Concordance for IBD among twins compared to ordinary siblings — A Norwegian population-based study

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Abstract

Aims: Comparing the risk to develop concordant disease among twins with inflammatory bowel disease (IBD) to ordinary siblings. Moreover, clinical characteristics of IBD and the association between perinatal factors and IBD, were evaluated.

Methods: Patients with IBD, enrolled from an incidence study between 1990 and 1994, and the twins were identified from the Norwegian national birth registry, which was established in 1967.

Results: Eight monozygotic and 16 dizygotic pairs, in which at least one twin reported a positive history of IBD were compared to 84 patients with Crohn's disease (CD) and 87 patients with ulcerative colitis (UC) from the incidence study. The relative risks for concordant disease in monozygotic pairs were estimated to 95.4 (95% CI: 76.3, 114.6) and 49.5 (95% CI: 35.7, 63.3) for CD and UC, respectively. The corresponding risks in dizygotic pairs were 42.4 (95% CI: 29.6, 55.2) and 0.0. Among ordinary siblings of CD and UC the risks for concordance were 22.7 (95% CI: 13.3, 32.1) and 4.6 (95% CI: 0.4, 8.7), respectively.

Stricturing disease was significantly higher in twins with CD compared to incidental cases. The first-born twin in pairs discordant for disease, 12 out of 19 (63.2%), tended to be affected by IBD \( (p=0.10) \).

Conclusion: Genetic factors influence the development of IBD and fibrostenotic disease in CD. The increased risk for concordant disease among dizygotic twins compared to ordinary siblings, at least in CD, might underscore the importance of shared environment in utero or in childhood.

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Keywords: Inflammatory bowel disease; Population-based; Twins; Ordinary siblings; Concordant disease

Abbreviations: AAD, age at diagnosis; CD, Crohn's disease; DZ, dizygote; IBD, inflammatory bowel disease; MZ, monozygote; PRR, population relative risk; UC, ulcerative colitis.

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1. Introduction

Twin studies have been used to explore the contribution of environmental and genetic factors in complex non-Mendelian diseases like schizophrenia and bipolar disease\textsuperscript{1,2} asthma\textsuperscript{3} and inflammatory bowel disease (IBD).\textsuperscript{4, 6} Monozygotic twins have the same chromosomal DNA sequence because they arise from the same zygote, while DZ twins arise from two separate eggs, fertilized by different sperms. Therefore, the classical twin study is based on the assumption that monozygotic (MZ) twins are genetically identical, while dizygotic (DZ) twins on average share 50% of their genes like ordinary siblings.

The degree of genetic contribution to a given phenotype can be explored by comparing the concordance for disease between MZ and DZ twin pairs,\textsuperscript{7} assuming that relevant environmental exposures do not differ between members of MZ and DZ pairs. Although the concordance rate for many common diseases is higher among MZ than among DZ twins, MZ pairs are often discordant for diseases, which have been attributed to the effect of unique environmental factors.

Several studies have provided evidence that environmental factors play an important role in the development of IBD.\textsuperscript{8} Exposures early in life, such as colonization of the gut,\textsuperscript{9} breast feeding,\textsuperscript{10} early infectious diseases,\textsuperscript{11} and smoking history\textsuperscript{12,13} may influence the development of IBD later in life.

The aims of this study were to assess the genetic and environmental influences related to development of IBD by comparing the population relative risk for concordant disease in Norwegian MZ and DZ twins to the risk of concordant disease among ordinary siblings of patients with IBD. Furthermore, we intended to compare clinical characteristics of IBD among twins and patients from population-based incidence cohorts. We also explored the role of perinatal factors in modifying the risk for developing IBD among twins.

2. Materials and methods

2.1. Twins

The data are based on a population sample of twins identified through the Norwegian Medical Birth Registry, which was established in 1967. A total of 13,774 twins, born during the period of 1967 to 1979, were included in a research program at the National Institute of Public Health, Oslo.\textsuperscript{14, 15} Postal questionnaires were sent in 1992 to the twins born between 1967 and 1974, and in 1998 to twins born between 1967 and 1979. The data presented here are based on the 1998 questionnaire, which was sent to 12,700 twins, of whom all pairs were still alive with addresses in Norway. All of them had previously indicated that they would like to participate in research (twins born 1967–75). Responses were received from 8,045 twins (63%) and the sample included 3,334 complete pairs and 1,377 single responders. The questionnaire included a checklist for 31 illnesses and symptoms, including a question asking: Do you have, or have you ever had inflammatory bowel disease, of which Crohn’s disease (CD) and ulcerative colitis (UC) were specified.

Data on birth weight and gestational age were obtained from the Norwegian Medical Birth Registry of Norway. Classification of zygosity was based on responses to seven questionnaire items, which have been verified against genetic markers, to correctly categorize zygosity with 97% accuracy.\textsuperscript{14} Twenty-four microsatellite markers were genotyped on 676 of the like-sex pairs in the sample. Results from these markers were used as dependent variables in a discriminant analysis with the questionnaire items as independent variables. Seventeen of these pairs (2.5%) were misclassified by the questionnaire data and were corrected. From these data, we estimated that, in our entire sample, zygosity misclassification occurred in 1% of pairs, a rate unlikely to substantially bias results.\textsuperscript{15}

One hundred and thirty-eight pairs were excluded because of incomplete data.

The final sample included 504 male monozygotic pairs, 379 male dizygotic, 746 female monozygotic, 635 female dizygotic and 932 dizygotic unlike pairs. Among these, there were 45 twin pairs of which at least one of the twins claimed to have IBD. Invitation letters were sent to these 45 twin pairs in 1999 and in 2008, as a part of a twin study of autoimmune disease of which IBD was included. Of these 45 pairs, 32 pairs (71%) consented to participate in this study. Hospital records and phone interviews disclosed that 8 of the 32 twin pairs did not suffer from IBD, but functional gastrointestinal diseases. The remaining study sample included 24 twin pairs, 8 MZ and 16 DZ pairs.

2.2. Comparison group

The control group included patients with UC or CD, identified in a Norwegian population-based incidence study of IBD (The IBSEN study) which was carried out from January 1, 1990, to December 31, 1993, in four counties of south eastern Norway.\textsuperscript{16, 17} A follow-up was performed by the same senior gastroenterologist at one, five and ten years after inclusion with a re-evaluation of the diagnosis and an assessment of the course of disease. At each follow up, the patients were asked if they had any 1st degree relatives with IBD, which was later confirmed by the patients.\textsuperscript{18}

The incidence study included 756 patients, of whom 519 patients were classified as UC and 237 as CD. Of these 756 patients, 171 patients were born after 1967, and identified in the Norwegian Medical Birth Registry. Of these 171 patients, 84 patients suffer from CD and 87 from UC.

2.3. Additional information

Clinical diagnosis and classification of the twins and incidental cases were performed according to international accepted criteria.\textsuperscript{19} The Vienna classification was used, as the Montreal classification did not exist at the time the study started.

Other clinical characteristics were: Immuno-modulatory therapy; Azathioprine, Methotrexate, systemic steroids, and smoking history. Smoking history before diagnosis, current smoker (daily smoking) or not, was chosen in preference to smoking history at the time of study inclusion, as this environmental factor could influence both the course and the phenotype of disease.
2.4. Ethics

Approval of the study was obtained from the Regional Ethics committee and the Norwegian Data Registry. Confidentiality of both patient identity and records was maintained, using guidelines set up by the National Health Department.

2.5. Statistical analyses

Data management and statistical analyses were carried out using SPSS, version 16. Comparisons of categorical variables were analysed by the Pearson chi square test, and Fischer’s exact test when appropriate.

We used Wilcoxon’s signed rank test to compare the within pair difference in birth weight between twins with and without IBD. Linear regression analysis was performed to study birth weight in the full sample of twins, to adjust for gestational age and sex. Birth weight is highly correlated with gestational age, and adjustment for sex is important due to the fact that male babies are approximately 128 g heavier than female babies.

Logistic regression analyses were performed when comparing the clinical characteristics between the twins with IBD and the patients with IBD included from the incident study.

Two analyses were conducted, one with and one without affected co-twins, because affected co-twins (two with stricturing CD and one with extensive UC) might bias the data.

Separated analyses were fit for UC and CD.

Results were considered statistically significant when the p-values were less than 0.05.

In one of the DZ twin pairs, the twins had mixed disease, one with UC and one with CD, which was counted twice in the analyses of concordant disease, once among UC and once in CD. Therefore, Table 1 comprises 25 twin pairs instead of 24 twin pairs.

To indicate whether the concordance for smoking history was stronger than expected by chance alone within twin pairs, the Cohen’s $\kappa$ statistic was measured. A $\kappa$ value of 1.00 indicates perfect agreement, whereas a value close to 0 indicates no agreement.

The prevalence of CD and UC among co-twins of twins with CD and UC was calculated by dividing the number of co-twins with concordant disease by the number of co-twins at risk. Likewise, the prevalence of siblings with concordant disease in the incidence study was estimated by dividing affected relatives by the number of siblings at risk.

Prevalence rates were calculated instead of concordance rates among twins in order to compare the data directly with the prevalence among ordinary siblings.

The median number of siblings at risk was one. This number was based on the response rate from 79% of the patients ($n=136$), which was used in the calculations for the whole cohort of patients ($n=171$).

To estimate the population relative risk (PRR) for co-twins and siblings at risk, the prevalence of affected co-twins in the twin cohort or the prevalence of affected siblings in the incident cohort, was divided by the prevalence of CD (262/100 000) and UC (505/100 000) in the respective background population. To calculate the 95% confidence intervals for the population relative risk we assumed that the number of cases among the co-twins and siblings followed a Poisson distribution.

3. Results

3.1. Twins and perinatal factors

Twenty four twin pairs, 8 MZ and 16 DZ pairs, in which at least one twin reported a history of IBD, were identified in the twin registry born during the period 1967 to 1979.

Of the 24 twin pairs, 13 and 15 twins suffered from UC and CD, respectively. In 20 of the 24 pairs, only one of the twins had a positive history of IBD. 3 pairs were discordant for disease, and one pair represented twins with mixed disease, one with CD and one with UC. This gave us the number of 28 twins with IBD.

The concordant pairs comprised one MZ male and one DZ female pair with CD, and one MZ male pair with UC.

The twins in the MZ male pair, who were discordant for CD, had their disease located in the terminal ileum, complicated with stricture. The one with the earliest age at diagnosis (AAD) (14 years) was operated with small bowel resection three times, compared to the twin with AAD of 20 years, who was operated twice. In the DZ female pair, concordant for CD, both twins had their disease located in the terminal ileum, complicated with stricture, the same AAD (19 years), and operated once with small bowel resection.

In the UC pair, both twins where operated by colectomy for extensive colitis.

The median age at the time of survey and the median follow-up time among twins were 33 years (range: 23–40) and 10 years (2–24), respectively. The corresponding data among incidental cases were 31 years (range 14–37) and 10 years (0–14). Data on perinatal factors like birth weight, gestational age and birth order were missing in two twin pairs, of which one had mixed disease; therefore only 25 of 28 twins with IBD were available in the analyses for perinatal factors.

The mean difference in birth weight between twins with IBD (25 twins) and the rest of the twin cohort (6382 twins) adjusted for sex and gestational age was: 18.3 g, 95% CI: –148.4, 184.9.
There was no within pair difference in median birth weight between twins in pairs discordant for disease (18 pairs), \( p = 0.18 \).

Among the first-born twins in pairs discordant for disease, 12 out of 19 (63.2%) were affected by IBD \( p = 0.10 \).

The prevalence of UC and CD in the twin cohort were 203/100 000 and 234/100 000, respectively.

3.2. Relative risk of CD and UC in twins and patients from the incidence cohort

The comparison group of IBD (171 patients) from the incidence cohort born after 1967, included 84 patients with CD and 87 patients with UC. In this cohort, 5 sib–sib pairs were concordant for CD, 2 sib–sib pairs were concordant for UC and one sib–sib pair had mixed diseases.

The relative risks for concordant disease in MZ pairs were estimated to 95.4 (95% CI: 76.3, 114.6) and 49.5 (95% CI: 35.7, 63.3) in CD and UC, respectively (Table 1).

The corresponding risks in DZ pairs were 42.4 (95% CI: 29.6, 55.2) and 0.0 (none affected co–twins among the dizygotic UC twins). Among ordinary siblings of CD and UC, the risks for concordance were 22.7 (95% CI: 13.3, 32.1) and 4.6 (95% CI: 0.4, 8.7), respectively (Table 2).

3.3. Classification of disease among twins and incidental cases

The median follow-up time was 10 years for the twins as well as for the incidental cases. Moreover, the median age at diagnosis and age at the time of survey were approximately the same among the twins and the incidental cases (Tables 3, 4). There were no differences between twins and incidental cases with UC regarding clinical characteristics (Table 3). These results did not change when the affected co-twin was included in the analyses (12 twins instead of 13 with UC). However, the proportion of non-smokers were significantly higher in UC compared to CD in the incidental group (OR=5.3, 95% CI: 2.3, 12.5), which could not be confirmed among the twins (OR=1.4, 95% CI: 0.2, 9.8). In the twin pair with mixed disease, the twin with CD was the one who was a current smoker.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Characteristics of twins from the Norwegian population-based twin registry (1998) and cases from the incident study (1990–1994) with UC.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Twins</td>
</tr>
<tr>
<td>No</td>
<td>13</td>
</tr>
<tr>
<td>Distribution</td>
<td></td>
</tr>
<tr>
<td>Proctitis</td>
<td>4 (30.8%)</td>
</tr>
<tr>
<td>Left sided</td>
<td>23 (26.4%)</td>
</tr>
<tr>
<td>Extensive</td>
<td>9 (69.2%)</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>23</td>
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<tr>
<td>Range</td>
<td>16–27</td>
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The agreement for smoking history was high \( (\kappa \text{-value}=0.62) \) within MZ twin pairs, which was independent of type of IBD, compared to DZ pairs \( (\kappa \text{-value}=-0.24) \).

The location of disease among CD patients differed between twins and cases from the incidence study (Table 4). In the analyses without affected co-twins including, 13 twins instead of 15, twins were less likely to be affected by ileocolonic disease (OR=0.25, 95% CI: 0.06, 0.96), but more likely to have their disease located only in terminal ileum, compared to incidental cases (Table 4) (OR=5.87, 95% CI: 1.71, 20.13). A nearly significant increase in the use of immunomodulatory therapy was shown for incidental cases (34.6%, 27/78) compared to twins (7.7%, 1/13) \( (p = 0.052) \). There was high,
but non-significant proportion of CD patients with strictureing disease among twins compared to CD cases in the incidental group. \( \text{OR} = 7.00, 95\% \, \text{CI}: 0.82, 59.49, p = 0.075 \). The comparisons between twins and incidental cases regarding strictureing disease, reached significant level when the two affected co-twins were included in the analyses \( \text{OR} = 8.7, 95\% \, \text{CI}: 1.05, 72.69, p = 0.045 \).

4. Discussion

One of the main findings in this study was that the risk for development of concordant disease among co-twins of twins with IBD was remarkably high as compared to the general population. The risk was higher among MZ twins compared to DZ twins and higher in CD compared to UC. These findings are in agreement with those from other twin studies in Scandinavia and Germany. Moreover, the risk of concordance for IBD among twins was several-fold higher than among ordinary siblings (Tables 1 and 2).

In this study we compared twin pairs, of whom at least one of the twins suffers from IBD, with siblings of patients with IBD enrolled in an incidence cohort of IBD between 1990 and 1994. Both cohorts were population-based, born after 1967 and identified in the Norwegian Medical Birth Registry of Norway.

The prevalence of UC (203/100 000) in our twin cohort was estimated to be about half of the prevalence in the background population (505/100 000), while the prevalence of CD (234/100 000) was nearly the same as in the background population (262/100 000). The similarity in prevalence of CD and difference in prevalence of UC between the cohorts could be explained by age effects. The median age of the twins and the incidental cases at the time of survey, was 33 years (range 23–40 years) and 31 years (range 14–37 years), respectively, while the peak of AAD for CD appears between 15 and 25 years, and for UC between 25 and 35 years in the background population.

The IBD prevalence in the twin cohort was based on twins born between 1967 and 1979, of whom 72.2% had an age between 23 and 35 years at the time of survey. The age of the twins and incidental cases born after 1967 had passed the highest period of risk for developing CD, but not UC. This is in contrast to the IBD prevalence in the background population which was based on all incidental cases of CD and UC diagnosed in the period between 1990 and 1994, not only those born after 1967.

The difference in incident peak of AAD between UC and CD is also reflected in the estimated PRR for development of concordant disease among ordinary siblings of UC, which was approximately half of the estimated PRR among siblings of UC in the original incident cohort, \( 4.6 \) versus \( 10.9 \). The corresponding PRR among siblings of CD in the present cohort born after 1967 was nearly the same as in the original cohort, \( 22.7 \) versus \( 25.7 \). Due to this truncation effect, the prevalence estimates of CD in the twin-population and the PRR for CD among twins and incidental cases are more reliable than those for UC.

The Norwegian twin register included twins born from 1967 to 1979, a birth cohort spanning only 13 years, which explains the low number of affected twins.

Only gender and low birth weight were associated with decreased possibility of participating in our study. The trend in birth weight difference was the same in all 500 g weight groups from <1550 g to >2500 g, and therefore unlikely to influence our analyses based on the full sample. However, the small number of twins is a limitation of this study and also the reason why we were not able to include any concordant pair with UC among the DZ twins. Therefore, a comparison of risks between DZ twins and ordinary siblings was only possible in CD, in contrast to UC.

The risk for development of concordant disease among DZ twins with CD was twice as high as the corresponding risk among ordinary siblings. Shared environmental factors between twins in utero as well as in early childhood might contribute to the higher risk for development of concordance disease among DZ twins with CD compared to ordinary siblings.

Although there are differences in blood supply between twins in utero, they are sharing the consequences of their mothers’ nutrition and health condition during pregnancy. Furthermore, twins are perfectly correlated for age of exposure and the types of environmental exposure they experience during early childhood when their immune systems are at the same phases of development. These shared environmental exposures include infectious diseases, water supply, pets and sanitary conditions, all of which have been considered as risk factors for the development of IBD.

There is increasing evidence that epigenetic mechanisms have an influence on the phenotypic differences and similarities among twins. Epigenetic mechanism refers to DNA and chromatin modifications that change and modify the expression of genes and thereby influence the phenotypic variation. The epigenetic information might be inherited or could occur during the lifetime of the individual through the influence of environmental factors intrauterine or later in life.

The effects of early environmental factors and nutrition in fetal life, has been analysed in several investigations. Nutritional state in critical periods of organ development during pregnancy has been shown to predispose to chronic diseases such as cardiovascular heart disease, non-insulin diabetes, and hypertension later in life. To explore the link between environmental factors and phenotypic differences in MZ twins, Fraga et al. compared the epigenetic profile among MZ twins in different age groups. They showed that the degree of discordance in epigenetic patterns, methylation and chromatin remodelling of genes, in identical twin pairs was related to differences in lifestyle between the twins and the amount of time they had spent together.

There was no difference in median birth weight between twins with and without IBD, which is in agreement with other investigations of relationship between perinatal factors and IBD. In support of the German twin study, which included 189 twin pairs, we found a trend \( (p=0.1) \) towards an association between being the first in birth order and development of IBD, which suggests the important role of environmental trigger factors at delivery, like gut colonization. Like others, we found that current smoking was associated with CD and not UC among the incidental cases, but not among the twins. The agreement for smoking history
was high ($\kappa$-value = 0.62) within MZ twin pairs, which was independent of type of IBD, compared to DZ pairs ($\kappa$-value = -0.24), which might underscore the heritability of smoking habit. These findings are supported by Kendler et al. who showed that there was a high interclass correlation for smoking history within MZ twin pairs who were reared together as well as in twin pairs who were reared apart.

Among incidental cases of CD, we found a higher proportion of colon inflammation compared to twins, which might explain why the immunomodulation therapy was reported in a higher extent in the first group. Twins with CD were more likely to have inflammation in the small bowel (terminal ileum) compared to incidental cases with CD, which was also reflected by the trend towards a higher proportion of strictureing disease among twins, in agreement with an increased genetic role for ileal and fibrostenotic disease.41

A Danish twin investigation showed an overall NOD2/CARD15 allele mutation frequency of 44% among twins with CD, compared to 6.9% in the background population in Denmark, which suggested a high genetic contribution among twins with CD, in agreement with our results.

There is considerable variation in the prevalence of NOD2/CARD15 mutations among European populations. The prevalence in Norway is low among the healthy population compared to what has been reported elsewhere in Europe, with no significant association for CD. This pattern is consistent with what has been demonstrated in Scandinavia and Finland. The fact that the incidence of CD has shown a steep increase since the 1990s in the Scandinavian countries and in Finland, all with low prevalence of the NOD2/CARD15 mutations, point at environmental factors or other genetic factors as important contributors to the development of CD.

However, NOD2/CARD15 mutations have been shown to be associated with ileal and strictureing CD in Finland and in Norway, which might support that these mutations represent a risk factor for development of CD also in these countries. A higher genetic contribution among twins with CD compared to the background population might also explain the substantially higher RR for concordant disease among MZ and DZ twins with CD, compared to the background population.

The median follow-up time was 10 years for both the twins and the incidental cases, which is important when comparing behaviour and distribution of disease, as we know that sub-phenotypes of CD are changing over time. However, our finding of higher proportion of ileal and strictureing disease among twins compared to incidental cases might be limited by the small number of twins.

In conclusion, the risk for development of concordant disease among co-twins of twins with IBD was remarkably high as compared to the general population. The risk was higher among MZ twins compared to DZ twins and higher in CD compared to UC, which underscore genetic influence in CD. Furthermore, our study might suggest an increased genetic role for ileal and fibrostenotic disease.

The risk of concordance for IBD among twins was several-fold higher than among ordinary siblings. The two-fold risk for concordant disease among DZ twins with CD compared to ordinary siblings indicates the importance of shared environment in utero or in childhood. However, future studies including more twins are needed for this comparison between DZ twins and ordinary siblings.

**Statement of authorship**

MBB carried out the study and data analyses and drafted the manuscript. GA has been involved in the design of the study and the data analyses. JH and MHV have taken part in the planning, design and draft of the manuscript.

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**Potential competing interests**

None.

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