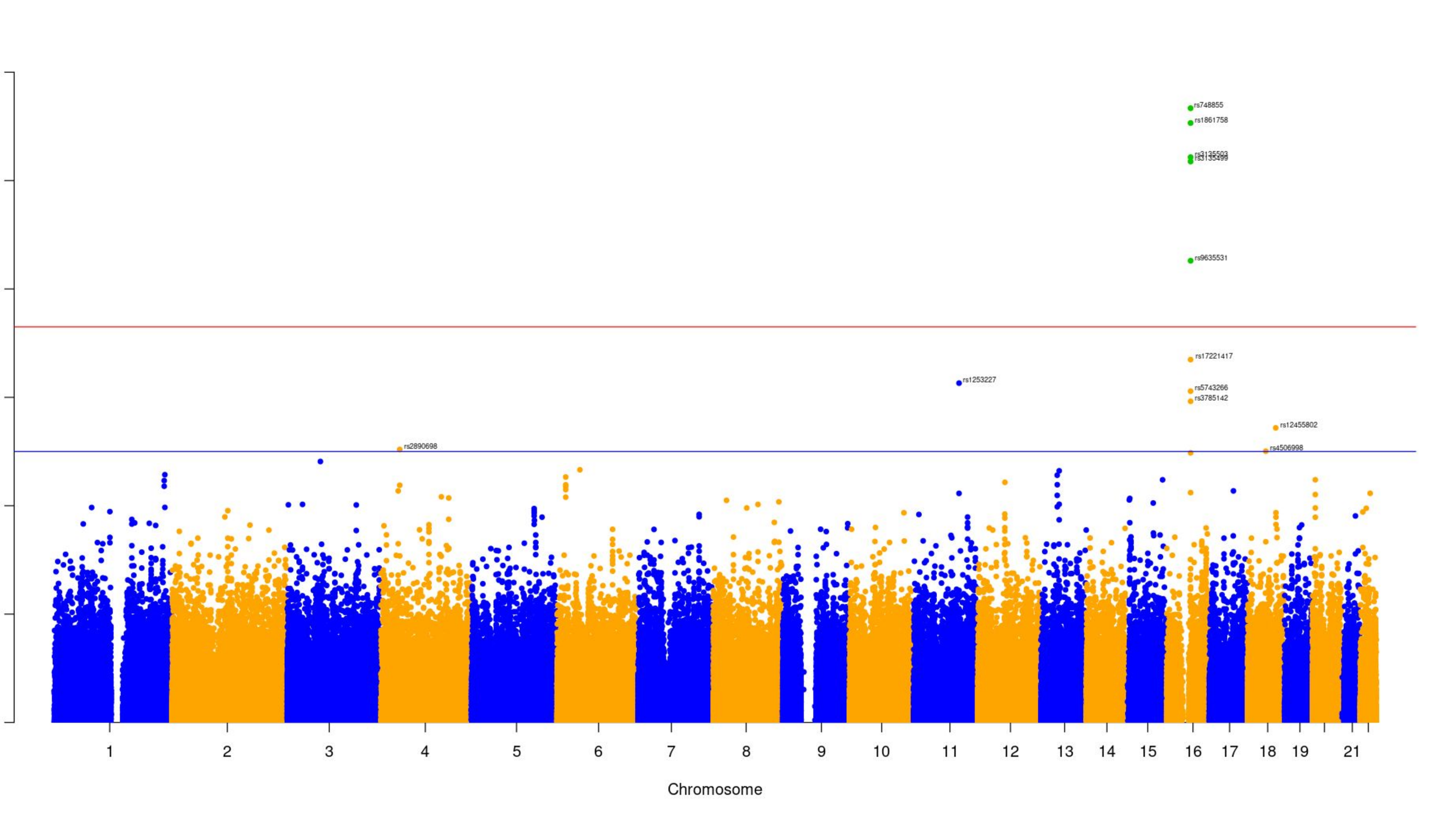
rs748855
rs1861758
rs3135503
rs3135499
rs9635531
rs17221417
rs1253227
rs5743266
rs3785142
rs12455802
rs2890698
rs4506998
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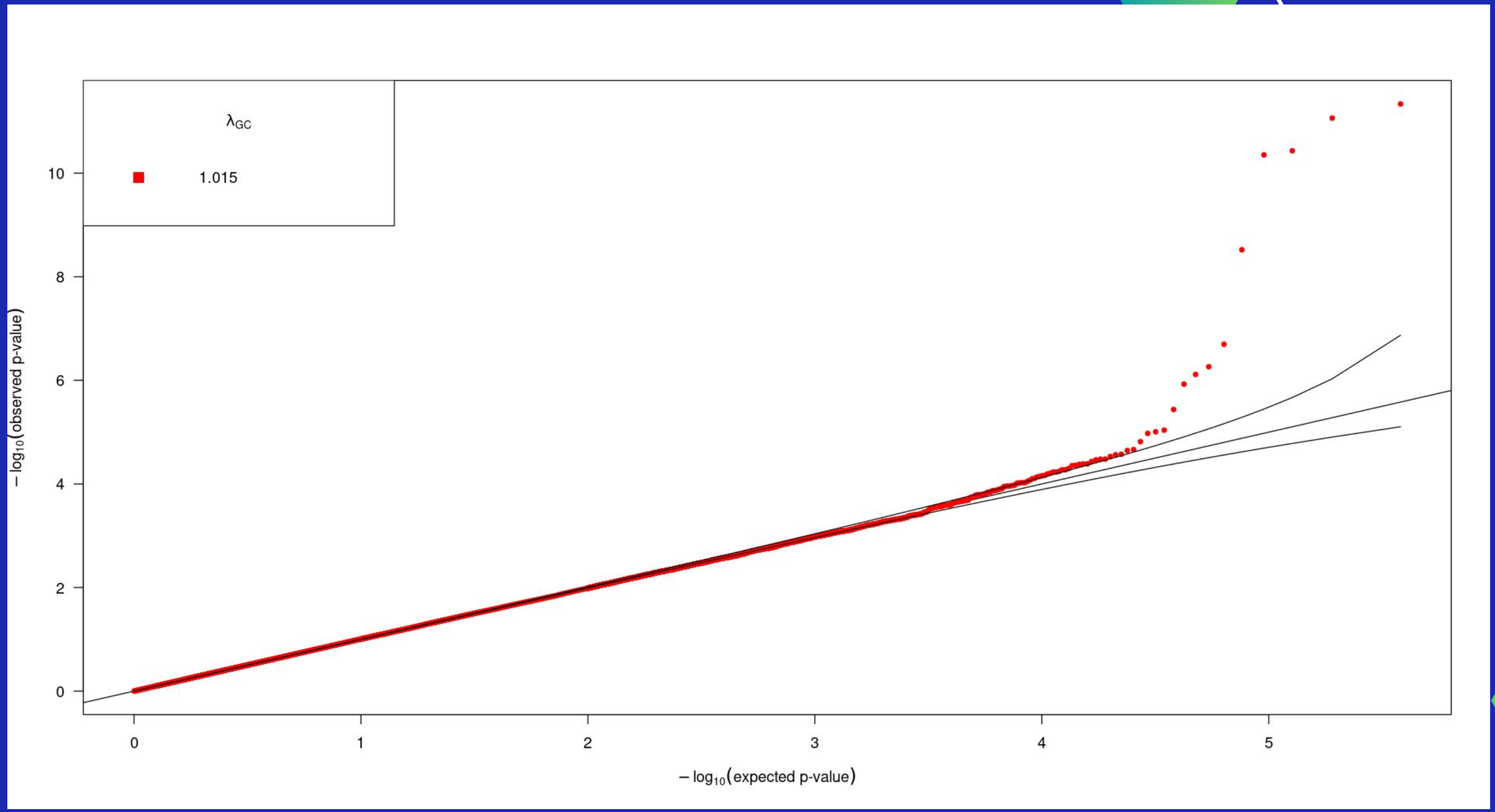
21
Chromosome

Genomic control



Preliminary hits

SNP	P-value	Chromosome	Gene	Position	A1	A2	Clinical SIgnificance	GWAS catalog status
rs748855	0.000000000005	16	*NOD2*	50751398	C	T	Possible	Not reported
rs1861758	0.000000000009	16	*NOD2*	50751787	T	C	Possible	Not reported
rs3135503	0.00000000004	16	*CYLD*	50791250	G	T	Not reported	Not reported
rs3135499	0.00000000004	16	*NOD2, LOC124903774*	50766127	G	T	More possible	Not reported
rs9635531	0.000000003	16	*LINC02168*	50841795	A	G	Not reported	Not reported
rs17221417	0.0000002	16	*NOD2*	50739582	C	G	Possible	Reported
rs1253227	0.0000005	11	*MAML2*	96029482	G	A	Not reported	Not reported
rs5743266	0.0000008	16	*NOD2*	50731096	T	C		
rs3785142	0.00000118211857916311	16	*CLYD*	50787147	G	A	Not reported	Not reported
rs12455802	0.00000365675300118725	18	*PIGN*	59582470	C	G	Not reported	Not reported
rs2890698	0.00000915661334099731	4	*SMIM14, UGDH-AS1*	39586775	T	G	Not reported	Not reported
rs4506998	0.00000983751181627577	18		38643072	A	G	Not reported	Not reported

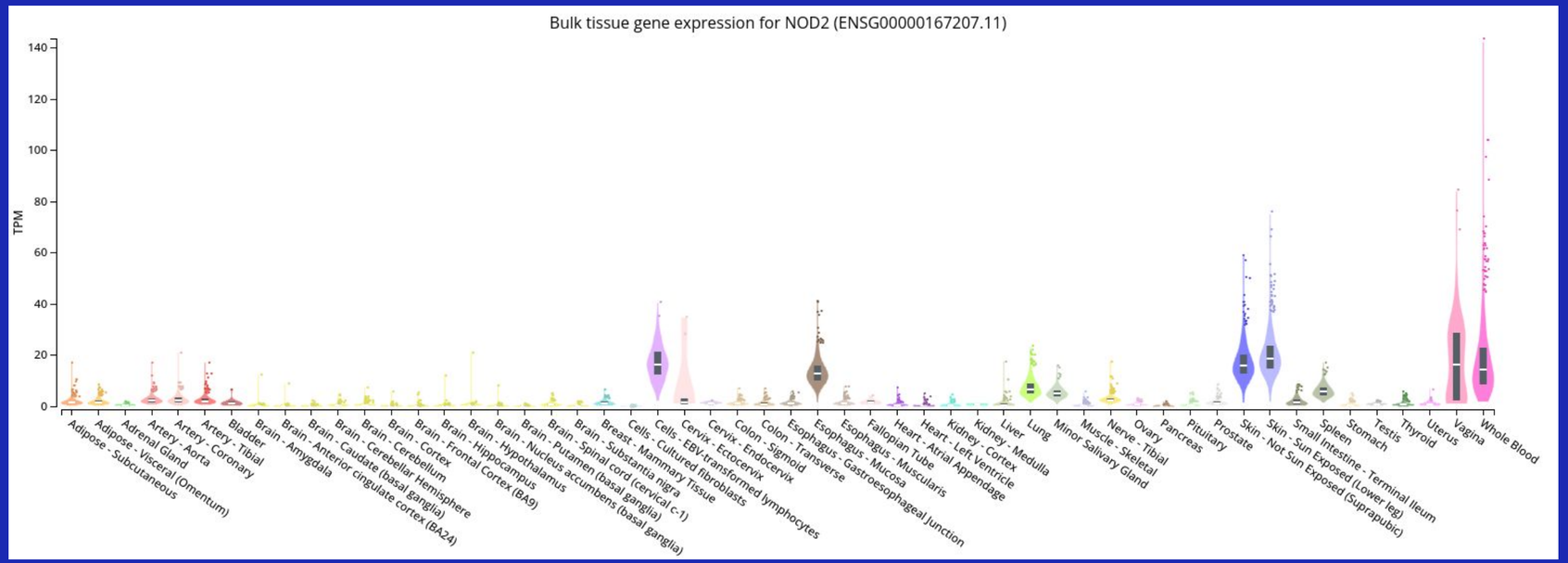
Final hits

SNP	P-value	Chromosome	Gene	Position	A1	A2	Clinical SIgnificance	GWAS catalog status
rs748855	0.000000000005	16	*NOD2*	50751398	C	T	Possible	Not reported
rs1861758	0.000000000009	16	*NOD2*	50751787	T	C	Possible	Not reported
rs3135503	0.00000000004	16	*CYLD*	50791250	G	T	Not reported	Not reported
rs17221417	0.0000002	16	*NOD2*	50739582	C	G	Possible	Reported
rs3785142	0.00000118211857916311	16	*CLYD*	50787147	G	A	Not reported	Not reported

The following databases were researched: **Bravo, ClinVar** (if the registry existed)**, dbSNP, GWAS catalog, GnomAD**

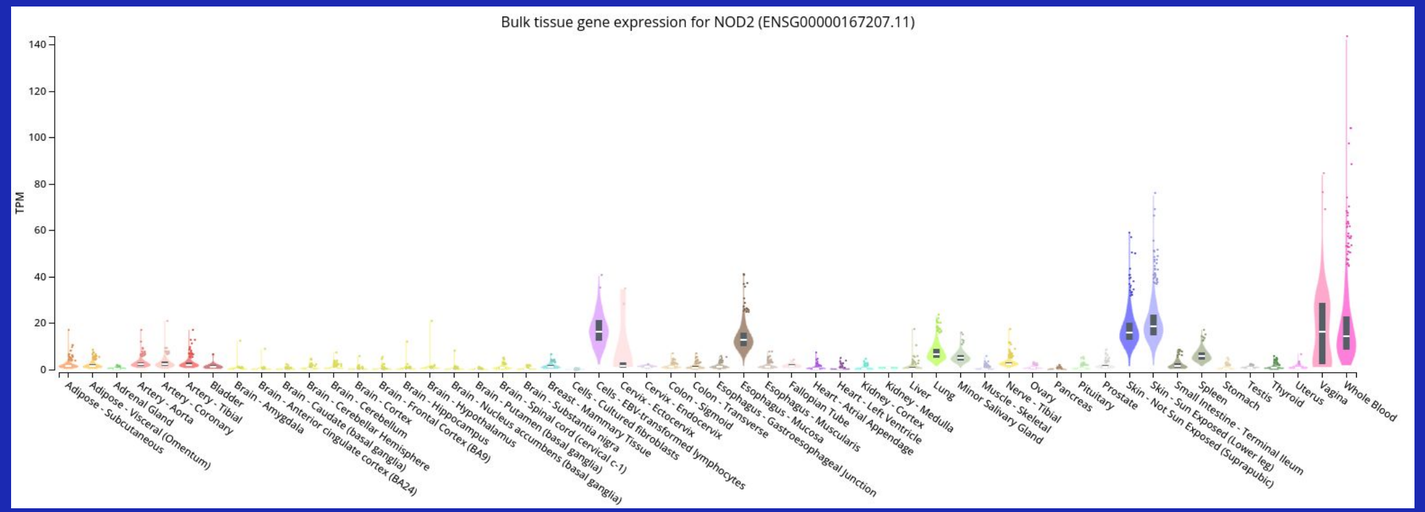
NOD2

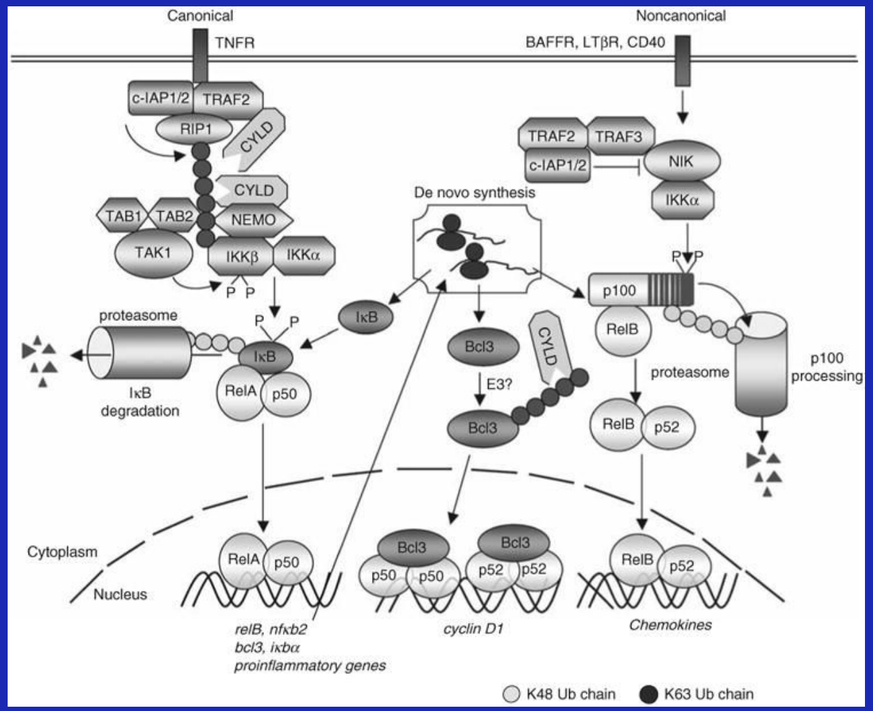
- Involved in **recognizing certain bacteria and stimulating the immune system to respond** appropriately.
- When triggered by specific substances produced by bacteria, the NOD2 protein **turns on** a protein complex called **nuclear factor-kappa-B.**
  - This protein complex regulates the activity of multiple genes, including genes that control immune responses and inflammatory reactions.
- Its variation is associated with Crohn's disease risk



CYLD

- Deubiquitinating enzyme that removes lys63-linked ubiquitin chains and acts as a negative regulator of NF-kappa-B signaling.
- *CYLD* deubiquitinates several NF-kappa-B regulators, including TRAF2 , TRAF6, and NEMO.
- Down regulated in IBD.
- Anti-inflammatory role.





CYLD

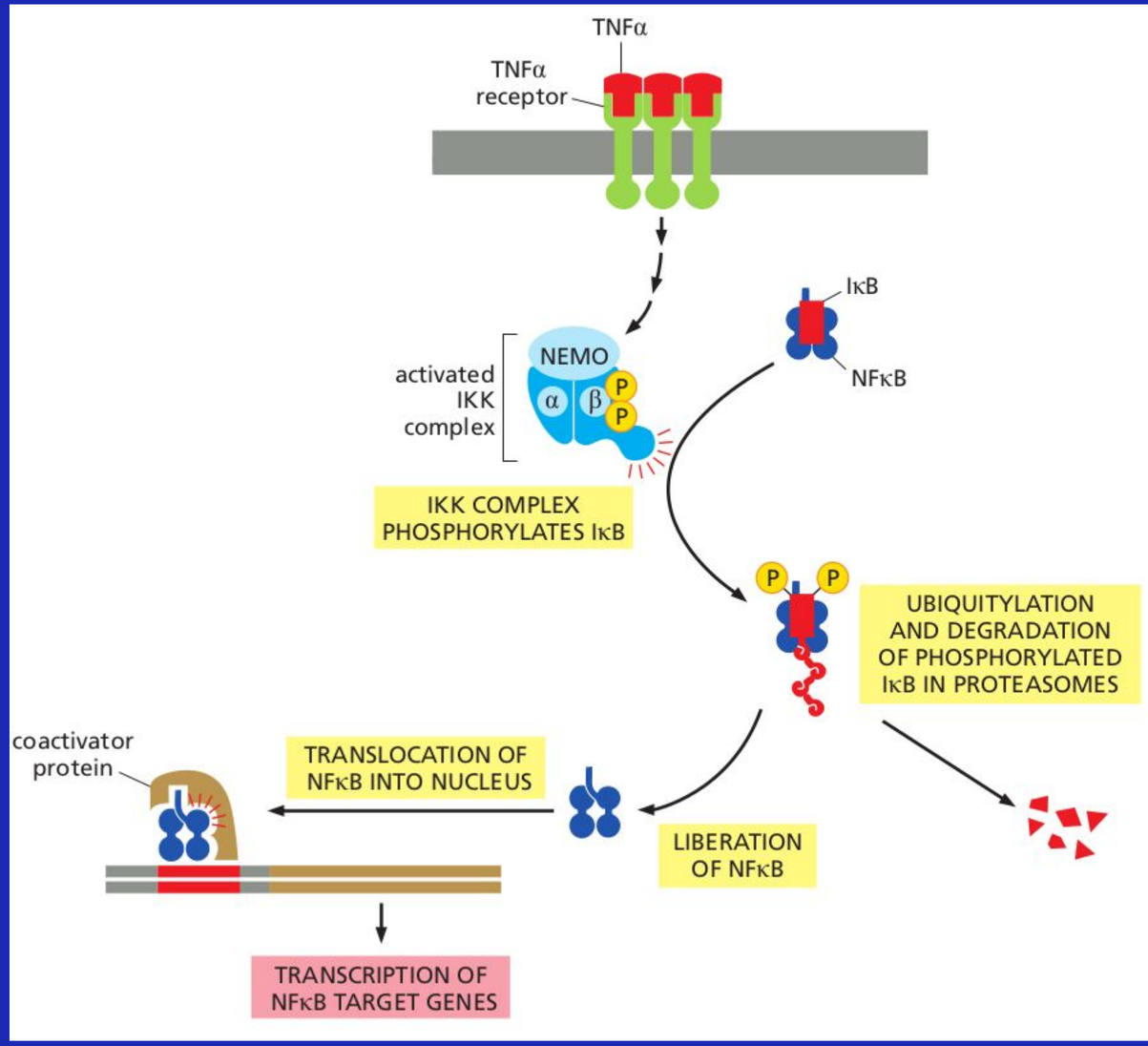


Figure 15–62 The activation of the NFκB pathway by TNFα. Both TNFα and its receptors are trimers. The binding of TNFα causes a rearrangement of the clustered cytosolic tails of the receptors, which now recruit various signaling proteins, resulting in the activation of a protein kinase that phosphorylates and activates IκB kinase kinase (IKK). IKK is a heterotrimer composed of two kinase subunits (IKKα and IKKβ) and a regulatory subunit called NEMO. IKKβ then phosphorylates IκB on two serines, which marks the protein for ubiquitylation and degradation in proteasomes. The released NFκB translocates into the nucleus, where, in collaboration with coactivator proteins, it stimulates the transcription of its target genes.

Conclusions

The susceptibility to IBD appears to be linked to various variations in the *NOD2* gene.

In the course of this study of association, there were identified three variants that had been reported previously.

Additionally, there were detected variants in the *CLYD* gene, previously unrecognized as pathogenic, suggesting their potential involvement in the development of IBD.

This investigation postulates that variants within the *CLYD* gene may contribute to the onset of inflammatory bowel disease.

Resources

- Biology Lectures (Director). (2019). NOD like receptor signaling pathway (NOD1/NOD2 signaling pathway). https://www.youtube.com/watch?v=_y9F8C_wXVg
- Hussain Biology (Director). (2019, February 18). NF-κB Pathway | Cell Survival Pathway. https://www.youtube.com/watch?v=8HWVhdSRvng
- Inflammatory Bowel Disease (IBD). (2022, May 16). https://www.hopkinsmedicine.org/health/conditions-and-diseases/inflammatory-bowel-disease
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- Roda, G., Chien Ng, S., Kotze, P. G., Argollo, M., Panaccione, R., Spinelli, A., Kaser, A., Peyrin-Biroulet, L., & Danese, S. (2020). Crohn's disease. Nature Reviews Disease Primers, 6(1), Article 1. https://doi.org/10.1038/s41572-020-0156-2
- Seyedian, S. S., Nokhostin, F., & Malamir, M. D. (2019). A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease. Journal of Medicine and Life, 12(2), 113–122. https://doi.org/10.25122/jml-2018-0075
- Sun, S.-C. (2010). CYLD: A tumor suppressor deubiquitinase regulating NF-κB activation and diverse biological processes. Cell Death and Differentiation, 17(1), 25–34. https://doi.org/10.1038/cdd.2009.43