ACKNOWLEDGEMENTS

The present Ph.D. thesis is based on studies, which were carried out during my employment as a research fellow at the Department of Medical Gastroenterology, Herlev Hospital, 2002-2005.

I sincerely thank my supervisors Vibeke Binder MD, DMSci, Pia Munkholm, MD, DMSci and Ebbe Langholz MD, DMSci for introducing me to the EC-IBD study group in January 2001. Your enthusiasm, engagement and patience have been outstanding. Thank you for the numerous hours of supervision and for your continuous support in the process of writing manuscripts. I shall miss our Tuesday afternoons with inspiring discussions on research, IBD and other aspects of life.

I wish to express my sincere gratitude to Pia, my daily mentor and “chief”. You introduced me to the international world of IBD and taught me that research is not only hard work but also lots of fun. Thank you for giving me the opportunity to travel round the world participating in congresses and meetings in the continuous search for new knowledge. Your enormous enthusiasm and unselfishness has been outstanding.

Thank you to the members of the EC-IBD study group for their contribution to this thesis. All of you have done a great job collecting data and commenting on manuscripts. Thank you for all the hospitality you have shown me during the many EC-IBD meetings.

Also a warm thank to all my office mates Tine Jess, MD, Lotte Dinesen, MD, Vibeke Wewer MD, Ph.D, Sarah Caspersen, Stud.med. The collaboration with you has been a great pleasure. We have had many rewarding and inspiring discussions and I hope we will continue working together.

I thank all the scientific staff at the Laboratory of Medical Gastroenterology, Herlev Hospital. Especially thanks to the laboratory technicians for their pleasant company and assistance in all my different projects.

Also I have to thank members of the YKG for hours of gossip and fruitful discussions (in the mentioned order). Special thanks goes to Ida Vind, colleague, travel companion, but most of all friend. Thank you for all your support and encouragement. We had fun.

I also wish to thank my parents and Behka for their invaluable, unconditional and unlimited help.

Finally I express my deepest gratitude to my husband Søren for his never-ending patience and back-up. Thank you to my children Nadja, Thomas and Ida for reminding me what life is all about.

The studies in this thesis were supported by grants from The European Commission (QLG-CT-2000-01414), The Danish Colitis-Crohn Foundation (Colitis-Crohn Foreningen) and The Vibeke Binder and Povl Riis Foundation.

Lene Buhl Riis
Herlev, December 2005

To Thomas, Ida and Nadja
PREFACE

The present Ph.D. thesis is based on five papers made during my employment at the department of medical gastroenterology, at Herlev Hospital 2002-2005. The thesis consists of an introduction followed by an outline of the methods used. Thereafter major results from the individual papers are presented individually, followed by an overall conclusion.

The thesis is based upon the following papers, which will be referred to by their roman numerals:

I:
Riis L, Munkholm P, Binder V, Skovgaard LT, Langholz E.
Intra- and Interobserver Variation in the Use of the Vienna Classification of Crohn’s Disease. Inflamm Bowel Dis 2005;11:657-661

II:

III:

IV:
Does pregnancy change the disease course? A study in a European cohort of patients with Inflammatory Bowel Disease. Accepted for publication in Am J Gastroenterol.

V:
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>1</th>
<th>INTRODUCTION AND AIMS</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>MATERIALS AND METHODS</td>
<td>8</td>
</tr>
<tr>
<td>2.1</td>
<td>The EC-IBD cohort</td>
<td>8</td>
</tr>
<tr>
<td>2.1.1</td>
<td>Data acquisition tools</td>
<td>9</td>
</tr>
<tr>
<td>2.1.2</td>
<td>Data collection</td>
<td>9</td>
</tr>
<tr>
<td>2.1.3</td>
<td>Data management and validation</td>
<td>9</td>
</tr>
<tr>
<td>2.1.4</td>
<td>Final patient population</td>
<td>10</td>
</tr>
<tr>
<td>2.1.5</td>
<td>Genetic and serological analysis</td>
<td>10</td>
</tr>
<tr>
<td>2.1.6</td>
<td>Definitions</td>
<td>11</td>
</tr>
<tr>
<td>2.2</td>
<td>Pregnancy consensus</td>
<td>12</td>
</tr>
<tr>
<td>2.3</td>
<td>Statistical methods</td>
<td>14</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Intra- and interobserver variation</td>
<td>14</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Population attributable risk</td>
<td>14</td>
</tr>
<tr>
<td>2.3.3</td>
<td>Comparison of groups</td>
<td>14</td>
</tr>
<tr>
<td>2.3.4</td>
<td>Correlation analysis</td>
<td>14</td>
</tr>
<tr>
<td>2.3.5</td>
<td>Cox regression analysis</td>
<td>14</td>
</tr>
<tr>
<td>2.4</td>
<td>Intra- and interobserver variation when assessing disease extent and behaviour</td>
<td>15</td>
</tr>
<tr>
<td>2.4.1</td>
<td>Method</td>
<td>15</td>
</tr>
<tr>
<td>2.4.2</td>
<td>Results (paper I)</td>
<td>15</td>
</tr>
<tr>
<td>2.4.3</td>
<td>Discussion</td>
<td>16</td>
</tr>
<tr>
<td>2.5</td>
<td>Ethical considerations</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>RESULTS AND DISCUSSION</td>
<td>17</td>
</tr>
<tr>
<td>3.1</td>
<td>Prevalence of genetic and serologic markers in a population-based cohort</td>
<td>17</td>
</tr>
<tr>
<td>3.1.1</td>
<td>Results (paper II)</td>
<td>17</td>
</tr>
<tr>
<td>3.1.2</td>
<td>Discussion</td>
<td>18</td>
</tr>
<tr>
<td>3.2</td>
<td>Phenotypes and genotypes in an unselected cohort of IBD patients</td>
<td>19</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Results (paper III)</td>
<td>19</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Discussion</td>
<td>20</td>
</tr>
<tr>
<td>3.3</td>
<td>Pregnancy, a factor influencing disease course and phenotype in IBD</td>
<td>22</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Results (paper IV)</td>
<td>22</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Discussion</td>
<td>23</td>
</tr>
<tr>
<td>3.4</td>
<td>Fertility and pregnancy outcome, important issues also in IBD</td>
<td>23</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Results (paper IV and V)</td>
<td>24</td>
</tr>
<tr>
<td>3.4.2</td>
<td>Discussion</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>CONCLUSION AND PERSPECTIVES</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>SUMMARY</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>DANSK RESUMÉ</td>
<td>29</td>
</tr>
<tr>
<td>7</td>
<td>REFERENCES</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>APPENDIX A</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>APPENDIX B</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------</td>
<td></td>
</tr>
<tr>
<td>ASCA</td>
<td>Anti-Saccharomyces cerevisiae antibodies</td>
<td></td>
</tr>
<tr>
<td>CARD</td>
<td>Caspase-Activation Recruitment Domain</td>
<td></td>
</tr>
<tr>
<td>CD</td>
<td>Crohn’s disease</td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
<td></td>
</tr>
<tr>
<td>ECCO</td>
<td>European Crohn’s &amp; Colitis Organization</td>
<td></td>
</tr>
<tr>
<td>EC-IBD</td>
<td>European Collaborative study group on Inflammatory Bowel Disease</td>
<td></td>
</tr>
<tr>
<td>EL</td>
<td>Evidence level</td>
<td></td>
</tr>
<tr>
<td>HC</td>
<td>Healthy controls</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
<td></td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
<td></td>
</tr>
<tr>
<td>LTFU</td>
<td>Lost to follow-up</td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
<td></td>
</tr>
<tr>
<td>pANCA</td>
<td>Perinuclear antineutrophil cytoplasmic antibodies</td>
<td></td>
</tr>
<tr>
<td>PAR%</td>
<td>Population attributable risk percent</td>
<td></td>
</tr>
<tr>
<td>PpPFU</td>
<td>Physician per Patient Follow-up form</td>
<td></td>
</tr>
<tr>
<td>PQ</td>
<td>Patient questionnaire</td>
<td></td>
</tr>
<tr>
<td>RG</td>
<td>Recommendation grade</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
<td></td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
<td></td>
</tr>
<tr>
<td>TLR</td>
<td>Toll like receptor</td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td>Ulcerative colitis</td>
<td></td>
</tr>
</tbody>
</table>
1. INTRODUCTION AND AIMS

Ulcerative colitis (UC) and Crohn’s disease (CD) are chronic inflammatory disorders collectively referred to as inflammatory bowel disease (IBD). The inflammation in UC is usually limited to the mucosa of the large bowel, spanning a continuum from proctitis with only rectal involvement to pancolitis. CD can affect any part of the gastrointestinal tract from mouth to the anal canal, although the most common sites to be affected are the terminal ileum and coecum. Unlike UC, the inflammation of CD is not necessarily continuous and areas of ulceration may be interspersed by relatively normal mucosa. The inflammatory process in CD is transmural, affecting all layers of the gastrointestinal wall, sometimes with a strictureing or penetrating behaviour.

UC and CD are heterogeneous diseases and heterogeneity exists even within the two disease entities. Within each disease there are subgroups of patients with different prognosis and different needs for treatment. Classifying patients according to disease phenotype are useful in daily clinical practice and research. Phenotypes tend to be homogeneous groups of patients with specific clinical and epidemiological characteristics, similar response to treatment and predictable prognosis. UC patients have traditionally been classified based on disease extent at diagnosis\(^1,2\) whereas CD patients are classified according to age at diagnosis, disease location and behaviour\(^3,4\).

The aetiology of IBD is still unknown. For decades it has been evident that a genetic factor must be involved since epidemiological studies have shown that cases of IBD cluster within families. In population based studies 5-10\% of all IBD patients report a positive family history\(^5,8\). The assumption of a genetic predisposition led to an intense search for genes involved in the development of IBD. Genome-wide scans have been performed in IBD resulting in a number of susceptibility loci\(^9\). Fine mapping of one of these loci (the IBD1 locus) led to the discovery of CARD15 in 2001, CARD15 being a susceptibility gene in CD\(^10\). Thus genetic epidemiology became important in the study of the role of genetic factors and their interaction with environmental elements in their occurrence of disease in human populations\(^11,12\).

Going back more than four decades, Denmark has a long tradition of conducting epidemiological studies on IBD\(^13-18\). Clinical epidemiological studies on large unselected cohorts offer the advantage of evaluating all spectres of IBD, which can be diffi-
2. MATERIALS AND METHODS

2.1 The EC-IBD cohort

In 1988, gastroenterologists from 20 centres in 12 European countries founded the European Collaborative study group on Inflammatory Bowel Disease (EC-IBD). The group collected a population-based prospective inception cohort during the period October 1991 – September 1993, consisting of 2201 IBD patients uniformly diagnosed according to Lenneard-Jones criteria. The study revealed that overall the incidences of UC and CD were slightly higher in the North European centres compared to the South European centres. However this difference was much lower than expected, and there was a considerable variation in the incidences across Europe: The highest incidence of UC was found in Iceland, but the second highest was in Crete. In 1998 the first steps were taken to conduct a ten year follow-up, financially supported by a grant from the European Commission. Centres participating in the original cohort were approached and encouraged to participate in the follow-up. The EC-IBD group formed “working groups” responsible for different aspects of IBD e.g. phenotype-genotype studies, course of disease, cancer in IBD, dissemination of messages and health care consumption and costs (figure 2). Working group 1, 2 and 3 had Ph.D. students assigned to coordinate and perform research activities.
2. MATERIALS AND METHODS

2.1.1 Data acquisition tools
For the data acquisition, two questionnaires were developed at the beginning of the follow-up study (2000-2001): the "Physician per Patient Follow-up form" (PpPFU) and the "Patient Questionnaire" (PQ). Members of the above-mentioned working groups all gave their input to the questionnaires, and final comprehensive questionnaires were eventually designed by group leaders and Ph.D. students in collaboration. The PQ was translated into nine languages by professional translators and was proof read by assigned members from the participating countries. The language of the PpPFU was English.

The questionnaires were transformed into electronic versions and made available to all members of the group through a username-password protected website on the internet.

The PpPFU was designed to obtain information on disease course from diagnosis until end of follow-up: vital status, cause of death, diagnosis, disease activity, disease extent and behaviour, use of medication, surgery, cancer. The PQ contained questions regarding family history of IBD, diet and smoking habits, fertility and pregnancy and quality of life (in all 747 questions).

2.1.2 Data collection
Thirteen centres from eight European countries and Israel participated in the follow-up. The centres that declined to take part did so primarily due to shortage of man-power. The population-based nature of the study was not compromised, since all participating centres originally fulfilled the population-based definitions, however to ensure that the individual cohorts remained on the whole population-based, a minimum follow-up rate of 60% was set.

The individual centres each had the responsibility for follow-up of the patients included in the original incident cohort. The following describes how data was collected in Copenhagen, but the other centres used similar procedures. The Danish patients all resided in Copenhagen County at time of diagnosis. For the follow-up patients vital status and current residential address were traced using the personal registration number (CPR-number). Hospital databases were used to trace where the patients (incl. those emigrated from the region) had been followed for their IBD since diagnosis. Patient-files were scrutinized for information regarding disease course and the following information was obtained: Vital status, date and cause of death, date of last visit, gender, IBD diagnosis, date and type of all disease recurrences, examinations and/or surgery performed (date, type, findings), medical treatment (in three month periods), extraintestinal manifestations (type, specialist confirmation), epithelial dysplasia, cancer (type, stage), use of healthcare (date and type of event: visit to outpatient clinic or emergency ward, hospitalization incl. number of days, examinations). All information was entered into the EC-IBD web-page. Figure B1-B4 in appendix B gives examples of screen-prints from the web-page used for data-collection.

After approval from the ethics committee, all living (and traceable) patients were approached by letter, inviting them to take part in the follow-up by participating in interview and blood sampling. Interviews were primarily performed at Herlev Hospital, where patients were given access to a computer in order to fill in the internet-based questionnaire. Patients not responding to the written invitation were approached in the out-patient clinic or contacted by phone, which improved the participation rate significantly. The interview also provided an opportunity to ensure that all information regarding disease course had been obtained (i.e. that hospital charts from all hospital visits had been evaluated) and whether the patients had used the general practitioner as primary consultant which could bias the number of reported recurrences.

Data management and validation
After data collection ended January 1st 2004, data management took place at “Centre for Data and Information Management” (MEMIC) in Maastricht, The Netherlands. Data was transferred from the internet program into a traditional database and data validation was performed. Logical checks were made comparing crude data on number of patients per centre, diagnosis, gender and age at diagnosis from the new database, with information from the original database collected 1991-1993. A logbook, describing discrepancies encountered, was forwarded to the participating centres and the members were encouraged to give solutions to the problems listed. Revisions were eventually incorporated into the final database, which was finally distributed to all members of the EC-IBD study group, January 5th 2005.
2. MATERIALS AND METHODS

2.1.4 Final patient population
Thirteen centres enrolled in the follow-up study rendering 1505 patients from the original cohort available for follow-up. Three centres did not reach the 60% follow-up threshold, thus leaving 10 centres from seven European countries and Israel in the final analyses (table 1). The PpPFU response rate was overall 86%, range 57-91%.

The cohort finally consisted of 1164 patients from Europe and Israel, 784 patients originally diagnosed with UC and 380 with CD. Patients were followed from time of diagnosis (between October 1st 1991 and September 30th 1993) until data inclusion between August 1st 2002 and January 31st 2004, or any date prior to that due to death or lost to follow-up. Of the 1164 patients 111 had died (9%) and 150 (13%) were lost to follow-up or misdiagnosed. The rest (903 patients) were contacted regarding participation in the patient interview and 777 (86%) accepted to participate. Overall PpPFU was available in 86% of the patients, well above the 60% threshold. DNA and/or serum were available in 687 of 777 (88%) interviewed patients. Blood samples from healthy controls matched for gender, age and ethnicity were collected at each participating centre, in all 692 samples.

<table>
<thead>
<tr>
<th>Total number of IBD patients in original cohort (n)</th>
<th>PpPFU response (n %)</th>
<th>PQ response (n %)</th>
<th>PQ response without deaths and “No IBD”b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almadaa (Portugal) 21 12 (57) 11 (52) 58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer Sheva (Israel) 60 46 (77) 40 (67) 76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copenhagen (Denmark) 147 132 (90) 103 (70) 84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cremona (Italy) 51 43 (84) 35 (68) 76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heraklion (Greece) 62 49 (79) 38 (61) 68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ioannina (Greece) 43 39 (91) 36 (84) 88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oslo (Norway) 379 332 (88) 226 (60) 69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reggio Emilia (Italy) 85 69 (81) 61 (73) 79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Limburg (Netherlands) 216 194 (90) 155 (72) 76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vigo (Spain) 100 81 (81) 72 (72) 77</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total 1164 997 (86) 777 (67) 75

Table 1. Follow-up rates for the participating centres. The physician questionnaire (PpPFU) was available on all patients who had answered the patient questionnaire (PQ).
a Almada only reached the 60% threshold for CD patients and are only represented in (II)
b A few patients were found retrospectively to have been misdiagnosed.

2.1.5 Genetic and serological analysis
DNA and serum for this study was obtained in connection with the patient interview. Whole venous blood was collected in one 7 ml EDTA tube and one 5 ml gel containing tube. The gel containing tube was centrifuged for 5 minutes at 3000rpm and 3 ml serum was transferred to tubes and frozen until shipment. Samples were shipped to the gastroenterological laboratory in Leuven, Belgium for analysis.

Genotyping
Genomic DNA was extracted from whole venous blood using the QIAamp DNA Blood Mini KIT (QIAGEN) according to manufacturer’s instruction. DNA was genotyped for three single nucleotide polymorphisms (SNP) in the CARD15 gene (Arg702Trp (referred to as SNP8), Gly908Arg (SNP12) and Leu1007fsinsC (SNP13)), and for the Asp299Gly SNP in TLR4. Genotyping analyses were performed using polymerase chain reaction restriction fragment length polymorphism techniques as described previously21-23.
2. MATERIALS AND METHODS

Serological analyses
Anti-Saccharomyces cerevisiae antibodies (ASCA) was measured by a standardized ELISA (Medipan Diagnostica Germany) according to the manufacturer’s instructions. Results were interpreted by calculating the binding index. ASCA IgG and IgA were considered positive if the binding index was higher than 1.0024.

Perinuclear antineutrophil cytoplasmic antibodies (pANCA) was determined by indirect immunofluorescence (IIF) using ethanol-fixed neutrophil slides (Inova Diagnostics, San Diego, CA, USA).^{25}

Definitions

Phenotype
In disease context, the word phenotype is used to describe a homogenous group of patients with similar clinical and epidemiological characteristics, who can be recognized and studied according to prognosis, disease course in long-term and response to a given medical therapy. A phenotype can be defined based on the clinical appearance at diagnosis, as well as the initial (within few years) disease course, localisation, demographic variables and genetic/serological markers.

Disease recurrence
All episodes with disease recurrence were reported on the PpPFU web-page. The definition on disease recurrence was: A consultation for increased disease activity and either 1) increase in dose of previously initiated anti-inflammatory medication and/or 2) initiation of new anti-inflammatory medication and/or 3) surgical intervention for increased disease activity.

Extent of disease
Results of all clinical, endoscopic and radiological examinations (X-ray, MR-scan, CT-scan) or surgeries performed during the entire follow-up were entered into the PpPFU web-page (see figure B1 in appendix B). Results were described as normal or abnormal for the following locations: Mouth, oesophagus, stomach, duodenum, jejunum, rest ileum, terminal ileum, ileocecal valve, coecum, ascending, transverse, descending and sigmoid colon, rectum and anus. Thus the final database contained detailed information on disease extent after each examination/procedure performed. UC and CD patients were then classified after each event into subgroups based on disease extent as defined in table 2.

<table>
<thead>
<tr>
<th>Ulcerative colitis</th>
<th>Crohn’s disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proctitis</td>
<td><strong>Terminal ileum</strong></td>
</tr>
<tr>
<td>Substantial</td>
<td><strong>Colon</strong></td>
</tr>
<tr>
<td>Pancolitis</td>
<td><strong>Ileocolon</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Upper GI</strong></td>
</tr>
</tbody>
</table>

Table 2. Definitions of disease extent in UC and CD patients.

*GI: gastrointestinal
2. MATERIALS AND METHODS

Disease behaviour
CD patients were also classified according to disease behaviour into inflammatory, stricturing or penetrating behaviour. Information on stenoses and fistulas/abscesses were reported on the PpPFU webpage (see figure B1 and B2 in appendix B). Based on the provided information we classified the patients after each examination/event. If a stenosis was recorded the patient was classified as having stricturing disease and penetrating disease was defined as the occurrence of fistulas (regardless of subtype) and/or abscesses. Inflammatory disease was CD without the presence of stricturing or penetrating disease.

Surgery
In paper III and IV, surgery was defined as total or subtotal colectomy for UC and small or large bowel resections in CD at any time during the first 5 years of disease course. The final data set also had information on fistulectomies, abscess drainage, pouch surgery etc. (see figure B2, appendix B).

Medical treatment
In paper III, immuno-modulating therapy was defined as treatment with systemic steroids, azathioprine, 6-Mercaptopurine, methotrexate, cyclosporine or infliximab at any time during the first 5 years after diagnosis. Information about the use of systemic or local 5-aminosalicylate, local steroids etc. (see figure B4, appendix B) was also available from the data set.

Pregnancy consensus
The material presented in paper V is not based on results from the EC-IBD collaboration. The paper constitutes chapter 11 in the “European Evidence-based Consensus on the Diagnosis and Management of Crohn’s Disease”. The consensus was made on the initiative of the “European Crohn’s and Colitis Organization” (ECCO).

The ECCO-governing board entrusted a chairman to perform a European consensus on Crohn’s disease. The consensus was reached according to the Delphi procedure, which is briefly described in the following. The chairman appointed 61 experts on IBD from 19 European countries to participate in the consensus. Working parties were formed, each responsible for an aspect of CD, one of them being fertility and pregnancy in CD. The working parties elaborated a questionnaire with questions focusing on current practice and areas of controversy within the topic. Table 3 shows some of the questions asked regarding fertility and pregnancy.

All 61 participants in the consensus were asked to answer the questionnaires based on their experience as well as evidence from the literature. The working party assigned Lene Riis as the first author of the final consensus paper which involved a systematic literature search in relevant databases and grading the evidence level for each paper according to the Oxford Centre for Evidence Based Medicine (table 4).

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>In quiescent or mild disease would you in choice of delivery recommend</td>
<td></td>
</tr>
<tr>
<td>Caesarean section</td>
<td>Yes  No  Uncertain</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>Yes  No  Uncertain</td>
</tr>
<tr>
<td>Is episiotomy recommended in Crohn’s disease?</td>
<td></td>
</tr>
<tr>
<td>Would you recommend abortion if your female patient, at time of conception, had been treated with</td>
<td></td>
</tr>
<tr>
<td>Sulfasalasine</td>
<td>Yes  No  Uncertain</td>
</tr>
<tr>
<td>5-Aminosalisylates</td>
<td>Yes  No  Uncertain</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Yes  No  Uncertain</td>
</tr>
<tr>
<td>Azathioprine or 6-Mercaptopurine</td>
<td>Yes  No  Uncertain</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Yes  No  Uncertain</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Yes  No  Uncertain</td>
</tr>
<tr>
<td>Infliximab</td>
<td>Yes  No  Uncertain</td>
</tr>
</tbody>
</table>

Table 3. Examples of the questions asked in the questionnaire sent to all participants in the ECCO consensus, prior to the consensus meeting.
2. MATERIALS AND METHODS

Based on the answers to the questionnaires and literature evidence, preliminary statements were written by the first author. These statements were then discussed during a weekend conference, first within the working group and thereafter at a common assembly with all working parties present. During the assembly the statements were iteratively revised until a final consensus was reached. Consensus was defined as agreement by >80% of participants. The recommendation was graded (RG) based on the evidence label (table 4). Finally the first author wrote the final consensus paper with the input from co-authors.

The paper was intended to be an independent chapter in the final consensus published as a supplementary to “Gut” but was eventually enrolled in a paper on “Special Situations in Crohn’s disease” together with subjects on post-operative recurrence, fistulizing disease, paediatrics, pregnancy, psychosomatics, extraintestinal manifestations and alternative therapy.

Appendix A, paper V, presents chapter 11 “The management of pregnancy in Crohn’s disease” as it will appear in the final consensus.

<table>
<thead>
<tr>
<th>Levels of evidence</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a Systematic review (SR) with homogeneity of Level 1 diagnostic studies</td>
<td>Systematic review (SR) with homogeneity of randomized controlled trials (RCTs)</td>
</tr>
<tr>
<td>1b Validating cohort study with good reference</td>
<td>Individual RCT (with narrow Confidence standards Interval)</td>
</tr>
<tr>
<td>1c Specificity is so high that a positive result rules in the diagnosis or sensitivity is so high that a negative result rules out the diagnosis</td>
<td>All or none</td>
</tr>
<tr>
<td>2a SR with homogeneity of Level &gt;2 diagnostic studies</td>
<td>SR (with homogeneity ) of cohort studies</td>
</tr>
<tr>
<td>2b Exploratory cohort study with good reference standards</td>
<td>Individual cohort study (including low quality RCT; e.g., &lt;80% follow-up)</td>
</tr>
<tr>
<td>2c “Outcomes” Research; Ecological studies</td>
<td></td>
</tr>
<tr>
<td>3a SR with homogeneity of 3b and better studies</td>
<td>SR with homogeneity of case-control studies</td>
</tr>
<tr>
<td>3b Non-consecutive study; or without consistently applied reference standards</td>
<td>Individual Case-Control Study</td>
</tr>
<tr>
<td>4 Case-control study, poor or non-independent reference standard</td>
<td>Case-series (and poor quality cohort and case-control studies )</td>
</tr>
<tr>
<td>5 Expert opinion without explicit critical appraisal, or based on physiology, bench research or “first principles”</td>
<td>Expert opinion without explicit critical appraisal, or based on physiology, bench research or “first principles”</td>
</tr>
</tbody>
</table>

Grades of recommendation

A Consistent level 1 studies
B Consistent level 2 or 3 studies or extrapolations from level 1 studies
C Level 4 studies or extrapolations from level 2 or 3 studies
D Level 5 evidence or troublingly inconsistent or inconclusive studies of any level

Table 4. Levels of evidence and grades of recommendation based on the Oxford Centre for Evidence Based Medicine. SR: Systematic review - RCT: Randomized controlled trials
2. MATERIALS AND METHODS

2.3 Statistical methods

All statistical analyses were performed using SPSS 10.1 (SPSS Inc., Chicago IL) or SAS version 9 (SAS Institute INC).

2.3.1 Intra- and interobserver variation

Kappa (κ) statistics was used to measure intra- and interobserver agreement. The κ coefficient estimates the agreement in excess of the agreement that would be expected by chance (chance-corrected agreement). It is estimated from the observed and expected frequencies on the diagonal of a square table of frequencies. κ is thus calculated as:

\[ \kappa = \frac{P_o - P_e}{1 - P_e} \]

where Po is the observed agreement and Pe is the by chance expected agreement.

κ has a maximum of 1.00 when agreement is perfect; a value of zero indicates no agreement better than chance. For this study, strength of agreement was considered to be poor if κ<0.2, fair if 0.21<κ<0.4, moderate if 0.41<κ<0.60, good if 0.61<κ<0.8, and very good if κ>0.827.

2.3.2 Population attributable risk

In order to estimate the excess rate of CD in individuals with a genetic mutation compared with those without, the Population Attributable Risk percent (PAR %) was calculated as described by Hennekens and Buring. In the calculation the frequency of all alleles in the control population were assumed to reflect that of the general population. PAR% was calculated using the following equation:

\[ \text{PAR} = \frac{P_e(RR-1)}{P_e(RR-1)+1} \times 100 \]

P_e = Proportion of exposed individuals in the population (in paper II the proportion of persons in the background population with a mutation). The relative risk (RR) is calculated as

\[ RR = \frac{a \times d}{b \times c} \]

where

- a= the number of patients who has a mutation and have CD
- b= the number who have a mutation but does not have CD
- c= the number who does not have a mutation but have CD
- d= the number who have neither mutation nor CD

2.3.3 Comparison of groups

Categorical variables were compared between groups using the chi-square or Fishers test, where appropriate, and quantitative variables were compared using Mann-Whitney test. A p-value <0.05 was considered statistically significant.

2.3.4 Correlation analysis

Correlation is the method of analysis to use when studying a possible association between two variables. To measure the degree of correlation the correlation coefficient (called r) is calculated. r can take any value from -1 to +1. The value of -1 or +1 is obtained if the points in a scatter diagram lies on a perfect straight line, corresponding to full linear correlation, a value around 0 indicates that there is no linear correlation between the two variables.

For non-parametric values, rank correlation was used to estimate the relation between two variables. Here subjects are ranked for each variable, orderings are compared and the Spearman’s rank correlation coefficient calculated.

2.3.5 Cox regression analysis

A Cox regression analysis was performed to investigate the influence of genotypes and serotypes on the following events: “increased disease extent”, “surgery”, “disease recurrence” and for CD patients also “change in disease behaviour”. Explanatory variables used in the model included CARD15, TLR4, ASCA, ANCA, age at diagnosis, gender, family history, smoking status, disease extent and behaviour. All models are tested for interaction between genotypes and serotypes. The assumption of proportional hazards was checked by plots of the logarithm of the integrated hazard. Results are presented as hazard ratios (HR) with 95% confidence intervals (CI).
2. MATERIALS AND METHODS

2.4 Intra- and interobserver variation when assessing disease extent and behaviour

In order to evaluate the validity of data collected in a multi-centre setting, we decided to investigate the intra- and interobserver variation associated with collecting data on disease location and behaviour. In paper I, the observer variation associated with collecting data from hospital charts and classifying patients according to strict definitions was evaluated by investigating the intra- and interobserver variation in the use of the Vienna classification. This classification is often used in clinical practice and research, and classifies CD patients according to disease location and behaviour.

2.4.1 Method

Hospital charts of ten randomly selected Danish CD patients were scrutinized to obtain information on disease course, including all examinations performed (radiology, endoscopy, histology, clinical observations), and medical and surgical history. The description of the examinations as provided in the patient file by the radiologist, endoscopist etc. were copied word by word. The descriptions along with clinical information, was sent by e-mail to 11 Danish gastroenterologists with a special interest in IBD. Participants were asked to classify patient cases according to the Vienna classification which was described in details on the data collection form. The cases were presented 3 times: baseline, after 8 weeks and 1 year after the first assessment respectively. Prior to the third evaluation the definitions in the Vienna classification were thoroughly outlined and the participants received instructions regarding possible pitfalls in the use of the classification.

2.4.2 Results (paper I)

The intra-observer variation when comparing results from the first and second round yielded a kappa (κ) value of 0.75 for location and 0.77 for behaviour, indicating a low intra-observer variation (figure 3). The mean overall inter-observer κ-value was 0.64 (range 0.12-1.00), slightly higher when classifying according to location compared to behaviour (table 5). After outlining definitions and possible pitfalls between round two and three, the κ-value improved for location but not behaviour.

However, evaluating the individual cases revealed, that all 11 participants agreed on location or behaviour in only one and three cases respectively.

<table>
<thead>
<tr>
<th></th>
<th>Location</th>
<th>Behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round 1</td>
<td>0.61 (0.32-1.00)</td>
<td>0.58 (0.12-1.00)</td>
</tr>
<tr>
<td>Round 2</td>
<td>0.65 (0.26-1.00)</td>
<td>0.63 (0.33-1.00)</td>
</tr>
<tr>
<td>Round 3</td>
<td>0.74 (0.40-1.00)</td>
<td>0.60 (0.22-1.00)</td>
</tr>
</tbody>
</table>

Table 5. Mean κ values (range) when classifying cases according to the Vienna classification. The κ value improved slightly from the first to the second round and for location from the second to the third round although large range and variation was present.

Figure 3. A, κ values for intraobserver study (rounds one and two). The line represents κ=0.60; values above this are regarded as good or very good agreement.

B, κ values for interobserver study, first round. The bars represent the mean κ value for each observer compared with the 10 other observers. The line represents κ=0.60; values above this are regarded as good or very good agreement. Note that some of the observers agreed well with themselves but poorly with the other observers (e.g., observer 7 and 8 have high intraobserver but low interobserver agreement).
2. MATERIALS AND METHODS

2.4.3 Discussion

The overall inter-observer variation when using the Vienna classification was acceptable, yielding a $\kappa$-value defined as “good”, even though looking into the individual cases revealed some disagreement. Classifying according to disease location resulted in the highest $\kappa$-value. The $\kappa$-value improved between the second and third round after specifying to the participants that location according to the Vienna classification should be assessed before the first resection. The difficulties thus seemed to be related to the interpretation of the definitions in the Vienna classification, more than the actual assessment of disease extent itself.

The inter-observer variation was higher for behaviour compared to location indicating that it was more difficult to classify according to behaviour. This was mainly due to difficulties in assessing the presence of a stenosis. The Vienna classification uses the definition “occurrence of constant luminal narrowing…… with prestenotic dilatation or obstructive signs/symptoms”. The term “obstructive signs/symptoms” implies subjective interpretation which presumably accounted for some of the inter-observer variation described.

When comparing disease location in patients from different centres one should be aware how the information on disease extent has been obtained. Recently capsule endoscopy has become a modality used in the diagnosing and managing of IBD patients. This has revealed that a large percentage of CD patients have disease proximal to the terminal ileum. The assessment of disease extent is thus only based on the information available to the observer. Furthermore, it should be noted that assessing disease extent is based on the interpretation of examinations and observations that are subject to inter-observer variation. Studies on the EC-IBD cohort have previously shown that the modalities used for diagnosing were comparable between centres and there is no reason to believe that practice has changed during the years of follow-up.

This is the first study to investigate the inter-observer variation in the use of the Vienna classification, despite a widespread use in clinical practice and research. Other classification systems used in CD have been evaluated coming to the same conclusions. Louis et al evaluated the validity of the Vienna classification when classifying the same patient group 5, 10 and 15 years after diagnosis. They showed that disease behaviour classified according to the Vienna classification varied over the course of disease, thus patient classification depends on disease duration. Disease location on the other hand was fairly stable over time.

In conclusion, we have shown that the use of the Vienna classification in CD is associated with certain problems, in spite a $\kappa$ value indicating a good inter-observer agreement. The study also revealed that some of the problems related to the use of the classification were due to difficulties in interpreting the definitions. For the EC-IBD study we therefore decided to record the results of the individual examinations or clinical observations leaving it up to the person analysing the data to classify patients, thereby eliminating one link in the inter-observer variation chain.

2.5 Ethical considerations

The study was approved by the local ethics committee of all participating centres, including the Ethics Committee for Copenhagen County (KA 02038m).

All participating patients gave their informed consent for blood sampling and interview. The Danish Data Protection Agency accepted the collection of data and blood, including the sharing of data with centres outside Denmark.
3. RESULTS AND DISCUSSION

3.1 Prevalence of genetic and serologic markers in a population-based cohort

The original study on the EC-IBD cohort published in 1996 described differences in the incidence in UC and CD across Europe\(^2\). The discovery in 2001 of CARD15 (also known as NOD2) being a susceptibility gene in CD raised the question whether the differences in incidence could be ascribed to differences in the prevalence of mutations in this gene.

CARD15 is located within the IBD1 locus on chromosome 16. CARD15 codes for a protein expressed in monocytes, macrophages, dendritic cells, epithelial cells and paneth cells\(^3\). The protein consists of two N-terminal Caspase-Activation Recruitment Domains (CARDs), a nucleotide-binding region and a C-terminal leucin-rich-repeat region. Many polymorphisms have been described within the gene, the three most common being Arg702Trp (SNP 8), Gly908Arg (SNP 12) and Leu1007fsinsC (SNP 13).

The discovery of CARD15 resulted in an increased interest in genes involved in the pathway of innate immunity and the molecules playing a role within this pathway. The family of the toll like receptors (TLR), of which TLR4 is associated with the gut, plays an important role in the innate immunity. TLR4 is up-regulated in the epithelial cells in patients with IBD, and it has been debated whether there is an association between the Asp299Gly polymorphism in TLR4 and susceptibility to IBD. Studies on the prevalence of the mutation in IBD cohorts have given conflicting results\(^3\)\(^-\)\(^4\)^\(^2\)\(^-\)\(^4\)^\(^1\).

The functional consequences of the polymorphisms in CARD15 and TLR4 are still not fully understood. In the healthy gut, CARD15 exerts antimicrobial activity through the production of defensins and prevents intracellular bacterial invasion. TLR4 signalling protects the intestinal epithelial barrier and promotes healing. Defects in CARD15 and/or TLR4 alter the signalling pathways and may stimulate various inflammatory responses leading to tissue injury and mucosal inflammation.

In the literature, antibodies against the yeast saccharomyces cerevisiae (ASCA) is reported to be present in 35-76% of CD patients and antibodies against neutrophils (pANCA) in 30-83% of UC patients\(^2\)\(^4\)^\(^-\)\(^4\)^\(^9\). The antibodies are not disease specific since a small proportion of CD patients are pANCA positive, and UC patients can be ASCA positive.

The genetic and serological markers described above have primarily been described in selected cohorts. The EC-IBD cohort provided a unique opportunity of investigating the prevalences of genetic and serological markers in population-based cohorts with different incidences of IBD.

Results (paper II)

Blood for genotyping and serological analysis was available from 687 IBD patients (454 UC and 233 CD) and 692 healthy controls.

The overall prevalence of at least one mutation in CARD15 was 23.9% (range 0-50%) in CD patients which was significantly higher than the prevalence observed among UC patients (9.6%, range 0-23.5%) (OR 2.89, 95%CI: 1.84-4.55, p<0.0001) and controls (14.4%, range 4.8-25.9%) (OR 1.87, 95%CI: 1.28-2.75, p=0.001). The prevalence was lower in the Scandinavian countries (Norway and Denmark) compared to the rest of Europe (12.1% vs. 32.8%). There was no correlation between the incidence of Crohn’s disease and the prevalence of mutations in CARD15 (figure 4).

The excess rate of CD in individuals with at least one mutation in CARD15 expressed as the Population Attributable Risk (PAR %) was 11.2% for the whole cohort, with large differences between the participating countries (range -13.7% - 44.6%). Overall the PAR% was lower in north compared to south Europe; 6.8% vs. 19.6%.

TLR4 was not significantly associated with IBD in this cohort, the prevalence in UC and CD was 9.0% and 6.7% respectively compared to 12.3% in controls.
ASCA was more common among CD patients (27.9%) compared to UC patients (6.6%) and controls (6.2%). The prevalence of ASCA was not different in Scandinavia compared to the rest of Europe (25.5% vs. 29.5%). pANCA was associated with UC since 25% of UC patients were pANCA positive compared to 8.4% in CD and 2.0% in controls. pANCA was more common in Scandinavia compared to the rest of Europe (35.3% vs. 16%, p<0.001).

3. RESULTS AND DISCUSSION

3.1.2 Discussion

The result presented in paper II is confirmative of other studies showing that the prevalence of CARD15 is varying considerably between different populations (table 6). The EC-IBD study is the only study performed on a population-based cohort, the rest being selected cohorts of CD patients.

The EC-IBD study showed one of the lowest reported prevalences of CARD15, and it was not correlated to disease incidence. When analyzing the Scandinavian countries separately the prevalence was even lower; 12.1% in CD. This difference between the Scandinavian countries and the rest of Europe was also present among healthy controls and probably reflects the differences in genetic origin. A population-based incident cohort from the Copenhagen area confirmed the low prevalence of CARD15 in Denmark: 14% in newly diagnosed CD patients and 11% in healthy controls (I. Vind, personal communication), despite the incidence of CD being 8.7/100.000, which is among the highest reported in Europe18.

When looking at the individual countries presented in paper II, some have a relatively high prevalence of mutations in CARD15, but the prevalence in the healthy population is correspondingly high. In order to estimate the excess rate of CD in individuals with a genetic mutation compared to those without we calculated the population attributable risk percent (PAR%).

In some countries the PAR% was negative, thus CARD15 did not seem to significantly increase the susceptibility to CD in those populations. However, conclusions from paper II must be drawn with some caution since calculations were based on a low number of patients in some centres. Nevertheless, the majority of CD cases cannot be ascribed to variations in CARD15 or any other currently known genetic variant and the major cause of the rising incidence in CD described the resent years is likely to be found in the environment.

The prevalence of the TLR4 Asp299Gly polymorphism was low and did not differ between UC, CD and healthy controls. This mutation was first described in a Belgian study, where the frequency of the allele mutation was higher in CD compared to UC patients38. Replication studies have shown conflicting results. The association with CD was confirmed in Dutch and Greek populations but not in Irish CD patients39, 41, 60. Török et al. found an

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of Crohn's disease patients</th>
<th>At least one mutation in CARD15 in Crohn's disease patients (controls) %</th>
<th>Allele frequencies of CARD15 in Crohn’s disease (controls) %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SNP8</td>
<td>SNP12</td>
</tr>
<tr>
<td>Greece50</td>
<td>120</td>
<td>82 (21)</td>
<td>10 (1)</td>
</tr>
<tr>
<td>Canada51</td>
<td>229</td>
<td>45 (9)</td>
<td>13 (5)</td>
</tr>
<tr>
<td>France52</td>
<td>205</td>
<td>38 (20)</td>
<td>12 (5)</td>
</tr>
<tr>
<td>Italy53</td>
<td>316</td>
<td>38 (15)</td>
<td>9 (4)</td>
</tr>
<tr>
<td>UK54</td>
<td>244</td>
<td>36 (16)</td>
<td>13 (5)</td>
</tr>
<tr>
<td>USA55</td>
<td>201</td>
<td>35 (-)</td>
<td>14 (4)</td>
</tr>
<tr>
<td>Spain56</td>
<td>204</td>
<td>33 (11)</td>
<td>14 (4)</td>
</tr>
<tr>
<td>EC-IBD(40)</td>
<td>213</td>
<td>24 (14)</td>
<td>9 (4)</td>
</tr>
<tr>
<td>Finland57</td>
<td>271</td>
<td>16 (7)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>USA58</td>
<td>416</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Japan59</td>
<td>483</td>
<td>0 (-)</td>
<td>0 (-)</td>
</tr>
</tbody>
</table>

Table 6. Allele frequencies for CARD15 in Crohn’s disease in published studies. The EC-IBD study is the only population-based study.
association with UC which still remains to be confirmed. These findings as in the case of CARD15 are indicative of the heterogeneity between populations.

The prevalence of the serological markers was lower in the EC-IBD cohort compared to previous reports. The study confirmed the association between UC and pANCA although the prevalence was very low in the south European countries. Opposite to CARD15, ASCA did not differ significantly between the participating centres.

The discussion whether ASCA is a genetic or environmental markers is ongoing. Family studies have shown that 20-25% of first degree relatives to patients with CD were ASCA positive. Healthy spouses being negative for these antibodies pointed towards a genetic origin of ASCA. The same did a Belgian study, where the prevalence of ASCA was higher in families with more than two affected members compared to families with only two affected members. However no differences were found in the prevalence between pure CD families and sporadic cases.

Recently, Halfvarson et al. described that there was no increased occurrence of ASCA in healthy twin siblings belonging to monozygotic twin pairs discordant for CD. The authors conclude that ASCA is not a genetically determined marker. Also pointing towards an environmental origin of ASCA is a study by Israeli et al. They detected ASCA in 31% of patients before clinical diagnosis of CD, with an increase in ASCA frequency the closer patients came to time of diagnosis.

ASCA is antibodies directed against brewer’s and baker’s yeast, dietary components consumed across Europe. One could speculate that the results presented in paper II points towards an environmental origin of ASCA since prevalences of the genetic marker CARD15 varies whereas ASCA is comparable between centres.

In conclusion this study confirmed that the prevalence of genetic and serological markers is varying between different European populations and not correlated to the incidence of CD. The markers were less present in this population-based setting compared to reports from selected cohorts. The described markers could influence disease phenotype, which would explain the differences in prevalences seen between selected and unselected CD populations.

### Phenotypes and genotypes in an unselected cohort of IBD patients

The population based cohort of patients diagnosed within a short time period of two years and followed for 10 years since diagnosis offers optimal conditions for studying prognostic factors influencing disease course. Paper III aims at defining clinically relevant phenotypes based on long-term disease course and furthermore to investigate phenotype-genotype associations.

#### Results (paper III)

A total of 873 patients were followed from inclusion till at least August 2002 (median follow-up 123 months, range 107-144), 142 patients were lost to follow-up at varying time during disease course (median follow-up 49 months, range 1-133), and 110 died during the follow-up period (median follow-up 57.5 months, range 1-133).

Of the 873 patients eligible for interview and blood sampling, 433 UC and 356 CD patients accepted to participate, the rest (84 patients, 10%) were not willing to participate. In both the CD and the UC group, patients lost to follow up or not willing to participate were not different from participating patients regarding age at diagnosis, gender, disease location or behaviour at diagnosis.

Prior to looking at the data set we created a number of clinically characteristic phenotypes that subsequently were applied to the data set.

We ended up defining 5 phenotypes for UC and 8 for CD based on disease course within the first 5 years after diagnosis (table 7). Many other phenotypes were created, but when applied to the dataset they comprised too few patients to allow further evaluation.

For UC patients, the defined phenotypes were based on maximum disease extent and recurrence rates within the first 5 years after diagnosis. In CD, phenotypes were based on age at diagnosis and disease location, behaviour and recurrence rates within the first 5 years after diagnosis.

After defining the phenotypes we examined whether demographic variables (age at diagnosis, gender, smoking status at diagnosis and family history of IBD) were prognostic factors for any of the phenotypes. For a closer description of phenotypes and prognostic factors please refer to paper III.
3. RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Ulcerative colitis</th>
<th>Crohn’s disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proctitis without progression</td>
<td>Age &lt;40 at diagnosis</td>
</tr>
<tr>
<td>Substantial colitis</td>
<td>Terminal ileum disease exclusively</td>
</tr>
<tr>
<td>Pancolitis</td>
<td>Colonic disease exclusively</td>
</tr>
<tr>
<td>No disease recurrence within 5 years</td>
<td>Ileo-colonic disease</td>
</tr>
<tr>
<td>Frequent disease recurrence</td>
<td>Perianal disease</td>
</tr>
<tr>
<td></td>
<td>Complicated disease behaviour</td>
</tr>
<tr>
<td></td>
<td>No disease recurrence within 5 years</td>
</tr>
</tbody>
</table>

Table 7. Phenotypes defined for ulcerative colitis and Crohn’s disease. Phenotypes are based on disease course within the first 5 years.

For CD, we looked at the distribution of patients according to phenotype in the Scandinavian countries compared to the rest of Europe. In Scandinavia, 23% of CD patients could be classified as “frequent disease recurrence” compared to 32% in the rest of Europe (p=0.17) and 72% of the patients were below 40 years at diagnosis compared to 73% in the rest of the cohort. Also, the phenotypes “complicated disease behaviour” and “terminal ileum exclusively” were equally distributed in Scandinavia compared to the rest of Europe; 39% vs. 40% (p=0.88) and 20% vs. 24% (p=0.56) respectively.

Phenotype-genotype correlations revealed that in UC the phenotype “proctitis without progression” was less pANCA positive compared to proctitis patients who progressed towards more extensive disease. The “frequent disease recurrence” phenotype had a higher prevalence of pANCA compared to patients with “no recurrence”. Thus, pANCA seemed to be associated with more extensive and active disease. In Crohn’s disease, the phenotypes “age <40”, “terminal ileum location exclusively” and “complicated disease behaviour” were associated with being ASCA positive, and “colonic disease location exclusively” with being pANCA positive. Furthermore there was an association between “terminal ileum location exclusively” and at least one mutation in CARD15. The associations were all statistically significant.

Cox regression analysis confirmed that pANCA positive UC patients had an increased risk of change in disease extent towards more extensive disease (HR 1.91, 95%CI:1.1-3.3, p=0.02). There was a trend towards that being pANCA positive will increase the overall risk of disease recurrence (HR 1.28, 95%CI:0.99-1.65, p=0.06), but not the risk of colectomy (HR 1.11, 95%CI:0.46-2.66, p=0.81). TLR4 did not influence disease extent or the risk of flare or surgery in UC.

In CD, the analysis confirmed that ASCA was associated with complicated disease behaviour since ASCA positive CD patients had an increased risk of changing from inflammatory to stenosing or penetrating behaviour (HR 1.8, 95%CI:1.0-3.2, p=0.05). Furthermore CARD15 positive CD patients had an increased risk of change in disease extent towards further involvement of the gastrointestinal tract (HR 2.4, 95%CI:1.3-4.5, p=0.007). CARD15, TLR4 or ASCA status did not influence on disease recurrence or surgery in CD.

Discussion

We defined clinically characteristic phenotypes, based on disease extent, behaviour and recurrence rates within the first 5 years after diagnosis. The phenotypes contain a relatively large number of patients and are recognizable in clinical practice. Although we had data from 10 years follow-up we chose to concentrate on disease course the first 5 years after diagnosis. Previous studies have shown that disease course the first years is predictive of the subsequent disease course65, 66. Therefore, it could also be argued that phenotypes based on 10 years disease course would be of little relevance in daily clinic where physicians would like to predict the immediate phenotype in order to counsel patients.

Age at diagnosis, gender, family history or smoking at diagnosis did not predict surgical events or disease recurrence within the individual phenoty-
3. RESULTS AND DISCUSSION

The phenotypic distribution of CD patients was not different in Scandinavia compared to the rest of Europe. One could have expected a milder disease course in the Scandinavian patients, where the prevalence of CARD15 is low. However this was not the case which once again confirms that the influence on disease course is multi-factorial.

The described phenotypes are based on variables not dependent on practice policy such as medical treatment or surgery, which could vary between the participating centres. It could be argued that disease location is also depending on practice policy since surgery is known to alter the natural history of CD and certain medical treatments especially the biological modifiers are thought to alter disease course. However, biological modifiers were not available during the initial phase of this cohort. In our cohort, disease location was relatively stable compared to previous reports; this could be due to the population-based nature of the present study. Previous studies on disease behaviour have been performed in referral centres where patients with complicated disease tend to be overrepresented.

A number of patients from this population-based cohort were lost to follow-up or had died during the 10-11 years since diagnosis. Furthermore, some patients declined to take part in the interview and blood sampling. This implies a risk of selection bias - patients participating in the study could be selected patients from an unselected cohort. The experience from contacting the Danish patient population is that neither mild nor severe cases are overrepresented among patients declining to participate. Patients with a mild disease course did not want to be reminded of having a chronic disease, and patients with a severe disease course were reluctant to pay an extra visit to the hospital where they had spent too much time already. Patients lost to follow-up or not willing to participate were comparable to patients participating regarding gender, age and disease location at diagnosis, indicating that the patients were not selected in a way that could influence results. Furthermore recurrence rates of 61% in UC and 70% in CD, and surgery rates of 6% and 32% in UC and CD patients after 5 years of IBD makes this cohort comparable to other population-based cohorts.

Genotype/phenotype correlations were confirmative of reports from tertiary referral centres. In addition we found, that pANCA was correlated with...
3. RESULTS AND DISCUSSION

a more severe disease course in UC patients, in terms of a higher risk of disease progression and recurrence. In CD, ASCA and CARD15 were predictive of a more aggressive phenotype with respect of complicated disease behaviour and disease progression. These findings could have implications for clinical practice. It is speculated whether initiating immuno-modulating therapy at an early point in disease course will alter the natural course of IBD. CARD15, ASCA and pANCA status seems to define subsets of patients at risk for a more severe disease course. These patients could perhaps benefit from a more intensive treatment regimen initiated soon after diagnosis.

3.3 Pregnancy, a factor influencing disease course and phenotype in IBD

As described in the previous section, genetic and serological markers correlate with disease course and phenotype in IBD. These markers, however, were only present in 20-30% of the patients in the described population-based cohort and the diversity in phenotypes within IBD cannot be ascribed to genotype-serotype differences exclusively. A number of factors thought to exert an effect on the immune system (e.g., appendectomy, use of oral contraceptives, smoking) have been investigated in relation to IBD79. Paper IV concerns the question whether pregnancy is a factor influencing disease course and phenotype in women with IBD.

Results (paper IV)

For the study on the influence of pregnancy on IBD, the patient cohort consisted of 249 female patients (165 UC and 84 CD) reporting in all 491 pregnancies. Furthermore we included 50 women (33 UC and 17 CD) who in the PQ answered that they “never intended to become pregnant”, the reason for this not specified.

To assess the influence of pregnancy on disease course and phenotype in CD, patients were divided in two groups: Pregnant during disease course (n=38), and patients not pregnant during disease course (n=63). At follow-up, 52% of the non-pregnant patients had developed a stenosis compared to 37% of the patients with a pregnancy during disease course (OR 1.89, 95%CI:0.83-4.30, p=0.13); hence in this CD population, pregnancy did not protect against the development of a stricturing phenotype. The two groups were also comparable concerning mean number of resections (0.52 vs. 0.66, p=0.37). In the group of patients only pregnant before CD (n=46) there was no correlation between parity and number of resections (figure 5).

The correlation between pregnancy and recurrence rate was evaluated in 11 CD and 29 UC patients followed for three years before and after pregnancy. Figure 6 is indicative of a reduction in the mean number of flares in the years following a pregnancy.

3.3.1

Figure 5. Correlation of number of pregnancies and number of resections in 46 CD patients who have only been pregnant prior to being diagnosed with IBD (p=0.74, Spearman rank correlation coefficient -0.05).

Figure 6. Mean number of flares/year for 3 years prior to pregnancy, during pregnancy and post partum and for the three years after pregnancy.
3. RESULTS AND DISCUSSION

3.3.2 Discussion

The influence of pregnancy on IBD has been investigated in a number of studies. Most studies have confirmed that female patients with quiescent disease at time of conception often remain in remission; however one third of patients relapse during pregnancy. Conversely, two thirds of patients with active disease at conception, will remain active during pregnancy\textsuperscript{80-84}. Castiglione et al. studied the clinical course in pregnant IBD women and found, that the number of relapses in the years following a pregnancy were lower compared with the year before pregnancy\textsuperscript{85}. In CD, Nwokolo et al.\textsuperscript{86} described how increasing parity reduced the need for surgical resections. It could be argued, that patients needing surgery are severely ill patients with impaired fertility or no wish to have children. However, subgroup analysis showed, that even patients who had been pregnant before CD was diagnosed needed fewer resections subsequently. The authors concluded that effects exerted on the immune-system during pregnancy were responsible for the reduced resection rates.

The results in paper IV indicated a milder disease course with fewer relapses during and after pregnancy, though not providing any insight into the factors causing this change in disease course.

The adaptation of the immune system to pregnancy can potentially affect the course of all diseases with an immunological patho-aetiology. In normal pregnancy, the Th1 response is down-regulated leading to a change in the Th1/Th2 balance towards a higher Th2 response. Other immunomodulating changes taking place during and after pregnancy could influence disease course depending on the immunological pattern dominating the disease in question. In several autoimmune diseases pregnancy is known to exert an effect on the disease course\textsuperscript{87-89}. In rheumatoid arthritis pregnancy is associated with improvement of clinical signs and symptoms and in multiple sclerosis with stability or improvement. How this effect is exerted is still largely unknown. In rheumatoid arthritis it has been reported that disparity in HLA class II antigens between mother and foetus resulted in clinical remission during pregnancy\textsuperscript{89}.

In IBD, Kane et al described how improvement of symptoms during pregnancy were associated with disparity in HLA II class between mother and foetus\textsuperscript{91}. How this HLA disparity can lead to a down-regulation of the immune response remains unclear.

In a recent study Agret et al. studied 70 pregnancies in CD women\textsuperscript{92}. They found an overall reduction in disease activity during pregnancy, expressed as a decrease in Harvey-Bradshaw activity index. When stratifying for smoking status they observed that the decrease in activity was present only in smoking women, concluding that the decreased activity during pregnancy could be ascribed to a reduction in tobacco consumption among smokers. The conclusions of this study, however, are limited by the fact that 27% of the women who smoked at time of conception continued smoking throughout pregnancy. The decrease in disease activity could therefore also be caused by other changes exerted during pregnancy.

Most studies investigating pregnancy outcome in IBD patients have described that patients with active disease during pregnancy have a higher risk of premature birth and small for date children\textsuperscript{81, 82, 93-97}. This stresses the importance of maintaining inactive disease throughout the pregnancy. Many centres follow pregnant patients closely, and inform patients that active disease offers a greater risk to the foetus than the medication used in the treatment of IBD. The reduced recurrence rates described in paper IV could therefore also be ascribed to optimized treatment and better compliance during pregnancy.

The optimal way of gaining more insight into the question regarding recurrence rates, and the impact of pregnancy on IBD, would be to perform a large scale prospective study comparing pregnant and non-pregnant IBD patients with the same age, disease duration and location followed during the same time period. The European Crohn’s and Colitis organization (ECCO) is currently conducting such a study in a multi-centre setting (www.ecco-ibd.org).

Fertility and pregnancy outcome, important issues also in IBD

IBD is often diagnosed when patients are in their fertile age. Many patients are concerned about their ability to conceive and the impact of IBD on fertility and pregnancy. Doctors often get questions from patients regarding these subjects and dealing with pregnant IBD patients is a part of the daily clinic. Nevertheless pregnancy in IBD is an area subjected to little research. Most of the evidence is classified as B, C or D (table 4) since intervention studies or randomized, placebo controlled trials on mother and foetus are difficult to
3.
RESULTS AND DISCUSSION

perform due to ethical considerations. Thus, many questions can only be answered by the exercise of judgement and opinion, leading to differences in practice between clinicians. The purpose of the consensus presented in paper V is to address these differences promoting a European perspective on fertility and pregnancy with special focus on CD. In the following section results from the EC-IBD study, regarding number and outcome of pregnancies in patients with IBD (IV), are held up against the consensus which is based on existing literature (V).

3.4.1 Results (paper IV and V)  
Patients experiencing their first pregnancy before IBD was diagnosed, were younger at time of conception compared with women who had their first pregnancy after being diagnosed with UC (23.9 vs. 28.2 years p<0.001) or CD (23.5 vs. 30.4 years, p<0.001). Women who had their first pregnancy prior to IBD had on average more pregnancies than women being pregnant for the first time after IBD were diagnosed. Before IBD was diagnosed, 6.5% of all known pregnancies resulted in spontaneous abortions, this number increased significantly to 13% in pregnancies occurring after IBD was diagnosed (OR 2.3, 95%CI: 1.3-4.2, p=0.005). The rate of elective abortions remained unchanged (5% vs. 6.2%). In 48.6% of pregnancies occurring after IBD-diagnosis was made, the female patient took medication at time of conception and in 46.9% of cases during pregnancy. Patients were mainly treated with 5-aminosalicylates and corticosteroids. No increase in the number of developmental defects occurred, and defects were not correlated to the intake of one particular type of medication. Mode of delivery was caesarean in 8.1% of the pregnancies occurring before and in 28.7% occurring after IBD diagnosis, a statistically significant increase (OR 4.5, 95%CI: 2.7-7.7, p<0.001). There was no difference between UC and CD patients regarding the frequency of caesarean section.

3.4.2 Discussion  
The scope of paper IV, when discussing pregnancy outcome and demographic variables, was not as in case-controls studies to compare with a healthy control-group. In the unselected cohort the influence of pregnancy is assessed in both mild and severe cases of IBD. Factors such as age at first pregnancy, mode of delivery and number of pregnancies per woman, are parameters not exclusively related to being diagnosed with IBD, but also highly dependent on social conditions. These variables are best evaluated in a prospectively collected case-control study, with IBD women and healthy controls being pregnant during the same time period. The outcome in the inception cohort described here, should primarily be seen as a contribution to the existing knowledge on fertility and pregnancy in IBD. Data on pregnancy and fertility was collected retrospectively and without doubt subjected to recollection bias since some pregnancies occurred decades ago. Nevertheless, the results presented are representative of the questions asked by the patients and the topics discussed in the consensus (paper V).

Fertility  
Fertility in non-operated UC patient is normal compared to the background population. In CD, fertility is slightly reduced especially at times of increased disease activity. A very important problem is the reduced fertility in female UC patients having an ileal pouch-anal anastomosis following colectomy. After an interval of 24 months of unprotected intercourse, 20% of patients with IPAA were pregnant, compared to 80% of the background population98, 99. This is a significant finding which should be discussed with the patients prior to colectomy.

The rate of spontaneous abortions in paper IV was significantly higher after IBD was diagnosed, however, still comparable to the background population where 10-15% of all known pregnancies results in spontaneous abortion100. It would have been relevant to compare patients with active and inactive disease at conception, but that was unfortunately not possible in this study.

Method of delivery  
In the EC-IBD cohort we found an increase in the number of caesarean section after patients were diagnosed with IBD. This probably reflects the change also seen in the background population where the rate of caesarean section has changed during the past 20 years100. It would have been relevant to compare patients with active and inactive disease at conception, but that was unfortunately not possible in this study.
3. RESULTS AND DISCUSSION

**Medical therapies**

Almost half of the pregnant women in the EC-IBD study took medication at time of conception or during pregnancy. All pregnant women are concerned about taking medication during pregnancy due to a possible risk of birth defects or in other ways harming the foetus. The Food and Drug Administration (FDA) pregnancy categories can be used when counselling pregnant IBD women (table 9). Please refer to paper V for a close description of the individual medication. The uncontrolled data in paper IV did not raise any concern regarding developmental defects in children born to women medically treated for IBD. The indications for treatment (medical as well as surgical) in pregnant IBD patients are to a large extent the same as in the non-pregnant.

Maintaining adequate disease control is of great importance for both maternal and foetal health, and should be performed in a multidisciplinary way, involving both gastroenterologists and obstetricians.

**11E Mode of delivery in pregnant women with Crohn’s disease**

The mode of delivery should primarily be governed by obstetric necessity and indication, but also in conjunction with the gastroenterologist.

Uncomplicated CD patients without perianal disease should deliver vaginally after obstetric evaluation has been performed [EL4, RG C].

Caesarean section should be preferred in active perianal disease [EL4, RG C]. For inactive perianal disease, caesarean section may be considered [EL5, RG D].

An ileoanal pouch in CD patients is regarded as an indication for caesarean section [EL4, RG C]. Colostomy/ileoanostomy patients can deliver vaginally [EL4, RG C]. If there is an increased obstetric risk, a caesarean section should be performed.

However caesarean section in this setting carries an increased risk of complications [EL4, RG C].

Episiotomy should probably be avoided, but is better than a spontaneous uncontrolled laceration.

However there are discrepancies in the literature [EL4, RG C].

*Table 8. Consensus statement 11E on the subject “Mode of delivery in women with Crohn’s disease”. EL: Evidence level. RG: Recommendation grade. For levels of EL and RG please refer to table 4.*

<table>
<thead>
<tr>
<th>Considered safe</th>
<th>Probably safe</th>
<th>Contraindicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Aminosalisylates (FDA B)</td>
<td>Budesonide (FDA C)</td>
<td>Methotrexate (FDA X)</td>
</tr>
<tr>
<td>Antibiotics (FDA B)</td>
<td>Thiopurines (FDA D)</td>
<td>Thalidomide (FDA X)</td>
</tr>
<tr>
<td>Anti-TNFα (FDA B)</td>
<td>Quinolones (FDA C)</td>
<td>Sulphonamides (FDA C)</td>
</tr>
<tr>
<td>Steroids (no rating)</td>
<td>Ciclosporin (FDA C)</td>
<td>Tetracycline (FDA D)</td>
</tr>
<tr>
<td></td>
<td>Tacrolimus (FDA C)</td>
<td></td>
</tr>
</tbody>
</table>

*Table 9. Food and drug administration (FDA) categories on medication frequently used in IBD. Please refer to V for a more detailed description of the individual medication. The letters ABCDX refers to the category assigned by FDA: A  Controlled studies show no risk B  No evidence of risk in humans C  Risk cannot be ruled out, animal studies revealed adverse effects on fetus D  Positive evidence of risk in humans, risk/benefit ratio should be considered X  Contraindicated*
4. CONCLUSION AND PERSPECTIVES

Genetic epidemiology has received immense attention the past years, not only in gastroenterology but within many fields of clinical medicine. The continuing identification of genetic factors influencing disease occurrence and course will in the coming years have major impact on clinical practice. In the future, information regarding a patient’s genetic profile will without doubt be used in diagnosing, assigning treatment and counselling the individual IBD patient.

In genetic terms, IBD is a complex disease, caused by interplay between several genes or genetic variants and environmental factors. The genetic factor in complex diseases is termed “susceptibility genes” since they are not causative, i.e., the presence of susceptibility genes does not imply development of disease.

The present study investigating the susceptibility gene CARD15 in a European IBD population have confirmed that the prevalence of CARD15 variant varies between populations and the impact in the Scandinavian countries is limited. The search for genetic markers continues in IBD and OCTN1 and 2 (novel organic cation transporters), DLG5 (named for its homology with a Drosophila Discs Large Homolog 5) and NOD1 have all been investigated in relation to IBD, especially CD101-106. However, these genes have primarily been tested in selected cohorts and their contribution in population-based cohorts needs to be confirmed.

The discovery of genes associated with IBD will provide an insight into the patho-aetiology of IBD since the investigation of gene products can help us understand the underlying disease mechanisms leading to IBD.

In the excitement of unravelling the genetic component we should not overlook the importance of environmental factors obviously involved in the development of IBD. Twin studies have shown that even genetically identical individuals can be discordant for disease occurrence, which is indicative of an environmental component107. The rapid increase in the incidence of IBD cannot be ascribed to genetic changes but must be due to environmental factors18.

In the search for causative factors we must not forget the patients already diagnosed with IBD. Describing disease course and outcome in large cohorts is pivotal in the clinical management of patients. Phenotypic classifications based on demographic variables and disease outcome should be evaluated and refined continuously. The use of new treatment modalities, e.g. biological modifiers, is thought to alter the “natural course” of IBD and perhaps result in new patient subgroups or at least to a change in distribution of phenotypic sub-classes. The present study showed that serotype and to some extent genotype can predict the disease course. The clinician can use this information to intensify treatment at an early time-point, thereby perhaps changing the aggressive disease course.

This thesis has described associations between genotype/serotype and disease phenotypes and as more susceptibility genes emerge in IBD we will see classification systems based on genotypes. However, it is not only the actual development of IBD that is caused by interplay between susceptibility genes and environmental factors. It is well known that smoking modifies the clinical course of Crohn’s disease, and in this thesis it has been described how pregnancy has a possible effect on the subsequent course of IBD. Thus, the phenotype is a puzzle made up by environmental and genetic pieces.

Genetic variations have been shown to have an impact on drug response and the occurrence of adverse events. The differences in response to treatment observed among patients treated with corticosteroids and azathioprine have been associated with genetic heterogeneity. Mutations in CARD15 were found not to have any impact on response to infliximab but the impact of CARD15 on infliximab dependency and resistance to other drugs remains to be investigated9, 108-110. There is no doubt that the field of pharmacogenetics will attract much attention the coming years.

In the future, genetic testing is likely to be used when examining individuals with symptoms compatible with IBD or in the differentiation between UC and CD. However, the specificity of currently available genetic markers is not presently high enough to warrant a routine test of genetic markers in patients suspected to have IBD, or in family members of IBD patients (at-risk individuals).

The European collaboration forming the basis for this thesis made it possible within a relatively short time-frame, to get insight into not only phenotype and genotype correlations but also disease course, mortality and health economics in an incidence cohort of IBD patient111-113. There is a continued need for national and internatio-
CONCLUSION AND PERSPECTIVES

nal cooperation to increase the understanding of these relatively rare disorders. The work of the “European Crohn’s and Colitis Organisation” continues. A consensus conference on UC is scheduled to take place in 2007 and will as in the case of CD be aiming at ensuring standardised treatment for these patients within Europe. The EC-IBD group is currently seeking funding to perform a research program involving east European countries. The aim is to start a new incidence cohort and at the same time conduct an educational program for young doctors employed within the participating centres.

On a local scale, the Danish Crohn Colitis Database has initiated a new inception cohort in Copenhagen County and City. This cohort will provide a solid platform for further research into the aetiology and “natural” history of IBD.
5. SUMMARY

Ulcerative colitis (UC) and Crohn’s disease (CD) are chronic inflammatory bowel diseases (IBD) with unknown aetiology. The incidence of IBD varies between populations and a continuous increase has been observed, especially in the western world. In 2001 researchers described the first susceptibility gene in CD, CARD15, and since then other genes associated with the occurrence of IBD have been identified. UC and CD are heterogeneous diseases and within each disease there are subgroups of patients with different prognosis and needs for treatment.

Identifying prognostic factors associated with disease phenotype would be valuable in the management of IBD patients. The aim of this study was to describe the prevalence of serological and genetic markers in European IBD patients. Furthermore, we aimed at defining clinically characteristic phenotypes based on the disease course the first 5 years after diagnosis and investigate phenotype/genotype correlations. Finally we evaluated pregnancy as a factor influencing phenotype and disease course.

The patients participating in this study came from the EC-IBD cohort, a population-based cohort consisting of IBD patients from 20 centres in Europe and Israel, diagnosed in 1991-1993. A 10 year follow-up was performed involving going through hospital files registering information on vital status, cause of death, diagnosis, disease activity, disease extent and behaviour, use of medication, surgery and cancer. Furthermore patients were asked to participate in an interview regarding family history of IBD, diet and smoking habits, fertility and pregnancy and quality of life. At the time of the interview patients gave a blood sample for genetic and serological testing. All information was registered electronically using web-based questionnaires.

Overall the prevalence of genetic and serological markers was lower in this unselected population-based cohort compared to reports from tertiary referral centres. We found that the prevalence of these markers varied between European populations.

The prevalence of mutations in CARD15 was lower in the Scandinavian countries compared to the rest of Europe, and no association with the incidence of CD was found. We defined phenotypes based on disease extent, behaviour and recurrence rates within the first 5 years after diagnosis. In UC we found that pANCA was correlated with a more severe disease course in terms of a higher risk of disease progression and recurrence.

In CD, ASCA and CARD15 were predictive of a more aggressive phenotype with respect of complicated disease behaviour and disease progression. These findings could have implications for clinical practice where serologic and genetic markers could help identify patients with a more severe disease course who could benefit from a more intensive treatment regimen.

The described markers were only present in 20-30% of the patients in this unselected cohort and the diversity in phenotypes within IBD cannot be ascribed to genotype-serotype differences exclusively. A number of factors are known to exert an effect on the immune system and we investigated whether pregnancy had an influence on disease course. Our results indicated that pregnancy did not change disease phenotype or resection rates in CD patients, but it seemed to alter the course of disease in IBD women by reducing the number of flares in the years after a pregnancy.

These findings could reassure patients, who are often diagnosed in their fertile age, that pregnancy does not seem to worsen the disease course. In general patients have many questions regarding fertility and pregnancy in relation to IBD. Some of these questions cannot be answered based on evidence in the literature, and answers are often provided based on experience and opinion leading to differences in practice between clinicians.

A European consensus, presented in the thesis, aimed at addressing these differences promoting a European perspective on fertility and pregnancy with a special focus on CD.

This thesis describes associations between disease phenotype and genotype/serotype. In the future, as more susceptibility genes emerges, we will see the use of genetic testing when examining persons suspected of having IBD, or predicting disease course or designing the best treatment for patients already diagnosed with IBD.
Colitis ulcerosa (UC) og Crohn’s sygdom (CD) er såkaldte inflammatoriske tarmsygdomme. Årsagen til at disse sygdomme opstår, er ukendt. Forekomsten af disse sygdomme varierer mellem forskellige populationer og er stigende specielt i den vestlige verden.

I 2001 blev det første gen forbundet med sygdomsopståen beskrevet for CD, det såkaldte CARD15 gen, og siden da er det blevet vist at flere andre gener er forbundet med udviklingen af CD.

Colitis ulcerosa og Crohn’s sygdom er heterogene sygdomme og selv indenfor den enkelte sygdom findes der undergrupper af patienter med forskellig prognose og behandlings-behov.

At kunne identificere faktorer af betydning for udviklingen af disse såkaldte fænotyper vil være afgørende for behandlingen af IBD patienter.

Formålet med nærværende undersøgelse var, at beskrive forekomsten af serologiske og genetiske markører i en Europæisk kohorte af patienter diagnosticeret med IBD. Baseret på sygdomsforløbet de første 5 år efter diagnosen ønskede vi at definere klinisk karakteristiske fænotyper og undersøge eventuelle genotype-fænotype relationer. Endvidere undersøgte vi graviditis betydning for sygdommens fænotype og sygdomsforløb.


I forbindelse med en 10-års opfølgning blev alle hospitals journaler gennemgået og følgende informationer blev indhentet: vitale status, dødsårsag, diagnose, sygdomsaktivitet, sygdomsudbredning og fremtrædelsesform, medicinforbrug, operationer og cancer.

Endvidere anmodede vi patienterne om at deltage i et interview der omhandlede disposition for IBD, kost og rygevaner, fertilitet og graviditet samt spørgsmål angående livskvalitet. I forbindelse med interviewet afgav patienterne en blodprøve med henblik på serologisk og genetisk testning. Alle oplysninger blev indtastet i et elektronisk internet-baseret spørgskema.

Forekomsten af serologiske og genetiske markører var lavere i denne uselekerede kohorte sammenlignet med de hyppigheder der rapporteres fra centrene med et selekteret patient materiale. Vi fandt at forekomsten af disse markører varierede betragteligt med i gennem Europa, både blandt patienter og raske kontroller. Forekomsten af mutationer i CARD15 var lavere i Skandinavien sammenlignet med de øvrige Europæiske centre, og der var ingen sammenhæng mellem incidensen af Crohn’s sygdom og forekomsten af CARD15.

Vi definerede forskellige fænotyper baseret på oplysninger om sygdoms-udbredning, fremtrædelsesform og tilbagefald indenfor de første 5 år efter diagnosen blev stillet. For colitis ulcerosa patienter fandt vi at pANCA øgede risikoen for større sygdoms-udbredning, og tilbagefald. For patienter med Crohn’s sygdom var ASCA og CARD15 prædiktive markører for en mere aggressiv fænotype kendteget ved stenoser/fistler og øget sygdoms-udbredning. Disse fund kan tyde på at serologiske og genetiske markører kan hjælpe klinikeren med at identificere patienter med et sværere sygdomsforløb og med behov for mere målrettet behandling.

I denne uselekerede kohorte var de beskrevne markører kun tilstede hos 20-30% af patienterne og de forskelle i fænotyper som er beskrevet indenfor IBD kan ikke kun tilskrives genetiske og serologiske forskelle. Det er velkendt at andre faktorer som influerer på immunsystemet kan påvirke sygdomsforløbet, og vi valgte at undersøge om graviditet kunne påvirke sygdomsforløbet hos IBD patienter. Vores resultater antyder at graviditet ikke påvirke fænotypen eller operations frekvens hos patienter med Crohn’s sygdom. Dog så det ud til at antallet af tilbagefald hos både colitis ulcerosa patienter og patienter med Crohn’s sygdom blev reduceret i årene efter en graviditet sammenlignet med årene forud for graviditeten.

Disse resultater kan berolige patienterne, som ofte bliver diagnosticeret i den fertile alder, med at graviditet ikke ser ud til at forværre sygdomsforløbet. Generelt set har patienterne mange spørgsmål angående fertilitet og graviditet i forbindelse med IBD.

Nogle af disse spørgsmål kan ikke besvares ud fra den foreliggende litteratur, og de svar der gives er derfor ofte baseret på klinikerens erfaring, hvilket kan føre til forskelle i behandlingsregimenerne. I denne afhandling præsenteres en Europæisk konsensus med specielt fokus på fertilitet og graviditet hos patienter med Crohn’s sygdom, udarbejdet med formålet at sikre en tilnærmelsesvist ensartet behandling af Europæiske gravide IBD patienter.
7. REFERENCES


7. REFERENCES


7.

REFERENCES


7. REFERENCES


77. Bernklev T, Jahnsen J, Aadland E, Saur J,
7. REFERENCES


7. REFERENCES


