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Risk Factors for Hirschsprung Disease

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Abstract

Background*: Hirschsprung's disease (HSCR) is a congenital disorder with an approximate incidence of 1:5.000 live births, usually also associated to trisomy 21. Clinically, HSCR manifests with ileus due to a functional intestinal stenosis caused by the lack of intestinal innervation in the affected segments of the gut, usually beginning between the 5th and 12th week of embryonic development. The aim of the current research was to assess familial and environmental risk factors which could be associated to the development of HSCR, particularly during the first trimester of pregnancy.*

Methods: *The families from HSCR patients were interviewed through specialized questionnaires aiming to evaluate data on medical and pregnancy history, including changing of habits (nutrition, diet, medications), drug consumption (alcohol, smoking, illicit drugs), maternal physiological background and preexisting diseases or medical/surgical therapies. The study consisted thus of a retrospective, observational control group-supported examination.*

Results: *HSCR mothers had a significantly higher baseline weight before the start of pregnancy (p=0.045) with a higher number of live-born children (p=0.036), while HSCR fathers were significantly younger than control group (CG) fathers (p=0.03). HSCR mothers had unhealthier eating habits and a higher self- (p=0.012) or partner-nicotine consumption measured as number of cigarettes/day (p=0.042), which were also not reduced during pregnancy. Interestingly, HSCR mothers had a significantly lower medication intake when compared to CG mothers (p=0.04).*

Conclusion: *The key to reducing maternal risk factors for HSCR is in our opinion a consistent counselling of expectant mothers before and during pregnancy. Parental lifestyle parameters which may predispose to the occurrence of HSCR such as maternal obesity, smoking and unhealthy eating habits as well as vitamin deficiencies can be influenced and need to be optimised during pregnancy, since other known risk-factors cannot be altered (such as paternal age).*

Keywords: *Hirschsprung disease, risk factors, pregnancy, age, nutrition.*

Introduction

Hirschsprung's disease (HSCR) is a congenital disorder which has an incidence of approximately 1:5.000 live births [1] and is characterized by a lack of ganglia cells and intestinal innervation in the Meissner and Auerbach plexi of the intestine, usually manifesting between the 5th and the 12th week of pregnancy [2]. The consequence of this faulty innervation is a functional intestinal stenosis which may be as severe as presenting as a clinically ileus in newborns. The Incidence in boys is up to four-times higher than in females and HSCR is often associated to chromosomal anomalies, particularly trisomy 21 (over 7%, [3], who have a 40-fold increased risk of HSCR when compared to healthy newborns [4]. Located in the chromosome 10, the receptor tyrosine kinase (*RET*) protooncogene [5] is abnormal either with mutations or polymorphisms in 50% of familial cases and in 20-30% of sporadic cases of HSCR [6], mostly because *RET* variants lead to a loss of protein function [7]. Additionally, a multigenetic inheritance is assumed [8] and HSCR is often associated with a multiple endocrine neoplasia (MEN) type 2 [9]. The expression of the disease can vary greatly, HSCR always originates from the anus and extends orally over a segment of the intestine of varying lengths, the short-segment aganglionosis of the rectosigmoid being the most common form [10]. The rare total intestinal aganglionosis (1%) affects the entire gastrointestinal tract and is associated with high lethality despite intestinal transplantation [11].

Although these risk factors have been already established and are well-known, they do not explain the remaining 50% of familial and 70-80% of sporadic HSCR cases that do not show any genetic variants. Accordingly, the aim of our study was to investigate further risk factors, particularly those related to maternal health, which may be associated with HSCR in patient's concomitant or independent of genetic abnormalities.

Methods

Design

This study was designed as a retrospective, observational control group-supported examination including mothers of children with HSCR which were treated at our University Hospital from 1st January 2003 to 30st June 2017. The local ethics committee approved this study (No. 450/15). The Data was gathered from hospital files and from specialized surveys conducted using questionnaires, which were then completed with personal interviews.

Inclusion criteria

Mothers of live-borns and histologically confirmed HSCR operated during the study period were included (n=19). As comparison, a control group (CG) of mothers were recruited from age-matched children to the HSCR group who were treated for other diseases in the paediatric surgery ward, excluding HSCR or other congenital malformations (n=26). All mothers completed the same questionnaires and surveys.

Exclusion criteria

Mothers of HSCR children who were born outside the study-period or children that had other malformations were excluded from the study. Likewise, CG mothers whose children were admitted for bowel-related surgeries or congenital malformations were excluded.

Patients and surveyed subjects

The International Classification of Diseases (ICD-9 and ICD-10) was used to identify the patients using the diagnostic code Q43.1 for HSCR. During the study period a total of 26.396 deliveries occurred in the catchment area of the University Clinic for Gynecology and Obstetrics, of which 18 mothers fit the inclusion criteria while one child was adopted.

Data Collection

All HSCR families (n=19) were initially contacted by phone. From those 16 families (84.2%) consented to participate in the survey while the other three families declined participation. In the CG group, 86.6% of the mothers fully completed the questionnaires. The survey was based primarily on a modified questionnaire our cooperation partners the Malformations Monitoring Center, Otto-von-Guericke-University in Magdeburg (Germany), which was sent per post to be filled in and returned. Later, all mothers were invited for a structured interview to personally evaluate the previous medical and social history and maternal prenatal records. Data on medical history, complications during pregnancy, hormonal treatment, changes in life habits (nutrition, diet, medication), body weight, drug consumption (alcohol, smoking, illicit drugs) as well as exposure to nuclear waste, poisonous substances or landfill waste were assessed.

Statistics

All obtained data was analyzed primarily with descriptive methods and the mean, standard deviation, median and range were reported in the case of quantitative parameters, while absolute and relative frequencies were described for qualitative parameters. Exploratory tests between subsets were selected based on the underlying parameters. Given the size of the subsets, the t-test and non-parametric tests such as Wilcoxon and Kruskal-Wallis were performed in addition to ANOVA, including post-hoc testing. Ordered logistic regressions for univariate and multivariate group differences and analyses of covariance were performed. Significance was established as $p \leq 0.05$. All statistical tests were analyzed using the IBM SPSS software, version 28 (IBM, Illinois, USA).

Results

Parental physical data

HSCR mothers showed a higher body mass index (BMI) compared to CG mothers (26.46 ± 8.8 kg/m^2 versus 23.8 ± 4.2 kg/m^2), yet this difference was not significant (p=0.15). Body weight prior pregnancy was significantly higher in HSCR mothers (p=0.045) and four of these (21.0%) suffered from obesity grades I° to III° compared to only one CG mother (3.8%; p=0.44). However, there was no difference in the weight gain during pregnancy (p=0.275). HSCR mothers were with 30.1±3.7 years slightly younger than CG mothers with 31.9 ± 6.1 years, yet not significantly (p=0.12). In contrast, HSCR fathers were significantly younger than CG fathers (30.1 ± 4.9 years vs. 34 ± 7.0 years, p=0.03). These data are summarized in Table 1.

Contraceptive Methods, assisted reproduction, number of pregnancies and spontaneous or ectopic termination of pregnancy

There were no significant differences in regards to the use of contraceptive methods (especially hormonal) (p=0.285), the number of pregnancies generated through assisted reproduction techniques (p=0.498) and the number of spontaneous or ectopic termination in previous pregnancies (p=0.056) between HSCR and CG mothers. HSCR mothers reported on average 2.65 ± 1.0 gestations, which was similar to CG mothers with 2.8±1.8 pregnancies (p=0.30), yet the number of live-born children was significantly higher in HSCR (2.53±0.72 vs. 1.8±0.8, p=0.03). Seven HSCR mothers (36.8%) experienced problems during pregnancy compared to 34.6% of CG mothers and there was no significant difference (p=0.831). Pregnancy check-ups including sonography were documented in 88% of HSCR mothers and showed prenatal abnormalities in 19% (n=3) while no CG mother reported altered pregnancy check-up results. An invasive prenatal diagnostic (amniocentesis) was performed twice in HSCR mothers as a consequence of suspected prenatal ultrasound findings, in which both detected a trisomy 21 (p=0.151). These data can be seen on Tables 1 and 2.

Table 1: Risk factors of HSCR compared to CG.

Risk Factors	M. Hirschsprung (n=19; %)	CG (n=26; %)	p-value
Physical data			
Body mass index prior pregnancy (BMI; kg/m^2)	26.46 mean; SD 8.80; range 19.0-48.6)	23.8 mean; SD 4.2; range 17.8-34.8)	0.244
Body weight prior pregnancy (kg)	74.56 mean; SD 31.83; range 50-170)	61.1 mean; SD 13.6; range 48-96)	**0.045**
Weight gain during pregnancy (kg)	11.7; SD 5.4; range 0.7-25	15.96; SD 6.09; range 7.8-34	0.275
Age of mother at birth (years)	median 30.1; (31.11 mean; SD 3.71; range 25-37)	median 31.00, (31.9 mean; SD 6,1; range 21-44)	0.12
Age of father at birth (years)	median 31.0; (31.11 mean; SD 3.71; range 25-37)	median 32.0, (34.0 mean; SD 7.0; range 25-50)	**0.03**
Data around pregnancy			
Number of pregnancies/ women (n)	2.0 median; (2.65 mean; SD 1.00; range 2-5); total 45 pregnancies	2.0 median; (2.8 mean; SD 1.8; range 1-8); total 59 pregnancies	0.42
Spontaneous or ectopic termination of pregnancy (absolute number)	3 (6.6%)	15 (25.42%)	0.056
Life born children (n)	2.0 median, (2.47 mean; SD 0.8; range 1-4)	2.0 median, (2.2mean; SD 1.4; range 1-7)	**0.036**
Pre-existing factors/ illnesses			
• allergy • obesity (>30 BMI) • hypothyroidism • HSCR in family • chronic diseases	2 (10.6%) 4 (21.0%) 2 (10.6%) 1 (5.3%) 5 (26.3%)	2 (7.6%) 1 (3.8%) 3 (11.5%) 0 (0.0%) 6 (23.1%)	1.00 0.44 1.00 1.00 0.337
Pre-existing medication			
Medication intake prior pregnancy	3 (15.8%)	14 (53.8%)	**0.04**
Medication intake during pregnancy	5 (26.3%)	10 (38.5%)	0.72
Dietary habits during pregnancy			
Daily vegetables/ fruits	15 (78.95%)	25 (96.15%)	0.520
Regularly meat products (>3x/week)	14 (73.7%)	25 (96.15%	0.560
Daily dairy products	15 (78.95%)	24 (92.31%)	0.276
Daily coffee, black tea, energy drinks	15 (78.95%)	20 (76.92%)	0.437
Drug consumption			
Smoking			
Mother prior pregnancy	4 (28.6%)	9 (34.62%)	1.000
Mother during pregnancy	4 (28.6%)	0 (0%)	**0.012**
Cigarettes/d (mother) during pregnancy	3.5 (range 0-20)	0.86 (range 0-10)	**0.048**
Passive nicotine consumption during pregnancy (partner smoking)	4 (28.2%)	5 (19.23%)	0.520
Cigarettes/d (father)	6.15 (range 0-20)	2.32 (range 0-20)	**0.042**
Alcohol			
Mother prior pregnancy	11 (78.57%)	22 (84.6%)	0.194
Mother during pregnancy	0 (0%)	2 (7.69%)	0.405

Table 2: Specialized pregnancy questionnaire for HSCR and CG parents.

Pregnancy associated factors	HSCR (n=19; %)	CG (n=26; %)	p-value
Hormonal contraception methods	7 (36.8%)	13 (50.0%)	0.285
Pregnancy after sterility treatment (n)	1 (5.3%)	3 (11.5%)	0.498
Abnormalities during pregnancy	7 (36.8%)	9 (34.6%)	0.831
Abnormalities via ultrasound detected (n)	3 (15.8%)	0 (0%)	0.545
Prenatal invasive diagnostics (n)	2 (10.5%)	2 (7.7%)	0.648
Detection of Trisomy 21 in amniocentesis (n)	2 (10.5%)	0 0%)	0.151
other factors			
Mother with German origin	10 (52.6%)	21 (80.8%)	0.563
Father with German origin	13 (68.4%)	21(80.8%)	0.520
Home <25km to large-scale agriculture	3 (15.8 %)	4 (15.4%)	0.510

Pre-existing risk factors/ illnesses

Although more HSCR mothers reported allergies (p=1.00), obesity with a BMI>30 (p=0.44) and chronic diseases (p=0.337) than CG mothers, there were no significant differences between the groups. However, the medication intake of HSCR mothers was significantly less prior to pregnancy when compared to CG mothers (p=0.04) (Table 1).

Dietary habits and drug consumption

The regular consumption of different food groups was evaluated in both groups and results showed that HSCR mothers consumed less vegetables/fruits (p=0.520), meat (p=0.560) and dairy products (p=0.276) than CG mothers, but slightly more coffee/tea/energy drinks (p=0.437). Questions of drug consumption were answered by 73.7% of HSCR mothers, therefrom alcohol consumption prior pregnancy (2-3 times per month) was seen in 78.6% of HSCR (n=11) and in 84.6% of CG mothers (n=22). While HSCR mothers stopped alcohol consumption immediately after pregnancy was ascertained, 7.69% of CG mothers continued to consume alcohol occasionally. Nicotine consumption prior to pregnancy was not significantly different between HSCR (n=4; 28.6%) and CG mothers (n=9; 34.6%; p=1.0), but the number of cigarettes per day was significantly higher in HSCR mothers (p=0.012) and they continued smoking (n=4; 28.6%) during pregnancy while all CG group mothers stopped smoking immediately. The use of illicit drugs during pregnancy was denied by all interviewed mothers. Passive nicotine exposure was present in HSCR mothers, since 28.2% of their partners smoked a higher number of cigarettes per day during pregnancy compared to only 19.23% of CG fathers (p=0.042). (Table 1).

Family history, socioeconomic status of parents and external risk factors

Pre-existing conditions were reported by 26.3% of HSCR (n=5) and 23.1% of CG mothers (n=6; p=0.337). Most importantly, there were no significant differences between both groups in terms of previous diseases or malformations in siblings (p=0.217) or other relatives (p=0.390). The parental ethnical background played no role in the analyses, since again there were no significant difference between the groups either for mothers (p=0.563) or for fathers (p=0.520). (Table 2).

Linear and multiple regression analysis for HSCR risk factors

Based on the presented data, we could demonstrate that several variables were observed in higher frequency in HSCR families, such as spontaneous or ectopic termination of pregnancy, sterility treatment, number of pregnancies, life born children, mothers' obesity, smoking, medication, vitamin deficiency, dietary habits, fathers' age and nicotine consumption, external factors like home <25km to large-scale agriculture. To better understand if these variables were associated with HSCR we performed a multiple regression analysis, which is shown in Tables 3 a and 3 b.

Table 3 a: Linear regressions for HSCR yes/no.

Factor	OR	Significance	Lower estimate	Higher estimate
Spontaneous or ectopic termination of pregnancy	0.184	0.054	-0.004	0.372
Sterility treatment	-0.150	0.571	-0.097	0.135
Number of pregnancies (n)	0.019	0.739	-0.425	0.020
Life born children (n)	0.265	**0.010**	-0.462	-0.068
Weight gain during pregnancy (kg)	0.014	0.478	-0.030	0.058
Mothers obesity (BMI>30)	0.020	0.426	-0.483	0.209
Mothers smoking during pregnancy	0.714	**0.004**	0.513	0.995
Medication prior pregnancy	-0.324	**0.041**	-0.633	-0.014
Vitamin deficiency	0.605	0.234	-0.405	1.615
Daily consumption of meat products	-0.037	0.829	-0.383	0.309
Daily consumption of vegetables/fruits	-0.055	0.761	-0.421	0.310
Fathers' age at birth	0.021	0.080	-0.003	0.046
Passive nicotine consumption (Father smoking)	0.055	0.761	-0.310	0.421
Home <25km to large-scale agriculture	-0.571	**0.001**	-0.890	-0.253

Table 3 b: Multiple regression with cofactors for HSCR yes/no.

Factor	OR	Significance	Lower estimate	Higher estimate
Spontaneous or ectopic termination of pregnancy	0.305	0.636	-1.345	1.965
Number of para (n)	-0.195	0.482	-0.897	0.506
Fathers' age at birth	0.020	0.728	-0.131	0.172
Medication prior pregnancy	0.052	0.922	-1.328	1.432
Body weight prior pregnancy (kg)	0.023	0.875	-0.354	0.400
Mothers obesity (BMI>30)	0.308	0.737	-2.068	2.684
Mothers smoking during pregnancy	0.014	0.985	-1.945	1.973
Smoking father cigarettes/d	0.131	0.849	-1.660	1.922
Home <25km to large-scale agriculture	-0.207	0.780	-2.123	1.710

Relative risk for HSCR 95% CI

The probability of all variables, prior or during pregnancy, to be associated with HSCR is shown in Table 4. The relative risk for HSCR was 2.9-fold higher in mothers with a vitamin deficiency during pregnancy, 1.8-fold higher for mothers after daily juice consumption, 1.7-fold higher for mothers with pre-existing chronical illnesses, 1.6-fold higher if mothers used a cortisone-inhaler frequently, 1.4-fold higher for daily coffee, tea or energy drink consumption during pregnancy and 1.2-fold higher after passive nicotine consumption. Quite surprisingly, a family history of HSCR did not associate with an increase in the risk for subsequent cases of HSCR.

Table 4: Relative Risk for HSCR (95%CI).

Factor	Relative Risk	Significance	Lower estimate	Higher estimate
Vitamin deficiency during pregnancy	2.941	0.382	0.241	35.061
Dietary habits during pregnancy				
• Daily multivitamin juice	1.846	0.595	0.106	32.005
• Daily coffee, tea or energy drinks	1.414	0.437	0.367	5.448
Pre- existing chronical illnesses	1.759	0.237	0.422	7.333
Cortisone Inhaler (maternal use)	1.615	0.633	0.093	28.117
Passive nicotine (paternal smoking)	1.267	0.520	0.289	5.558
Trisomy 21	1.050	0.568	0.954	1.155
Family member with HSCR	0.929	0.412	0.803	1.074

Discussion

Parents of HSCR children are often distressed by questions of whether their behaviour and habits were responsible for the disease. In this case, it is essential to distinguish between factors that can be influenced and those that cannot such as the genetic background. Should additional potential risk factors be associated with HSCR, these should be optimally minimized, thus the main objective of the present study. Since the embryological development of HSCR takes place between the 5th and the 12th gestational weeks, it is essential to identify potential external influences which may act very early in the development [12].

Higher maternal age as a risk factor for HSCR has not been confirmed in studies by Best and Karim, and our data also support these results [13], [14]. Further evidence of an association of parental age and disease has been shown by Zhu et al., in which the prevalence of malformations of the limbs and multi-organ syndromes were more frequent as paternal age increases [15]. However, our own group has shown that younger maternal age is associated with other congenital malformations like abdominal wall defects [16], and with the current data we were able to show for the first time that this may also apply for HSCR, since HSCR fathers were significantly younger than controls. This observation has not been described before and therefore requires further investigation. The use of contraceptive methods and assisted pregnancy techniques (IVF, artificial insemination) were not confirmed as risk factors for HSCR, either in our study or in those by others such as Patel et al. who studied couples undergoing intrauterine insemination with abnormal sperm morphology (less than 4% normal forms). Additionally, Ryan et al. demonstrated that the incidence of HSCR is significantly lower in firstborns, a finding we also support as the parity of HSCR mothers in our cohort was significantly higher than controls [17]. These data are also in agreement with those of Granström et al., which have shown that a parity of ≥ 3 increases a child's risk for HSCR [18]. Lastly, in term of gestations Brown et al. and Tsonis et al. have shown that congenital anomalies detected via prenatal ultrasound lead to (spontaneous) termination of pregnancy more often than normal fetuses, yet we found no higher rates of miscarriages in HSCR mothers [19], [20]. Higher pre-pregnancy body weights of HSCR mothers in our cohort are similar to the findings of Chen et al., who showed that paternal pre-pregnancy BMI ≥25 kg/m^2 was associated with a higher risk of birth defect in an IVF offspring, particularly in the musculoskeletal system [21]. However, our data were independent of IVF, and thus suggest a direct link of body weight and HSCR occurrence. This has already been shown by Granström et al. identified maternal overweight as a risk indicator for HSCR [18]. We fully support these results since the incidence of II° and III° obesity was strikingly higher in HSCR mothers in our study. Additionally, maternal obesity was identified by Almatrafi et al. to increase the incidence of congenital anorectal malformations [22]. A possible explanation for this association lies again within the *RET* protooncogene, since mutations that are responsible for defective migration of enteric ganglia and nerve fibers may additionally influence the regulation of neuronal connections associated to weight gain and appetite [23]. These new data are certainly interesting and may account for the association seen in ours and previous studies. Lastly, it is our opinion that behavioral counseling should be a critical component of the routine care for overweight and obese women of reproductive age, aiming to achieve a healthy body weight and assure adequate intake of folic acid through diet and supplements both before and during the pregnancy. This type of intervention may reduce the incidence of poor maternal and fetal outcomes and could be delivered in both clinical and community-based settings, beginning with routine follow-ups and continuing throughout pre- and postnatal visits [24]. In another group of possible risk factors, it has been established that dietary habits influence the incidence of congenital malformations like neural tube or and congenital heart defects [25], primary congenital glaucoma [26] or congenital defects of male reproductive tract [27].

In terms of HSCR, both Granström et al. and Heuckeroth et al. described an influence of nutrition on the development of an aganglionosis [28], [18]. We did not, however, observe similar findings in our cohort, perhaps due its relatively small size. In animal models, it has already been shown by Fu et al., that vitamin A deficiency leads to aganglionosis of the distal intestinal segments [29] and that biotin improves the migration of the ganglion cells of the enteric nervous system, consequently biotin deficiencies can lead to clinical aganglionosis [30]. Our data also suggest that vitamin deficiency may influence the incidence of aganglionosis, which is also in agreement to studies that showed that vitamin supplementation reduce the incidence of congenital malformations like non-syndromic omphalocele [31] and genital organ and limb defects [32]. Finally, our data suggests that smoking during pregnancy and more importantly passive nicotine consumption (partner smoking) are associated with HSCR, although this is not in agreement with the results of Granström et al. who did not identify smoking as a risk factor for HSCR [18]. It has been previously well-established that the interaction of several factors (external, genetic and socio-economic) should be regarded as causative for the occurrence of congenital malformations [33]. In the same token, the Influence of ethnicity on congenital malformations has also been established [34], such as a higher incidence of congenital heart defects in Asian and Black ethnic groups [35]. An association in HSCR may be related to variants in the *RET* protooncogene is possible, since in a trans-ethnic meta-analysis a European- and Asian- specific variant of *RET* loci exist [36]. We could not confirm these results for our HSCR cohort, but it is possible that investigations with a larger cohort may reveal the same pattern.

Conclusion

In conclusion, we believe that there are several non-genetic risk factors that may be associated with both sporadic and familial HSCR, such as vitamin deficiency (2,9-fold increase in the relative risk (RR)), daily multivitamin juice consumption (1,8-fold RR), pre-existing chronical illnesses (1,7-fold RR), regular use of cortisone inhaler (1,6-fold RR), daily coffee, tea or energy drink consumption (1,4-fold RR) passive nicotine consumption through smoking fathers (1,2-fold RR). The key to reducing maternal risk factors for HSCR is consistent counselling before and during pregnancy. Influenceable parental lifestyle factors such as maternal obesity, smoking and unhealthy eating habits as well as vitamin deficiencies need to be detected and corrected, since they may interact synergistically with additional uninfluenceable factors such as paternal age and genetics potentiating the risk for the occurrence of HSCR.

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Disclosure statements

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Ethics approval and consent statement: Parents have given their written informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol has been approved by the research institute`s committee on human research. The local ethics committee of the University Hospital Ulm approved this study (No. 450/15).

Conflict of interest: No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this article

Authors' contributions: MK organized the study, performed the clinical examinations and was a major contributor in writing the manuscript. SF contacted the parents, organized the database and analysed the data. AS supervised the project and interpreted the patient data. All authors read and approved the final manuscript.

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