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Clinical and genetic factors associated with disease course in inflammatory bowel disease

Spekhorst, Lieke Maaike

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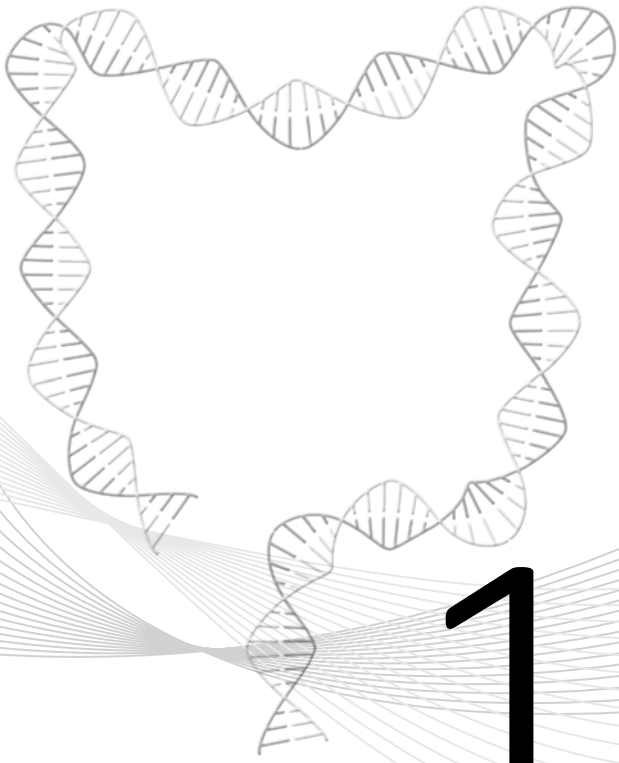

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Conclusions, Discussion and Future Perspectives

Conclusions

The aim of my thesis was to identify clinical and genetic predictors for specific IBD disease phenotypes.

In order to identify factors associated with a specific disease behaviour, it is necessary that patient phenotypes are described consistently. The Montreal classification is a classification system for sub-phenotypes of both CD and UC that is widely used in the clinic, but for which almost no data on its reliability and reproducibility was available. In **chapter 2**, we therefore validated the Montreal classification among 30 observers with different professions (gastroenterology specialist in IBD, gastroenterologist in training, and IBD-nurses) in 20 de-identified medical records. We found good to excellent inter-observer agreement for all Montreal items except disease severity in UC, for which agreement was poor. This validation step was important for **chapter 3**, which provides an overview of the phenotypic characteristics currently present in the IBD Parelsnoer cohort, where Montreal classification is an important phenotype. This cohort was founded by a collaboration of the eight University Medical Centers (UMCs) in the Netherlands, and consists of 3388 IBD patients. Chapter 3 describes the design and baseline characteristics for 225 IBD-related items present in the IBD Parelsnoer cohort. This chapter also describes the IBD Parelsnoer cohort's potential to facilitate clinical and basic science and to improve clinical care. Data presented in chapter 3 is used in subsequent chapters to assess clinical parameters associated with phenotypic differences in patients with IBD.

As patients with IBD can suffer significantly from disease symptoms or flares, they are at risk for work disability. In **chapter 4**, I assessed potential (clinical) risk factors for work disability in IBD, and found that work disability was associated with female sex, a lower education level, extraintestinal manifestations, an age > 55 years, an age > 40 years at diagnosis, a disease duration > 15 years, smoking, surgical interventions, anti-TNFα use and immunomodulator use. In CD patients, long-term full work disability (defined as > 80% work disability for > 2 years) was associated with a lower education level. In UC patients, long-term full work disability was associated with complications (osteopenia, thromboembolic events).

The incidence of IBD in developing countries may be increasing due to industrialization and Western lifestyle changes, but these changes cannot explain the phenotypic heterogeneity within and between regions. In **chapter 5**, I therefore assessed the impact of ethnicity and country of birth on IBD phenotype. What I found was that patients of non-Caucasian descent (non-CEU) with CD more often had upper gastro-intestinal disease (L4) and anal stenosis than patients with CD from West- and Central-European Caucasian descent (CEU). Non-CEU patients with IBD used more anti-TNFα agents and immunomodulators than CEU patients. The most interesting finding

was related to country of birth: non-CEU IBD patients born in Europe were diagnosed at a younger age than non-CEU IBD patients born outside Europe, implying that Western lifestyle might trigger IBD onset more early in life.

Treatment strategies in IBD are still applied equally to male and female patients. If we want to move further towards personalized treatment it is important to be aware of differences in IBD disease course and disease phenotypes between the sexes. In **chapter 6**, I focused on differences between males and females with IBD, comparing phenotype, clinical manifestations, disease course, medical treatment and other healthcare consumption. What we found was that early onset CD (< 16 years) was more frequently seen in males, male patients with CD more often had ileal disease and male patients more often underwent small bowel and ileocaecal resection. Male patients with CD also more frequently suffered from osteopenia and used prednisone more often. Extraintestinal manifestations (EIMs), on the other hand, were more often observed in female IBD patients. IBD-specific healthcare costs did not differ between male and female IBD patients.

In Part II of this thesis I identified genetic risk loci associated with disease behaviour. Part II starts with a review (**chapter 7**) describing the clinical presentation of IBD and gives an overview of the progress that has been made in the field of IBD genetics, from linkage and candidate studies to GWAS and Immunochip studies. Chapter 7 then describes the genetic and biological pathways of IBD and its overlap with other immune-mediated disease. In the last part of the review, I focus on genetic findings and how we can translate these findings to clinical practice.

Hidradenitis suppurativa (HS) is a chronic inflammation of the apocrine glands that is often followed by sinus tract formation and scarring. Although HS is not yet a well-recognized EIM in IBD, the prevalence of HS in IBD is much higher than in the general population, which suggests a shared pathogenesis. In **chapter 8**, I identify genetic and clinical parameters associated with HS in IBD. Female sex, patients with CD, smoking, a higher body mass index, and younger age were independently associated parameters for HS in IBD. The within-cases allelic association analysis revealed a suggestive protective association with the *ELOVL7* gene and a suggestive risk association with the genes *SULT1B1* and *SULT1E1* for HS in the context of IBD.

The need for surgery for fibrostenotic disease in patients with CD is an indicator of a severe disease course. In a within-cases allelic association analysis, we identified the *WWOX* gene as a disease-modifying gene associated with recurrent fibrostenotic CD disease (**chapter 9**). Functional studies showed an enhanced colonic expression of Transforming Growth Factor-beta (TGF-β) in *WWOX* risk-allele carriers, supporting our hypothesis of *WWOX* as having a role in fibrosis formation.

Anti-TNFα agents are widely used for inducing and maintaining clinical remission in patients with CD. However, some patients develop anti-drug antibodies (ADAs) to anti-TNFα, resulting in loss of response. In **chapter 10,** I identified genetic risk variants that play a role in the development

of ADAs to anti-TNFα (infiximab and adalimumab) by comparing IBD patients who developed anti-TNFα ADAs to IBD patients without these ADAs. I was able to replicate the association of the HLA-DQA1*05 allele associated with ADA formation to anti-TNFα. However, I was not able to replicate the association with the HLA-DQB1*03 allele, which is also known to be associated with anti-TNFα ADAs. I further identified eight suggestive association signals in non-HLA regions, which will need to be replicated in larger cohorts.

Discussion and Future Perspectives

Future of patient care - Precision medicine

The goal of precision medicine is to tailor medical decisions or practice to the needs of an individual patient using epidemiological, genetic or other molecular or cellular analysis. Precision medicine should create the ability to classify individuals into subpopulations, to weigh the chance of an individual developing a particular disease, to predict that individual's disease course/prognosis, and to know which medication or intervention that patient will benefit most from. In precision medicine the right group of patients will benefit from the right treatment at the right moment, while other patients will be protected from the side-effects or from adverse outcomes.

The concept of precision medicine is not novel. General practitioners have been using precision medicine in their daily practice for some time now. For example, the ten-year-risk of a cardiovascular event is assessed for each individual patient depending on the patient's clinical characteristics and parameters such as age, sex, smoking status, systolic blood pressure, and TC/HDL ratio. The combination of these risk factors predicts the risk of a cardiovascular event for the individual patient in the coming ten years. Depending on this risk score, a patient will then receive either solely lifestyle advice or medical treatment with statins. This is an example of a precision medicine model in which only clinical parameters have been included. Inclusion of other layers, for example genetic status, could further improve the performance of risk prediction models.

In 2015 Barack Obama, former president of the United States (US) announced the launch of Precision Medicine Initiative later called "All of Us" Research Program. The National Institute of Health (NIH) leads this initiative to build a national large-scale research enterprise with extensive information about lifestyle, environment and genetics. Participants will be from diverse ancestral, racial/ethnic, geographic, social and economic backgrounds, and from all age groups and health statuses, reflecting the diversity of the US population. The idea is to enrol more than one million US citizens. The main objective of the "All of Us" Research Program is to enable scientific research for common and rare disease and increase our knowledge of an individual's chances of staying healthy throughout life. Although we don't have a national Biobank initiative of this size or scope

in the Netherlands, we do have Lifelines, which is comparable to the "All of Us" Research Program in several ways. Lifelines is a population-based prospective cohort that includes more than 167,000 participants from the three Northern provinces of the Netherlands. This group of participants consists of three generations and will be followed-up over the coming thirty years. The main objective of Lifelines is to enable research to monitor the process of ageing and to identify factors related to health and disease. Detailed phenotypic and environmental factors are collected by questionnaire every 18 months. Health status parameters are measured every 5 years. For 10% of the participants, genetic information is available. In addition to this large population-based cohort, 1500 participants were asked to participate in the Lifelines DEEP cohort. Where Lifelines is comparable to "All of Us" Research Program, Lifelines DEEP takes it to the next level by including many more molecular layers. For the 1500 Lifelines DEEP participants, exhaled air (analysis of volatile organic compounds), faecal samples (microbiome and biomarker assessment), and additional blood (genetics, methylation and transcriptomics analyses) were collected. This rich dataset enables us as researchers to combine several molecular layers and address questions related to ageing from different perspectives (demographics, genotype, microbiome, transcriptome and methylation).

Another example of a cohort suitable for translational research is the IBD Parelsnoer cohort that I worked with during my PhD project. The IBD Parelsnoer cohort aims to facilitate the discovery of predictors (both epidemiological risk factors and biomarkers) for individual disease course and treatment response in IBD. Major strengths of the IBD Parelsnoer cohort are its prospective design, its extensive uniform information model comprising 225 data items, and the participation of all eight UMCs in the Netherlands. In addition, biomaterials such as serum, DNA, stool samples and, if available, biopsies from endoscopy and resection tissue are collected. This enables researchers to discover new biomarkers by the integration of molecular and clinical phenotypes. Another strength of the IBD Parelsnoer cohort is its wide collaboration with international IBD research groups.

An example of how precision medicine has made great progress by adding molecular levels comes from the field of oncology. Almost all tumours are now screened on a molecular level, producing information that allows medication to be adjusted to the individual patient and allows for chemotherapy—which is both physically and financially taxing—only to be prescribed to patients who will benefit from it. Severe side-effects in patients who don't benefit from these drugs and high medical costs can thus be prevented.

Precision medicine in IBD and other complex diseases

For complex multifactorial diseases such as IBD, precision medicine is still in its infancy, but it is starting to evolve. Precision medicine in IBD begins with the identification of clinical parameters that could influence IBD risk. Having had an appendectomy, for example, is protective against the development of UC, while smoking can increase the risk of developing CD. Since the influence

of genetic factors became evident in IBD (heritability in monozygotic twins), we have added the first molecular layer—genetics—by performing linkage studies and candidate gene association studies. In 2000, we started with GWAS analysis and by 2012 had identified 163 loci associated with IBD risk. However, these genetic risk variants only explained 20% of the genetic disease risk. To identify new genetic risk variants (which probably have much smaller effects) by GWAS, an immense increase in statistical power was needed. As most of the samples available in the Western World had already been included in previous studies, a thousand non-Caucasians samples were added. With the inclusion of these samples, we were able to identify more genetic variants associated with disease risk and bring the total number of genetic loci for IBD risk up to 242. However, increasing the number of risk loci did not add to the explained heritability, which remained at slightly over 20%. Furthermore, these identified genetic risk variants explain only about 10% of the total disease risk, suggesting that environmental and other unidentified molecular factors play a role in the pathogenesis of IBD. Although finding new genetic risk variants associated with IBD was the main goal of GWAS and Immunochip studies, this goal has now shifted towards identifying environmental factors that influence disease risk, and finding genetic variants associated with a specific disease phenotype.

As the name 'precision medicine' indicates, the goal is to create an algorithm that can predict disease course, the patient's individual response to therapy and any adverse drug response, as opposed to solely determining risk to develop IBD. Therefore, the second part of my thesis focuses on the identification of genetic variants associated with specific disease phenotypes (hidradenitis suppurativa in IBD in Chapter 8, and recurrent fibrostenotic CD in Chapter 9) and immunogenicity to anti-TNFα (antibody development against anti-TNFα treatment in Chapter 10). Unfortunately, my sample size in Chapter 8 and Chapter 10 was too small to gain enough power to detect an association signal at genome-wide statistical significance level (p-value < 5×10^{-8}). One way to increase power is by adding more samples. Luckily, large consortia are now collaborating to unravel the genetic background of disease behaviour and response to drugs. These international consortia have, for example, found that the *HLA-DQA1-HLA-DRB1* haplotype confers susceptibility to thiopurine-induced pancreatitis. Patients who are homozygotic for genetic variant rs2647087 have a 17% risk of developing pancreatitis after administration of a thiopurine in comparison to heterozygote patients, who have a 9% risk.

In addition to better understanding drug toxicity, we also aim to identify factors that predict drug response. Two new biological drugs, vedolizumab and ustekinumab, have been registered in the Netherlands as drug therapy for refractory disease in IBD. Both target a different pathway than well-known anti-TNFα agents (infliximab, adalimumab, golimumab). Vedolizumab has been shown to be more effective in biological-naïve patients compared to biological-exposed patients. We are now taking mucosal biopsies from biological-naïve patients and anti-TNFα-exposed patients before

and after treatment with vedolizumab. When we have identified which immune cells in the mucosal layer are associated with response to vedolizumab treatment, we can compare these to profiles of mucosal infiltrates present in pre-treatment biopsies. By doing so, we will be able to personalize vedolizumab treatment by selecting those patients who are likely to respond to vedolizumab treatment. An important question remains, however: Will it be cost-effective to determine these risk variants in every patient before starting treatment? What could make this financially feasible is if many specialties in the UMCG together develop a "response to drug" panel that includes variants associated with drug toxicity and variants associated with drug response. Testing every patient with a chronic disease before start of treatment might then be cost-effective.

Thus far, GWAS and Immunochip studies have identified 242 loci associated with IBD risk. Some of these risk variants are disease-specific, but most are shared between IBD and other immune mediated diseases. These associated-variants are unlikely to be the causal because they are quite common in the general population (minor allele frequency > 1%). Moreover, these variants mark genomic loci, and these loci can contain several genes in the human genome that influence disease risk. The effect size of genetic risk variants on disease risk tends to be much smaller in complex disease such as IBD, as compared to Mendelian or oligogenic diseases, where rare genetic variants often confer larger risk effects. An example of an oligogenic disease is very-early onset IBD (VEO-IBD), which is characterized by a severe disease course that is unresponsive to conventional therapy. Whole exome sequencing (WES) has identified mutations in the *IL10RA/B* genes in patients with VEO-IBD. This suggests that these rare genetic variants are highly penetrant and have a large effect size that causes this Mendelian-like or oligogenic form of IBD. VEO-IBD patients with these mutations are generally refractory to standard immunosuppressive therapy, which means that hematopoietic stem cell transplantation should be considered as a potential curative treatment option early in the disease course. Through WES we can identify rare genetic variants with large effect sizes that are only present in a small number of patients, and therefore not detected with genotyping arrays such as the Immunochip. In addition, these severe disease-causing variants are much more likely than low-impact variants to be located in the protein coding part of genes that is covered by WES. WES can help us to identify rare or novel genetic variants in Mendelian-like or oligogenic forms of IBD.

As discussed above, the genetic risk variants identified so far only explain 10% of the risk for disease, which suggests that environmental factors and other unidentified factors also have a role. We now know that the microbiome plays an important role in IBD because there is a clear difference in the microbiome between patients with IBD and healthy controls. This would suggest that it is possible to create a model that can distinguish healthy people from individuals suspected of having IBD by characterizing their gut microbiome. Furthermore, differences in microbiome profiling between IBD patients with active disease and IBD patients in remission could be used to

monitor therapeutic targets. The microbiome is also a relatively easy accessible treatment target. Medication, dietary changes or even faecal microbiome transplantation (FMT) that target the right bacteria could shift the microbiome towards a more 'healthy' gut. FMT has already changed the standard of care for *Clostridium difficile* infection. In IBD, however, the role of FMT is still emerging, and there are no definite conclusions as of yet. For now, the heterogeneity of the disease makes results from different studies difficult to compare and to interpret. For the future, with the right selection of microbial consortiums, microbiome profiling could be a promising development for 'precision medicine'. Other upcoming research fields, such as transcriptomics and methylation studies, are beyond the scope of this thesis. Hopefully, in the coming years, it will be possible to take these layers of molecular information into account in the precision medicine model.

To conclude, IBD is a complex disease. Environmental factors, the microbiome and genetic factors all have an effect on disease risk, disease behaviour, and response to therapy. Combining these factors into risk models for the individual patient is the goal of precision medicine, a goal that will hopefully become feasible in the coming decade.

Precision medicine in this thesis

Throughout this thesis I have used data from the IBD Parelsnoer cohort. Its prospective design, extensive phenotype collection and standardized biomaterial collection, make IBD Parelsnoer very suitable for translational research. With the data available in this biobank, it will be feasible to identify modulators that influence disease course. Biomarker discovery research to assess response to treatment or adverse outcomes is also possible. The data collection process is the main focus of future improvement. Currently, clinical information is collected during each patient visit. Data obtained through web-based questionnaires at standard time points (for example every three months) would be a highly valuable addition. These Patient Reported Outcomes Measurements (PROMS) could give us more insights into time-to-event related questions. For example, if a patient presents with a flare at the patient clinic, we could examine if their PROM shows any signs that pointed towards the development of this flare, e.g. an increase in diarrhoea frequency. This would be an even more reliable way to identify modulators that influence the disease course.

Another important point that needs to be addressed is treatment target goals in IBD. For decades clinic care and clinical trials have focused on the induction of clinical response and maintaining clinical remission. However, treatment strategies focused on clinical remission have not altered the disease course of IBD. Therefore, it is important that we look beyond just the treatment of symptoms toward achieving better outcomes by preventing bowel damage and disability. Mucosal healing and deep remission are currently suggested as good candidates for disease modification, but it has not yet been proven that achieving and maintaining deep remission will alter the disease course of IBD. I think that the IBD Parelsnoer cohort is a very suitable resource

for addressing this 'treatment target' goal, and that we should use this as a clinical endpoint when we assess modulators that can influence the disease course in IBD.

I was the first person to get the opportunity to work with the data available in the IBD Parelsnoer cohort, and this has taught me several important lessons. While working with the data, it became evident that clear definitions are of the utmost importance, as are internal data checks. For example, a clinician should not be able to fill out a Harvey Bradshaw Index for a patient with UC, as this index is only meant for CD patients. To make the data more reliable, we should be able to go back to the patient records. On the other hand, information security guidelines such as the international ISO 27.001 guarantee patient privacy, which is of utmost importance. The IBD Parelsnoer cohort collects phenotypes and biomaterials in a standardized manner, making international collaboration feasible. The IBD Parelsnoer cohort is also part of the Biobanking and Biomolecular Resources Research Infrastructure of the Netherlands (BBMRI-NL), which is the Dutch national node of BBMRI-ERIC, the largest research infrastructure project in Europe. The IBD Parelsnoer cohort is quite young and has the opportunity to grow. I think that choosing to add more molecular layers to the IBD Parelsnoer cohort, e.g. microbiome data, is a valid alternative to increasing the sample size. I believe that adding more molecular layers will help us built a precision medicine logarithm model. I think the IBD Parelsnoer cohort is a great initiative that creates opportunities for research.

Conclusions of this thesis

- The Montreal classification is a reliable tool for the assessment of sub-phenotypes in IBD with the exception of disease severity in UC.
- Female sex, a lower education level, extraintestinal manifestations, an age > 55 years, an age > 40 years at diagnosis, a disease duration > 15 years, smoking, surgical interventions, anti-TNFα use, and immunomodulator-use are associated with work disability in IBD.
- A lower education level in patients with CD and complications (osteopenia, thromboembolic event) in patients with UC are associated with long-term full work disability (> 80% work disability for > 2 years).
- Compared to patients of West- and Central-European Caucasian descent, patients of non-Caucasian descent more often have upper gastro-intestinal disease and anal stenosis and are prescribed more anti-TNFα and immunomodulators.
- Patients of non-Caucasian descent born in Europe are diagnosed at a younger age than patients of non-Caucasian descent born outside Europe.
- Early onset CD (< 16 years) is more frequently seen in males.
- Male patients with CD are more likely to have ileal disease and more often undergo small bowel and ileocaecal resection than females.

- Male patients with CD more frequently suffer from osteopenia and use prednisone more often than female CD patients.
- Extraintestinal manifestations are more often observed in female IBD patients.
- IBD-specific healthcare costs do not differ between male and female IBD patients.
- Female sex, patients with CD, smoking, a higher body mass index, and younger age are independently associated parameters for hidradenitis suppurativa in IBD.
- For hidradenitis suppurativa in IBD, we found a suggestive protective association with *ELOVL7* and suggestive risk association with the genes *SULT1B1* and *SULT1E1*.
- The *WWOX* gene is a disease-modifying gene associated with recurrent fibrostenotic CD disease.
- I have replicated the association of the HLA-DQA1*05 allele previously associated with anti-drug antibody (ADA) formation to anti-TNFα in IBD patients.
- I was not able to replicate the association with the HLA-DRB1*03 allele previously associated with ADA formation to anti-TNFα in IBD patients.
- A genome-wide association meta-analysis identified eight suggestive association signals in non-HLA regions associated with anti-TNFα ADAs in IBD patients.