Chapter 8

General discussion
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The diagnostic process for celiac disease (CeD) has improved over the last decades as anti-tissue transglutaminase antibodies have become available as a diagnostic marker. Nonetheless, population-based serological studies suggest that at least half of patients with CeD are not diagnosed\(^1\). \textit{Part I (Chapters 2 and 3)} of my thesis aimed to better describe the clinical features of CeD by exploring an adult patient cohort with regards to the presenting symptoms at diagnosis, comorbidities and co-occurrence of immune-mediated diseases. The goal of \textit{Part II (Chapters 4-6)} was to find new predictive biomarkers for CeD and to explore whether miRNAs could be suitable candidates for this purpose. In \textit{Part III (Chapter 7)}, I incorporated the lessons, technology and analyses set up in \textit{Part II} to study circulating miRNAs in Multiple Sclerosis (MS). Finally, despite all the advances that have been made in the last years, there are still many questions and challenges left in understanding the pathophysiology of CeD, the role of circulating biomarkers in CeD and the care for patients with CeD. In this \textit{General Discussion}, I highlight the results of this thesis and discuss remaining questions, future opportunities, challenges and recommendations.

Summary of results

Part I: The clinical features of celiac disease in adults

A substantial percentage of the individuals who have CeD are not diagnosed. Part of this gap can be explained by a lack of symptoms or because the clinical spectrum of CeD is not recognized\(^4\).

In \textit{Chapters 2 and 3}, we presented our efforts to better characterize the clinical features of CeD in adults in a cohort of adult CeD patients who visited a university hospital (University Medical Center Groningen (UMCG)) or a non-university hospital (Medisch Spectrum Twente (MST, Enschede)). \textit{Chapter 2} gives a comprehensive overview of the clinical presentation of 412 adult CeD patients, including symptoms at time of diagnosis and concomitant diseases. Males in this cohort were diagnosed significantly later in life (median age: 47.8 years) than females (median age: 35.8 years). The classical symptoms that were most frequently reported at time of diagnosis are diarrhea, fatigue, abdominal pain and weight loss, each present in around \(1/3\) of patients. Around 10% of the patients did not report any symptoms at diagnosis. Immune-mediated concomitant diseases were present in 25% of our cohort, and the most prevalent diseases were type I diabetes mellitus, microscopic colitis and thyroid disease. In our cohort, we also observed cancer types that are very rare in
the general population: eight cases of enteropathy-associated T cell lymphoma and one case of jejunal carcinoma.

In Chapter 3, we searched for possible reasons why males were diagnosed later in life than female patients in our adult cohort. This difference in age at diagnosis is in line with other reports. Hence, in a questionnaire study, we collected additional data that could not be retrieved from the medical case records, including the diagnostic delay. Our results show that diagnostic delay is long in many adult patients: 2 out of 3 patients reported a delay of more than a year between the start of their symptoms and diagnosis, and this delay was not associated with the age of diagnosis. Patients with one or more classical symptom and/or a positive family history of CeD were diagnosed earlier in life. Upon correcting for classical symptoms and family history, sex was not an independent predictor of age at diagnosis. I therefore concluded that CeD manifesting later in life is accompanied by a clinical CeD phenotype that occurs more often in males and does not present with the classical symptoms. I also observed that a longer diagnostic delay is associated with a prolonged recovery time of symptoms after starting a gluten-free diet. This underlines the importance of recognizing and treating CeD soon after onset of symptoms.

In summary, Part I of my thesis will contribute to more awareness about the heterogeneous signs and symptoms of CeD in adults, thereby contributing to quicker diagnosis of CeD. Recognizing CeD soon after onset is important, but it would be even better to detect CeD at an earlier stage. However, the current diagnostic workflow is designed for and only capable of diagnosing CeD at the time villous atrophy is present. Therefore, in Part II of this thesis, we searched for new biomarkers that are predictive of the development of CeD and detectable before the diagnostic antibodies (anti-tissue transglutaminase) can be detected in blood.

Part II: Finding new biomarkers for celiac disease

In Chapter 4, we give an extensive overview of how existing and potential new biomarkers for CeD could contribute to better or quicker diagnosis and improved follow-up. The review provides an up-to-date overview of current and novel molecular biomarkers for CeD. The disadvantage of the currently available serological and histopathological diagnostic tools is that they only diagnose CeD reliably when villous atrophy is already present. It is not (yet) possible to predict whether individuals are going to develop CeD in their lifetime or when they will develop disease.

Therefore, in Chapter 5, we searched for new predictive biomarkers that might identify CeD at an earlier stage than the currently available serological tools, focusing
on miRNAs circulating in blood. The longitudinal design of the PreventCD study (see the Introduction for an overview of all cohorts used in this study) allowed us to search for markers that can be detected in the systemic circulation before seroconversion (the first sample with positive anti-transglutaminase antibodies, reflecting the CeD diagnosis). In total, 53 miRNAs were prioritized as potential markers for CeD development. This is the first study to indicate that changes in the circulating miRNA profile occur before seroconversion, and for some microRNAs this even occurs years before seroconversion. Moreover, the level of some of these miRNAs normalized upon treatment with a gluten-free diet. These biomarkers need to be investigated in more detail but provide promising candidates for earlier diagnosis of CeD, which could lead to preventive strategies to mitigate the consequences of fully developed CeD.

To study the origin of the identified circulating CeD-associated miRNAs, we also investigated the diseased tissue: the small intestine. Some of the biomarker candidates we identified were indeed differently regulated in duodenal samples of CeD patients compared to controls, suggesting that these miRNAs could originate from the small intestine. To obtain more insight into the potential function of CeD-associated miRNAs in the small intestine, we analyzed duodenal biopsies in more detail in Chapter 6. Mature miRNAs can downregulate target mRNA transcripts (see also Figure 3 of the Introduction)\textsuperscript{9}. In the context of established CeD, a few miRNA-transcript pairs had been identified\textsuperscript{10,11}, but there was no comprehensive high-throughput study available that had searched for miRNA-target transcript pairs. We therefore performed next-generation sequencing of both miRNAs and RNA-sequencing on small-intestinal biopsies from CeD patients and controls. The miRNA-target transcript pairs associated with CeD in the small intestine were identified and overlayed with previously identified miRNA-target pairs from public datasets. The resulting network of 2030 miRNA-target pairs suggests that the miRNAs deregulated in CeD play a role in modulating metabolic pathways (especially lipid metabolism) and cell cycle pathways that are important in maintaining small-intestinal barrier integrity and could lead to villous atrophy. CeD-associated miRNAs also appear to be involved in regulating immune pathways such as interferon signaling, one of the pathways involved in CeD development. Similar pathways had been implicated in CeD by other studies (e.g. \textsuperscript{12,13} and Chapter 4). Moreover, the network analyses also suggest that the expression of transcripts is affected by both CeD-associated genetic risk factors and CeD-associated miRNAs, suggesting a cooperation of both systems.

It is relevant to integrate the role of miRNAs in the pathophysiological processes as this could help to identify biomarkers that are relevant to the disease process, and
miRNAs are important to consider in the search for pathways important in disease pathophysiology in order to find novel therapeutic options for CeD.

Part III: Finding biomarkers for multiple sclerosis

In Chapter 7, I used the knowledge and framework from Chapter 5 to analyze circulating microRNAs and applied it to find new biomarkers for MS in different compartments: the systemic circulation and cerebrospinal fluid. In addition, proteomics data was collected. In MS, biomarkers that would distinguish the relapsing-remitting phase from the progressive disease forms would be helpful as they could help in making earlier treatment decisions. In this study we identified several proteins and miRNAs that are potential biomarkers in the systemic circulation that are significantly different between relapsing-remitting MS and secondary progressive MS. In the prospective part of the study, participants with relapsing-remitting MS were followed for around 3 years, during which time 12 participants converted to secondary progressive MS. In these longitudinally drawn samples, granzyme B, A and H proteins peak around the time of conversion. Single-sample enrichment analysis of serum microRNA profiles revealed that the peak in granzyme levels around conversion coincides with enrichment for microRNAs that are enriched in CD4+, CD8+ and NK cells. Overall, in addition to proposing novel biomarker candidates for the subgroups of MS, the findings in the longitudinal data suggest that specific immune cell–driven processes may contribute to the conversion of relapsing-remitting MS to secondary progressive MS.

Remaining questions, future opportunities, challenges and recommendations

Remaining questions – Is there an association between the clinical features of CeD, sex-differences and genetic factors?

Previous data and our data in Chapters 1 and 2 indicate that the clinical features of CeD, including presenting symptoms and age of presentation, is different in males compared to females. In our cohort, the age difference could not be explained by the delay between onset of symptoms and diagnosis. It should be noted that diagnostic delay was a self-reported measure in our study. The point of seroconversion, the point at which the anti-transglutaminase antibodies become positive, would better approximate the onset of CeD, but we would need longitudinal data in adults to study this phenomenon in more detail. It would thus be interesting to see whether seroconversion occurs later in life in adult males than in females.
That said, there are indications that differences between sexes are not caused only by behavioral or psychological differences between males and females, nor by the testing bias that we know exists as physicians are known to test for CeD more often in (younger) females than males\textsuperscript{14,15}. CeD is more common in females than males, in our cohort the ratio is 1:2, as in most patient cohorts with confirmed CeD (range: 1:1.5–1:2)\textsuperscript{2}. A female predominance was also found in a recent meta-analysis that analyzed population-based studies (in total almost 300,000 participants) that used serology to screen for undetected CeD\textsuperscript{16}. This was also shown in the pediatric populations in two cohorts, the PreventCD cohort and the TEDDY cohort, that followed children at high risk of developing CeD from birth, which showed that girls are at higher risk of developing CeD than boys in the first decade of life\textsuperscript{17,18}. Moreover, in the PreventCD study, girls with a double dose of HLA-DQ2.5 had a severely increased risk of early CeD development, whereas this HLA effect was not observed in boys\textsuperscript{18}. Interestingly, a few smaller cohort studies have also suggested that HLA-DQ2.5 homozygosity is associated with more classical symptoms\textsuperscript{19–21}. The HLA dose-effect could have an immunological basis as, out of all the CeD HLA types, DQ-2.5 have the highest affinity with deaminated gluten peptides. A double dose can induce a stronger induction of gluten-specific T-cells, which then set in motion the further immunological cascade that leads to CeD\textsuperscript{22,23}. Unfortunately, in our cohort, the data on HLA status is not available for most patients as this is not part of the standard diagnostic procedure, but it would be very interesting to see whether HLA status impacts the clinical phenotype.

In all, these results show that there is much to discover about the relation between genetic, immunological and environmental factors and clinical phenotype and that it is important to also look at the interaction of sex in these studies.

**Remaining questions – Are circulating microRNAs as potential biomarkers for CeD?**

The results presented in Chapter 4 suggest, for the first time, that circulating miRNAs are early biomarkers for CeD, with some markers present 2 years before seroconversion. These results are very promising as this hopefully paves the way towards earlier biomarkers for CeD. However, I do want to emphasize that circulating miRNAs in the context of CeD are still in the exploration phase and there are still many steps to take before miRNAs can be implemented as a diagnostic or predictive tool in clinical care. Although the differences between cases and controls seem to be present on group level, the findings should first be validated in independent studies. Analyses on the clinical predictive value of a biomarker (e.g. sensitivity/specificity) should preferably not be performed in the set of samples that are used for the “discovery” phase of the study, but should be confirmed in independent cohorts.
that we did not include in the analyses in Chapter 4. Based on the overlapping confidence intervals of the levels of miRNAs in our study, the sensitivity/specificity of these markers on individual level are not on a level that can be used in diagnostic care. It still would be very interesting in future to combine different markers to see whether a combination of markers (miRNAs and/or other markers) could increase the diagnostic power. Potential confounders, such as age and sex could influence the circulating miRNA profile, and therefore should also be considered when studying the circulating miRNA profile. Moreover, the disease-specificity of the markers should be tested.

Chapter 6 provided us the opportunity to study circulating miRNAs in another immune-mediated disease: MS. Both diseases are immune-driven, but the disease manifestation and localization are different. From a clinical perspective, the clinical presentation of MS and CeD are very distinct, and we therefore do not need additional biomarkers to distinguish these two diseases. Still, it is interesting to compare the results from Chapter 4 and Chapter 6 as this could provide some insights into whether miRNAs could be disease-specific. Overlaying the 53 biomarker candidates for CeD proposed in Chapter 4 with the 51 miRNA candidates in MS versus controls from Chapter 6 there is only an overlap of two miRNAs that are differentially expressed in both diseases (miR-16-5p increased and miR-150-3p decreased). The highly distinct profiles suggest that the profiles are, at least to some extent, disease-specific. Note, however, that there are methodological differences between these studies that could influence these results: the analyses in MS were performed in adults and the analyses in CeD in children.

After the biomarker candidates pass both the exploration and validation phases, high-throughput techniques (such as miRNA-sequencing) should be adapted so that the relevant markers can be tested using techniques that can be more easily, and cost-effectively, implemented in the clinics. Markers used in daily clinic ideally should be reliable and consistent, but also easy to interpret.

Remaining questions – Can we link differences in miRNA expression to pathophysiology?
In my thesis I give suggestions about the processes that microRNAs could be involved in in CeD, these results are very interesting as they seem to point to important pathways, but they are only the starting point for future studies. I would therefore like to share some lessons that I learned in my search for the functional role of miRNAs in CeD.
There are several ways to go from a microRNA to the potential pathway these miRNAs have an impact on. A quick and easy way to get from miRNA to pathway is to extract all predicted and/or functionally validated target transcripts of the miRNAs of interest and perform pathway analysis on that gene list.

As this first approach includes all potential target transcripts, it also includes genes that are not expressed in the tissue of interest and genes that are not differentially expressed between CeD and controls, which are less likely to be involved in disease pathophysiology. Therefore, we performed miRNA and mRNA-sequencing in parallel in duodenal biopsies to find those pairs that are anti-correlated (the higher the miRNA level, the lower the mRNA level, and vice versa) and included only differentially expressed miRNAs and target transcripts to add disease-specific information.

Another consideration is that the public databases that we used include both functionally validated miRNA-transcript pairs and predicted pairs based on the “seed” sequence that should match with miRNA binding sites on the mRNA to be able to function. Some of these predicted miRNA-target transcript interactions might not be functional. Crosslinking-based methods such as HITS-CLIP, in which miRNAs are crosslinked to target transcripts and subsequently sequenced, are the best way to find functional miRNA-target transcript pairs.25,26

Future studies that could give us more insights into miRNA regulation include studies zooming in on what happens in a specific cell type or even at single-cell level. The most important reason for this is that miRNAs are highly cell-type-specific.27,28 In Chapter 5, we show that cell type–composition can partially explain the differences in miRNA expression between CeD and controls in small-intestinal biopsy samples. Thus, while the analyses we performed on tissue level in Chapter 5 could lead to global pathways involved in CeD, these analyses do not necessarily reflect the regulation going on at cell-type- or even single-cell-level. Exciting new developments might allow us to perform such analyses in the future. These developments include single-cell miRNA-sequencing, although this technique is not yet as developed and widely used as single-cell RNA-sequencing.29,30 Single-cell co-sequencing of mRNA and miRNAs might be even more suitable to get insights into regulation, although the technique is currently limited by the throughput as single cells need to be handpicked and individual libraries need to be prepared.31

Lastly it might also be necessary to consider that miRNAs can be transferred from one “donor” cell to a “recipient” cell, playing a role in intercellular communication within a tissue, or even between tissues, acting as “micro-hormones”.32,33 This also
raises questions about whether the miRNAs in circulation could have roles in the pathophysiology of diseases. In my opinion, however, we still need to wait for further functional evidence that extracellular miRNAs that circulate in the bloodstream do indeed have a significant function in target tissues before we draw conclusions about the function of these miRNAs from pathway analyses performed on differentially expressed miRNAs in circulation.

Future opportunities – Well-characterized cohorts and biobanks are the cornerstone in clinical research

Well-characterized (pre-)clinical cohorts were crucial in the research projects presented in this thesis. Setting up such cohorts included careful curation of the participants included in the studies, clinical characterization including the disease classification and subcategories and uniform collection of biomaterials. During my PhD, I was involved in both the clinical characterization as well as the collection of data (Chapter 2 and Chapter 3) and biomaterials for CeD patients. I experienced that these studies take time and effort from many individuals, including patients and their families, their physician and many members of the research team. Data needs to be collected, processed, stored, analyzed, interpreted and published. Although time- and resource-consuming, the collection of multiple layers of data and of longitudinal cohorts are crucial for current and future studies on CeD biomarkers and pathophysiology in order to develop new strategies to treat or even prevent CeD in the future.

The value of combining different data types is illustrated in Chapter 5, where both miRNA and mRNA data were collected for duodenal biopsies and combined with information from public databases. This resulted in the CeD miRNA-target transcript interaction network, which provides insights into in which potential pathways CeD-associated miRNAs are involved. Another example is shown in Chapter 6, where combining extracellular miRNA data and proteomics data suggested that immune-driven events lead to the conversion from relapsing-remitting to progressive MS. Thus, collecting multiple layers of data for the same individuals can help us start to untangle the relationships between biomarkers and the potential pathophysiological mechanisms they are involved in.

Collecting multiple layers of clinical data and biomaterials is an efficient way to provide data for numerous research opportunities, as shown by the vast amount of research output from the population-based biobanks such as Lifelines and the UK-biobank (https://www.lifelines.nl/over-lifelines/resultaten; https://www.ukbiobank.ac.uk/learn-more-about-uk-biobank/our-impact. During my PhD I helped to set up
the Celiac Disease Northern Netherlands (CeDNN) study. In this multicenter study in the Netherlands, multiple layers of data will be collected for 500 CeD patients (adult and pediatric patients), 1000 relatives (genetic controls) and 500 non-related controls (e.g. partners). CeDNN will contribute to further unraveling multiple aspects of CeD pathophysiology and could also aid the search for CeD biomarkers. The development of a CeD-specific intestine-on-chip model in CeDNN will provide opportunities to study the interaction between disease factors, but it could also help to find new treatments for CeD.

The availability of longitudinal data for the same individuals enabled the search for disease biomarkers present before the development or progress of disease in Chapters 4 and 6. Longitudinal data is necessary in the search for such biomarkers, but this data also provides the opportunity to study the effect of certain risk factors (e.g. genetic or environmental risk factors) on the development of disease. Initiatives that follow infants, such as the PreventCD cohort, TEDDY, CD-GEMM and Lifelines NEXT, will provide future opportunities to study these aspects of disease.

Future challenges – Biomarkers as screening tools for CeD

Diagnosing and treating CeD is important as untreated CeD leads to reduced quality of life, symptoms and complications. Although the incidence of diagnosed CeD has increased in the last decades, CeD remains undiagnosed in many patients. CeD-specific antibodies (tissue transglutaminase and endomysium) are non-invasive and have a high diagnostic value (as discussed in the Introduction and Chapter 4) and are therefore the most important screening tool currently available. As case-finding based on symptoms alone can be challenging because of the wide clinical spectrum of CeD (as illustrated in Part I of this thesis), active case-finding, or even wider screening of certain populations, could help to decrease the gap between undiagnosed and diagnosed CeD. One of the dilemmas of the coming years is in which populations CeD screening should be implemented. It is probably not feasible, or necessary, to implement anti-tissue transglutaminase testing for the whole population on the short-term. Therefore, strategies need to be developed to increase the yield of CeD screening by selecting those individuals with the highest risk of developing CeD.

One active case-finding screening strategy currently being investigated in the Netherlands in the context of the GLUTENSCREEN study is to select children visiting primary preventive care (“consultatiebureau”) who have symptoms and perform serology. Studies like GLUTENSCREEN will give us the crucial insights into the
benefits and costs of such screening programs that are necessary to decide whether to implement these practices as standard care.

Active case-finding could be an important first step towards more detection of CeD compared to standard care in symptomatic children. As children are often asymptomatic at time of seroconversion, it might also be necessary to screen asymptomatic individuals to further increase the diagnostic rate\textsuperscript{17,41}. An example here are children with a first-degree family member with CeD who have HLA types compatible with CeD. Current guidelines do recommend testing first-degree family members for CeD but do not include advice on the frequency of re-testing\textsuperscript{42}. Longitudinal studies such as the PreventCD study show the importance of longitudinal screening due to the high incidence of CeD and propose a screening interval based on prediction of the development of CeD in the years following a negative anti-transglutaminase sample\textsuperscript{18}. It is important to note that these recommendations are only for an already selected population, and future challenges include cost-benefit analyses of the use of such screening tools in the general population, where the incidence of CeD is lower. Selecting the individuals at highest risk of developing CeD could also be improved in future by including other risk factors for CeD, for example genetic risk factors beyond the HLA region\textsuperscript{43,44}.

Screening programs that use anti-transglutaminase antibodies as described above are developed to detect individuals who already have villous atrophy. Markers are needed that can be detected at an even earlier stage because these markers could help to develop measures to prevent overt CeD. Examples for these “early” markers are microRNAs (proposed in \textit{Chapter 4}), gene expression profiles in peripheral blood monocytes, the lipidome and recently also certain circulating cytokines\textsuperscript{45–48}. These studies are, like the miRNA study, still in the exploration phase and still far from clinical application. Cytokines might be especially promising because they are relatively easy to measure and therefore easier to implement in the clinic. These include cytokines such as IL-2 and IL-8, which are thought to play a role in the immunological pathways leading to villous atrophy in CeD. Previously, it has been shown that a peak in cytokines can be measured in blood after a single bolus gluten challenge in patients with CeD who had already started a gluten-free diet\textsuperscript{49,50}. Very recently another study in part of the PreventCD cohort showed that increased levels of IL-2 can be measured before seroconversion\textsuperscript{51}.

All these studies on earlier markers for CeD are promising as they indicate that processes preceding antibody production give off signals that have the potential to be picked up. These findings also show the importance of longitudinal sampling
and hopefully are the basis for future studies that focus on finding earlier markers for CeD. Early markers that can be used to select those individuals who will develop CeD can then be used to develop strategies that help to prevent full-blown CeD.

Lastly, if screening programs in children will be implemented in the future, the proportion of undetected CeD in adults should also decrease. Still, it is recognized that onset of CeD can also occur in adults. Retesting the whole (adult) population every few years might not be feasible, as retesting a large proportion of the adult population yields few new cases. Therefore, it will still be important for gastroenterologists seeing adult patients to be aware that CeD onset can occur in adults and which signs and symptoms to look for.

**Recommendations and future challenges - Follow-up in adult CeD**

Results in this thesis also raise questions about how we can improve the long-term follow-up of adult patients with CeD. In the most recent Dutch guideline from 2008, an annual follow-up by a medical specialist is advised. In our cohort however (Chapter 2) a large proportion of patients (35% (n = 74/211)) were not followed-up annually. We did not investigate why a large proportion of our cohort were lost to follow-up, but studies in the UK and the US have also reported similar rates of patients lost to follow-up. The current European guideline advises an annual or biannual follow-up of CeD patients that focuses on dietary adherence, presence of (new) symptoms that could point to low dietary adherence, refractory CeD or malignancies and screening for comorbidities (immune-mediated diseases, osteoporosis).

There are a couple of ways to optimize the follow-up in CeD and help to achieve better follow-up rates. Currently one of the reasons for routine checkups is to find out dietary adherence, as (unintended) gluten exposure is often the cause of persistent symptoms but is also thought to be associated with persistent villous atrophy, which is associated with higher risk of CeD-associated complications. Tools to assess dietary adherence in CeD are dietary questionnaires and serology. While these are the best tools currently available, they have been shown to not be sensitive enough to detect dietary lapses. Hopefully these measures can be replaced using more suitable biomarkers. Examples here are the measurement of immunogenic gluten peptides in urine or feces, as these are a much more direct measure of gluten intake, and point-of-care measurements are currently in development (described in Chapter 3).

Another reason for routine checkups is to screen for comorbidities associated with CeD such as osteoporosis and immune-mediated diseases (e.g. thyroid disease and type I diabetes). Although we know that these comorbidities have a high prevalence
in CeD, the question remains how often we should screen in order to detect them at an early/pre-symptomatic stage to decrease disease burden. Data on how often we find such comorbidities in routine screening would be helpful but are currently not available. One example of a comorbidity that might be less relevant for screening adults is type I diabetes, as the incidence decreases after 15 years of age and diabetes often precedes the onset of CeD in children\textsuperscript{65,66}.

Finally, the ongoing discussions on the way of follow-up (gastroenterologist/general practitioner/dietician and electronic or “live”) are very important to improve follow-up\textsuperscript{54,60}. Together with the medical team (including dieticians) and patients, we can design strategies that allow for efficient follow-up and reduced loss of patients to follow-up.

**Take home messages**

The work presented in this thesis contributes to a better understanding of the clinical features of CeD and proposes novel early biomarkers for CeD. I would like end by sharing some thoughts on lessons that I learned during my research:

- Two thirds of adult CeD patients report a delay of more than a year between the start of their symptoms and diagnosis. It is important to be aware of the wide variety of symptoms and comorbidities, especially as a longer diagnostic delay is associated with slower improvement of symptoms after start of treatment.
- There are clinical differences between males and females with CeD. Awareness of these clinical differences is needed to be able to recognize CeD quickly after onset of symptoms. It is important to study the pathophysiological mechanisms that can cause different clinical features as different underlying mechanisms might ask for different approaches to prevent or treat CeD.
- Differences in the circulating miRNAs in CeD can be shown years before seroconversion, making them early biomarker candidates for CeD development. These biomarkers should be validated in independent longitudinal studies, and the factors influencing the biomarker profiles need to be studied before these can be implemented for clinical use. It is promising that miRNA profiles in CeD and MS differ, since this indicates disease-specificity.
- miRNA profiles can be linked to pathophysiological pathways. Although there are some methods that predict pathways directly from a list of miRNAs, other data types should also be collected to gather additional evidence and further narrow down the “true targets” of the miRNAs. Cell type–composition also influences the miRNA profile and needs to be considered while interpreting these results.
• Studies storing in-depth clinical data, biomaterials and multiple data layers are very valuable to study many aspects of disease. Longitudinal study designs are crucial to find markers for development or progress of disease.
• Multidisciplinary teams including both fundamental researchers, bioinformaticians/statisticians and clinicians are of great value in all aspects of the research: from formulating hypotheses, study design, efficient collection and storage of data and biomaterials, to the analyses and interpretation of results. In my opinion one of the things my research profited from most were the discussions we had in the team to formulate the research problems and the different solutions to answer them.
• Another aspect that transformed the way I think about research problems was learning “R” programming skills and performing analyses. These skills helped me to see different potential solutions to problems, but also helped me to visualize results, which helped in discussions with other team members.
References


General discussion