Gastrointestinal motility disorders in children: etiology and associated behaviors
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Summary and Discussion
Summary

Gastrointestinal motility disorders may affect children of all ages. The most commonly found gastrointestinal motility disorders in the pediatric population are gastro-esophageal reflux disease, infantile hypertrophic pyloric stenosis and functional defecation disorders. Little is known about the exact pathophysiology of these gastrointestinal motility disorders in children. More knowledge of the pathophysiological mechanisms involved in these disorders may also contribute to the development of new therapeutic strategies.

In this thesis, we aimed to further unravel the role of genetic, environmental and behavioral factors in the etiology of the most common pediatric gastrointestinal motility disorders. The most salient findings are summarized in this chapter.

Part I - Gastro-esophageal reflux disease

Gastro-esophageal reflux disease (GERD) is a very common problem in children. Twin studies and familial aggregation studies have shown evidence for a genetic background of GERD. Several genetic loci have been found to be possibly associated with GERD, but, up till now, usually no genes have been confirmed to be associated with GERD in subsequent populations. This might be due to heterogeneity of studied populations with a similar heterogeneous etiology of GERD. Therefore, in Chapter 1, after detailed phenotyping, we performed genome-wide linkage analysis in a large multi-generational Dutch family with multiple family members affected with GERD. We assumed that the genetic background of GERD in this family would be homogeneous in affected family members. In this way, we identified several new genetic loci associated with the GERD phenotype in this family. Subsequent exome sequencing of the linkage regions however, has not yet lead to the identification of a variant likely to be associated with GERD in this family. Results of further studies in this family are awaited.

Part II - Infantile hypertrophic pyloric stenosis

In Chapter 2, we provided an extensive overview of the current knowledge of genetic factors involved in the pathophysiology of infantile hypertrophic pyloric stenosis (IHPS). Although nowadays this motility disorder can be treated with a relatively simple surgical procedure, its pathophysiology is still poorly understood. The results of twin studies and familial aggregation studies clearly point to a genetic involvement, with high estimated heritability rates. Linkage studies in IHPS and one GWAS study identified several loci and specific genes, but nevertheless, no genes have been confirmed to be associated with IHPS in different populations, suggesting genetic heterogeneity. The role of nitric oxide synthase (NOS), a mediator of smooth muscle cell relaxation in the gut and frequently suggested to be associated with IHPS, still remains controversial. Next to this, we demonstrated that there are many clinical syndromes going along with IHPS, caused by...
mutations in genes affecting many different pathways possibly important in the etiology of IHPS. This indicates that both isolated and non-isolated forms of IHPS have a similarly heterogeneous background.

Next to genetic factors, environmental factors may be associated with the development of IHPS. These environmental factors may have a direct influence on health, but may also have an indirect influence by means of changes in methylation of the genome and thereby differences in gene expression. There is a continuing enigma about the presence of seasonal variations in the incidence of IHPS. Many studies have been performed in very different populations with large differences in environmental factors such as climate, cultural habits, and nutrition patterns but also in genetic background. These factors can make it challenging to identify common specific etiological factors in case seasonal variation is found in different studies. Therefore, in Chapter 3, we performed a retrospective study in two different Dutch regions of the Netherlands to assess seasonality in the incidence of IHPS. Furthermore, we correlated the incidence of IHPS in both regions to several local climate factors. Seasonality in the incidence of IHPS was found only in one region, the province of North-Holland, correlating weakly with specific local climate factors. A peak in incidence was found in children born in winter months. In the other investigated region, such a seasonal pattern in the incidence of IHPS could not be demonstrated. No correlation with climate factors was found in this region. These results indicate that other environmental factors might be of influence in the seasonal variation in the incidence of IHPS found in one of the regions. These factors might have a direct influence on the health of the infant (probably in combination with a certain genetic constitution) or might act indirectly on the genome by changing methylation patterns.

Part III; Functional defecation disorders

Little is known about the genetic background of childhood constipation. However, in clinical practice, parents of children often tell that functional constipation “runs in their family”. In chapter 4, we provided an extensive overview of studies investigating the genetic background of constipation. Familial aggregation studies and twin studies performed in families with constipation clearly point to a genetic involvement. Association studies and direct gene sequencing studies have failed in identifying genes that could be confirmed to be associated with constipation. Many genes have been found to be associated with Hirschsprung disease in children, but sequencing of these genes in individuals with constipation did not lead to the discovery of variants also associated with constipation. In this chapter, we also pointed out that constipation can be part of many clinical syndromes of which causative mutations are frequently known. We showed that these mutations are found in genes affecting all aspects of normal defecation physiology, providing evidence for a heterogenetic background of non-isolated forms of constipation.
We could assume that isolated constipation is likely to be similarly heterogeneous, which might have complicated the identification of causative genes in previously performed genetic studies in constipation.

Next to a certain genetic constitution, behavioral factors are likely to be involved in the pathophysiology of functional defecation disorders. Behavioral problems have been found to be frequently present in constipated children. However, up till now, the addition of behavioral therapy to the regular treatment protocols has not proven to lead to better outcomes for children with constipation. There might be other factors influencing the behavior of a child with a functional defecation disorder that have not been identified to this date. As functional defecation disorders have proven to be more common among children with autism spectrum disorders, in Chapter 5, we were the first to investigate the prevalence of symptoms of autism spectrum disorders prospectively and systematically in a population of children presenting with functional defecation disorders. To our surprise and concern, we found that in nearly 30% of children visiting our specialized outpatient clinic, symptoms of an autism spectrum disorder were present. In 5% of the total study population, the diagnosis of an autism spectrum disorder had already been made. Given a prevalence of autism spectrum disorders in the general population of 0.6 to 1.0%, these results require further investigation. We stressed that clinicians should be alert for symptoms of autism spectrum disorders when evaluating a child with a functional defecation disorder.

Becoming toilet trained is an important milestone in a child’s early life and a step towards independency. In general, the majority of children become toilet trained for stools and urine before the age of 4 years. It is known that the presence of a functional defecation disorder may delay the moment of completion of toilet training. However, the possible influence of the presence of symptoms of autism spectrum disorders on the moment of achievement of toilet training in these children is unknown. Therefore, in Chapter 6, we compared the moment of completion of toilet training for stools and urine between three groups of children; children with a functional defecation disorder, children with a functional defecation disorder and concomitant symptoms of an autism spectrum disorder and controls from the general population. We found that children with a functional defecation disorder and concomitant symptoms of an autism spectrum disorder were later toilet trained for stools and urine compared to children with a functional defecation disorder only and healthy controls. We suggested that clinicians should be alert for symptoms of autism spectrum disorders in children presenting with a functional defecation disorder and toilet training problems.

The presence of a functional defecation disorder might not only affect a child but also his/her family. On the other hand, the family environment of the child might also have
an influence on the pathophysiology of functional defecation disorders in children. In Chapter 7, we described a study in which we investigated the psychological and physical health status, personality and child rearing practices of parents of children with and without functional defecation disorders. Higher levels of neuroticism, psychological distress and more physical complaints were found in parents of constipated children compared to the parents of controls. Remarkably, most differences could be attributed to differences between the mothers of the two study groups. These parental characteristics might hypothetically hamper the success of the standard treatment regimen for childhood constipation, consisting of providing structure in toileting by regular toilet sits, neglecting fecal incontinence and praising positive defecation behavior. We stressed that in children with functional constipation not responding to intensive conservative treatment parental factors possibly affecting the treatment should be evaluated and a more family-based multidisciplinary treatment strategy should be considered.

Symptoms of constipation may vary widely in intensity and severity among children. We have demonstrated that the pathophysiology of childhood constipation is very heterogeneous. Next to genetic factors, behavioral factors may also have an influence. When defining the efficacy of a new treatment strategy for a group of patients with constipation, it is extremely useful to phenotype the patients in such a way that the study group is as homogeneous as possible. In Chapter 8, we described that the application of a new experimental therapy, sacral neuromodulation therapy, led to spontaneous defecation without the need of laxatives and a significant decrease in abdominal pain and school absenteeism in a homogeneous group of female constipated adolescents. These patients all suffered from severe functional constipation and had not responded before to maximal intensive conservative treatment. Sacral neuromodulation therapy appeared to be an effective and relatively safe treatment option for female adolescents with severe functional constipation.
Discussion and future perspectives

The results of this thesis have further stressed the role of genetic, environmental and behavioral factors in the complex pathophysiology of pediatric gastrointestinal motility disorders. Little is known about the genetic background of pediatric gastrointestinal motility disorders as replication studies following association and linkage studies usually failed to confirm previous findings. Still, gastrointestinal motility disorders tend to run in families and an important role of genes in their pathophysiology is supported by familial aggregation studies. Next to this, this thesis demonstrated that non-isolated, syndromic forms of motility disorders in children are commonly found. We showed that there are many Mendelian disorders with very different genetic and biological backgrounds going along with gastrointestinal motility disorders in childhood, indicating genetic heterogeneity. This suggests that isolated gastrointestinal motility disorders in children are likely to have a strong heterogenic background as well, which may well explain the lack of repeatability of linkage and association study results in subsequent patient cohorts. Genes and proteins, almost without exception, are part of large biological pathways. Therefore, as shown in Chapters 2 and 4, studying non-isolated forms of motility disorders is valuable as it may identify pathophysiological pathways possibly associated with a gastrointestinal motility disorder. Other genes acting in the same pathway may be identified in disorders resembling the disorders under study. In this way, not single genes, but rather groups of genes coding for proteins functioning in (related) biological pathways might eventually come to light and learn us more about the genetic background of isolated motility disorders. We made a first attempt to group non-isolated phenotypes of both childhood constipation and infantile hypertrophic pyloric stenosis according to their presumed pathogenesis in Chapters 2 and 4. Hopefully, in the future, the results of our review studies may help other researchers in the field with the interpretation of results from association studies, linkage studies and total exome sequencing analyses.

The presence of significant heterogeneity further underlines the need for detailed phenotyping in genetic and therapeutic research in pediatric gastrointestinal motility disorders. Genome wide association studies in large groups of unrelated individuals with homogeneous clinical characteristics are therefore less likely to succeed in the identification of genes associated with pediatric motility disorders. Linkage analysis in trios (father, mother, and sibling) and well-defined multigenerational families, on the other hand, have a high chance to reveal specific genes associated with motility disorders in the population under study. Although it is unlikely that genes identified in this way account for the same phenotype in large groups of unrelated individuals, studying these identified genes and their role in biological pathways may learn us more about the complex pathophysiological mechanisms related to gastrointestinal motility disorders.
In the near future, much can also be expected from whole exome sequencing in determining the extent to which rare alleles explain the heritability of complex heterogeneous diseases such as motility disorders. To increase the efficiency of total exome sequencing in future research, a family-based approach (trios) or an extreme phenotype approach can be recommended. In an extreme phenotype approach, patients who are at both ends of a phenotype distribution can be selected for exome sequencing. The frequencies of alleles associated with the disease are enriched at both sides of the spectrum, making it more likely to identify novel candidate alleles. In the next years, the next generation sequencing field will gradually move to whole genome sequencing, whenever bioinformatic systems are ready to handle the large amount of data generated by this technique. This will also allow for variants in non-coding regions of the genome to come to light. The increasing accessibility of large local genomic databases such as ‘GoNL’ will further facilitate researchers in the gastrointestinal motility field to explore the tremendous possibilities brought by next generation sequencing.

Still, when interpreting the results of such studies, the role of environmental factors should be taken into account. Environmental factors such as nutrition patterns, cultural habits and climate may have a vast influence on health. Furthermore, the influences of certain agents such as medication or teratogenic factors on health should not be underestimated, especially in combination with an individual’s genetic and ethnic background. Indirect influences of environmental factors through methylation changes and thereby changes in gene activation may pass from generation to generation and may have large impact on one’s health. In the near future, it will become easier to investigate the effect of certain methylation patterns in study populations with gastrointestinal motility disorders as data on the ‘methylome’ of a general Dutch population will soon become accessible.

Next to this, it might become useful to start biobanks in specialized motility centers to be able to collect genetic material for research purposes of large numbers of patients visiting the outpatient clinic. Obviously, constructing and controlling such a biobank goes along with many important ethical and legal considerations and therefore such a project should be carried out with the greatest carefulness. However, the presence of a biobank could enable genotyping after careful phenotyping of specific subgroups of patients, which may provide us more insight in the pathophysiology of motility disorders. Eventually, in the future, this might result in a situation in which phenotyping and genotyping of an individual patient may predict the chances for success of specific treatment options.

Behaviors of both children and their parents, associated with functional defecation disorders in childhood, deserve further investigation. The strikingly high percentage of symptoms of autism spectrum disorders found in children with functional defecation disorders, as described in this thesis, provides further evidence for a possible association between the two disorders. Still, it remains a challenge to explain a possible association. Behaviors associated with autism spectrum disorders might lead to problems during the
toilet training period, which is known to be a critical phase in the development of functional defecation disorders. There might also be a neurodevelopmental link to motility disorders in children with autism spectrum disorders affecting gastrointestinal motility in either an indirect or more direct way. For instance, autism spectrum disorders and functional defecation disorders could be part of a contiguous gene syndrome yet to be discovered. On the other hand, the presence of a functional defecation disorder and the frustrations going along with it might induce certain behaviors.

It is very important to keep in mind that children included in the studies described in this thesis were recruited from a tertiary referral center for functional defecation disorders, probably resulting in a bias in our study population towards more severe cases. Next to this, in our studies, no definite diagnosis of an autism spectrum disorders was made, but only symptoms of autism spectrum disorders were investigated. Future studies should include more extensive standardized observational tests to be able to assess the true prevalence of autism spectrum disorders in children with functional defecation disorders. In order to further unravel the possible etiological association between autism spectrum disorders and functional defecation disorders, it would be interesting to perform gastrointestinal motility testing in children with both disorders, such as barostat studies combined with MR imaging of the brain. Sensory and motor thresholds following rectal distension in children with autism spectrum disorders could be assessed in this way. However, of course, there are many practical and ethical objections to perform such studies in this vulnerable group of children.

In clinical practice, we stress the need for clinicians to be alert for symptoms of autism spectrum disorders in children with refractory functional defecation disorders and problematic toilet training.

In this thesis, the question of cause and effect remains similarly unanswered regarding specific characteristics of parents of constipated children. The differences we have found in parents (especially mothers) of constipated and non-constipated children could be a consequence of the presence of a chronic and often frustrating disorder in their child. One might also hypothesize that the presence of certain parental characteristics might trigger defecation problems in their children, especially in combination with a genetic predisposition or the presence of other environmental factors. Future research should focus on longitudinal prospective studies to make the point of cause and effect more clear. In these studies, the role of mother and father in the family should be taken in to account, next to the health of other siblings and child-parent interactions as these probably important factors were not part of the current investigations.