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„Parental age effect on health and reproduction: Parental age and risk of Multiple Sclerosis."

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Abstract

Background: The parental age at conception has been rising in the last decades, primarily in developed countries. (1) Therefore, the parental age effect on the offspring had been taken into consideration more closely. Both, maternal and paternal age at conception, equally may have an adverse impact on the offspring's health. It's well known that advanced parental age is associated with diseases of complex aetiology and with autosomal disorders in offspring. (2) Multiple studies showed an association between the paternal age effect and the risk of schizophrenia and autism. (3–5, 2) Whereas advanced maternal age is strongly associated with chromosomal abnormalities, like Down syndrome. (6)

Multiple sclerosis (MS) is an inflammatory autoimmune disorder of the central nervous system (CNS) with demyelination. (7) The aetiology of this disease is not completely explored yet. So far, the risks for the disease seem to include external factors such as environmental exposures and genetic susceptibility. (3)

Some studies have already been taking a closer look at the possibility of an association between an advanced parental age at birth and the risk of Multiple sclerosis in offspring, with different results. Montgomery et al. (2004) observed an increased risk of Multiple sclerosis with advanced paternal age, whereas Ramagopalan et al. (2010) could not find an association between advanced parental age and a susceptibility to Multiple sclerosis.

Methods: We used the Wisconsin Longitudinal data set to investigate, if advanced parental age at birth has an impact on the risk of multiple sclerosis in offspring. For that we used generalized linear models.

Results: We found that age of father at birth of an individual is significantly positive associated with the diagnosis of MS, whereas age of mother is significantly negative associated. So, the risk of MS increases with fathers age and reduces with mothers age.

Conclusions: Parental age at birth is associated with susceptibility to MS.

Zusammenfassung

Hintergrund: Das Alter von Eltern zum Zeitpunkt der Geburt ihrer Kinder stieg in den letzten Jahrzehnten, insbesondere in Industriestaaten, stetig. Dies induzierte eine genauere Betrachtung möglicher Auswirkungen eines höheren Elternalters auf den Nachwuchs, bzw. des sogenannten elterlichen Alterseffekts. Sowohl ein höheres Alter der Mutter als auch des Vaters zum Zeitpunkt der Konzeption können sich negativ auf die Gesundheit der Nachkommen auswirken. Ein fortgeschrittenes Alter der Eltern geht bekannterweise mit autosomal rezessiv vererbten Erkrankungen, sowie mit Krankheiten komplexer Ätiologie der Kinder einher. In multiplen Studien wurde zum Beispiel eine Assoziation zwischen einem väterlichen Alterseffekt und einem erhöhten Risiko der Erkrankung an Schizophrenie oder Autismus der Nachkommen nachgewiesen. Bezüglich des mütterlichen Alterseffekts ist mit steigendem Alter der Mutter ein erhöhtes Risiko für chromosomale Anomalien der Kinder, wie in etwa das Downsyndrom, ubiquitär bekannt.

Multiple Sklerose ist eine autoimmune, chronisch-entzündliche Erkrankung des zentralen Nervensystems, welche mit einer Demyelinisierung der Nervenfasern und axonalen Schädigungen einhergeht. Die Ätiologie dieser Erkrankung ist weitgehend unbekannt. Mit hoher Wahrscheinlichkeit tragen jedoch Umweltfaktoren, sowie eine genetische Prädisposition zum Erkrankungsrisiko bei.

In einigen wenigen Studien wurde bereits die Möglichkeit eines Zusammenhangs zwischen einem elterlichen Alterseffekt und einem höheren Risiko der Nachkommen an Multiple Sklerose zu erkranken in Erwägung gezogen und untersucht. Montgomery et al. (2004) konnten bei steigendem väterlichem Alter ein höheres Risiko für eine MS-Erkrankung der Kinder nachweisen, während Ramagopalan et al. (2010) diese Assoziation in ihren Forschungsergebnissen nicht bestätigen konnten.

Methode: In dieser Studie wurden Daten der Wisconsin Longitudinal Study verwendet, um einen möglichen elterlichen Alterseffekt in Bezug auf das Risiko an Multiple Sklerose zu erkranken zu prüfen. Hierfür verwendeten wir ein allgemeines lineares Modell.

Ergebnisse: Unsere Ergebnisse zeigten, dass ein höheres Alter des Vaters zum Zeitpunkt der Geburt eines Kindes signifikant positiv assoziiert ist mit der Diagnose einer Multiple Sklerose der Nachkommen. Im Gegensatz dazu besteht bei einem fortgeschrittenen Alter der Mutter eine signifikant negative Assoziation mit der Diagnose einer Multiple Sklerose des Kindes. Ein höheres Alter des Vaters geht mit einer höheren Wahrscheinlichkeit der Nachkommen an Multiple Sklerose zu erkranken einher, während ein höheres Alter der Mutter die Suszeptibilität der Kinder an einer Multiplen Sklerose zu erkranken verringert.

Conclusio: Die Wahrscheinlichkeit an MS zu erkranken steht in Zusammenhang mit dem Alter der Eltern bei der Geburt.

Introduction

Multiple sclerosis is a chronic inflammatory disease of the central nervous system. MS is the most common cause of neurological disability in young adults with an age of onset between 20 and 40 years. (8) The prevalence of MS differs geographically and ethnically, with an increased accumulation in North America and Europe. (9) Since the aetiology of the disease is still mostly unknown, many studies have been conducted to get some clarity. So far, MS seems to be caused by an interaction of environmental and genetic risk factors. (8) Current studies are taking a closer look at causal biologic pathways and hope for an outcome that can help to prevent the disease or rather slow the progression, or give some clues for a better therapy of multiple sclerosis. (10)

The parental age effect describes the biological consequences of an advanced parental age at conception on offspring. Several studies indicated a negative effect on offspring‘s longevity due to an advanced parental age at breeding. As advanced maternal age has been linked to a reduced offspring’s lifespan and it has been shown that advanced paternal age contributes negatively to juvenile viability and adult reproductive performance in offspring, an impact of advanced paternal age (APA) on intra-population divergence is suggested. (11) So far, the evidence of parental age effects in humans is mostly correlational. Although the paternal age effect in human beings seems to be mediated by genetic mutations and epigenetic factors.

Multiple studies showed an association between the paternal age effect and the risk of schizophrenia and autism. The risk of autism in offspring of men at the age of 40 or older is 5.75 times higher than in children of men younger than 30 years. (12, 5, 13, 6) Other neurodevelopmental diseases such as sporadic cancers of the nervous system and brain have also been associated with APA. (12)

It’s well known that advanced parental age is associated with diseases of complex aetiology and with autosomal disorders in offspring. Advanced maternal age is strongly associated with genetic diseases like Down syndrome. Immune function changes age-related, therefore maternal age may have an impact on infectious exposures in young offspring as well as a direct influence on the fetus in utero. (12)

Additionally, other human genetic diseases including bipolar disorder, epilepsy, achondroplasia, the Apert syndrome, the Marfan syndrome and fibrodysplasia ossificans progressiva, were reported to be associated with advanced paternal age. (6) Another study showed that advanced parental age is connected with an increased risk of retinoblastoma and leukaemia. (14)

According to Montgomery et al. (2004) the risk of MS increases with father’s age, up to 2.00 for 51- to 55-year-old fathers, but not mother’s age. The paternal age effect may be associated with an increased risk of accumulating germ cell mutations among older men. Some de novo

germline mutations may increase the risk of MS in offspring. (12) Not only DNMs but also epigenetic changes are associated with aging. (3)

Background

Parental age

The evolution of the gene pool is shaped by demographic characteristics, including genetic drift, gene flow and natural selection. Kong et al. (2012) revealed that especially a paternal age effect shows an impact on the mutation rate, which is considerable for genomic change. (13)

A paternal age effect has first been suggested in the late 1800 and the first been described for achondroplasia in the 1950s. (15) The so-called “Lansing effect” describes a reduced longevity in offspring of older parents. Noguera et al. (2018) postulated that the mechanism of the Lansing effect is determined by paternally driven pre-natal telomere reduction. (16) Wylde et al. (2019) also support the opinion, that accumulation of germline-mutations might be the cause for parental age effects. Although they are open to consider non-genetic factors as well. Epigenetic pathways, like DNA methylation might be connected to age related genome changes too. A phenomenon also known as the “epigenetic clock”. (11) An experimental study in mice confirmed the hypothesis that delayed fatherhood is associated with reduced life span in offspring, mediated by epigenetic changes. (17) Other explanations might be given by transmitted altered microRNAs or transmitted proteins via gametes. (11)

The high number of germ cell divisions in aging men is associated with epigenetic changes that occur within spermatocytes. This affects especially modifications to histone and DNA methylation in spermatogenesis of older men and results in increased risk of diseases in offspring. (7) Epigenetic changes, like age-related DNA methylation mutations, take place at the zygotic stage and disrupt the physiological developmental process, therefore they are accompanied by an increased risk of disorders. (18) For instance, Curley et al. 2017 conducted a study to investigate the role of epigenetic mechanisms and suggested, that epigenetic changes in sperm cells might be contributed to chronic exposure to alcohol or cocaine. (19)

In addition to neurodevelopmental diseases, a recent US-study showed that advanced paternal age, meaning father’s aged 45 years or older, is also associated with an increased risk of premature birth, low birth weight and low Apgar score. They concluded that higher paternal age is accompanied by negative effects on mothers (gestational diabetes and pre-eclampsia) and offspring. (7) Many studies have reported an increased risk for mental health issues in the offspring of both younger and older parents compared to intermediate aged parents. (20)

A chinese study showed a positive relationship between parental age and intelligence of offspring. Children born of young parents, at the age of 26–28 for mothers and 30–32 for fathers, tend to be less intelligent than the offspring of more mature parents. Whereas a paternal age above 45 is associated with reduced IQ in the offspring. One explanation for this effect is a rising socioeconomic status with higher parental age resulting in increased resources for the education of the offspring. The quality of sperm may also influence the offspring, as spermatozoa attain their maximum values in men at 30-35 years of age. (6) Carslake et al. (2017) came to the same conclusion. Their results suggested, that an advanced maternal age that is accompanied with greater life experience and higher social position, may have a positive influence on the intelligence and social status of their offspring. (1)

Primary in developed countries parental age at conception has increased over the last decades. In a population based cohort study Khandwala et al. (2018) postulated that the female age at first births in the US has approximately risen about 2% annually since the 1970s. Simultaneously the number of fathers, aged more than 40 years at the conception of their first child, has doubled, to 9%. (7) Figure 1) shows an increase of 30% in delayed parenthood, including fathers over 35 years old, from 1980 to 2008. (15) Long term implications of such demographic shifts might involve a health risk for subsequent generations. (21)

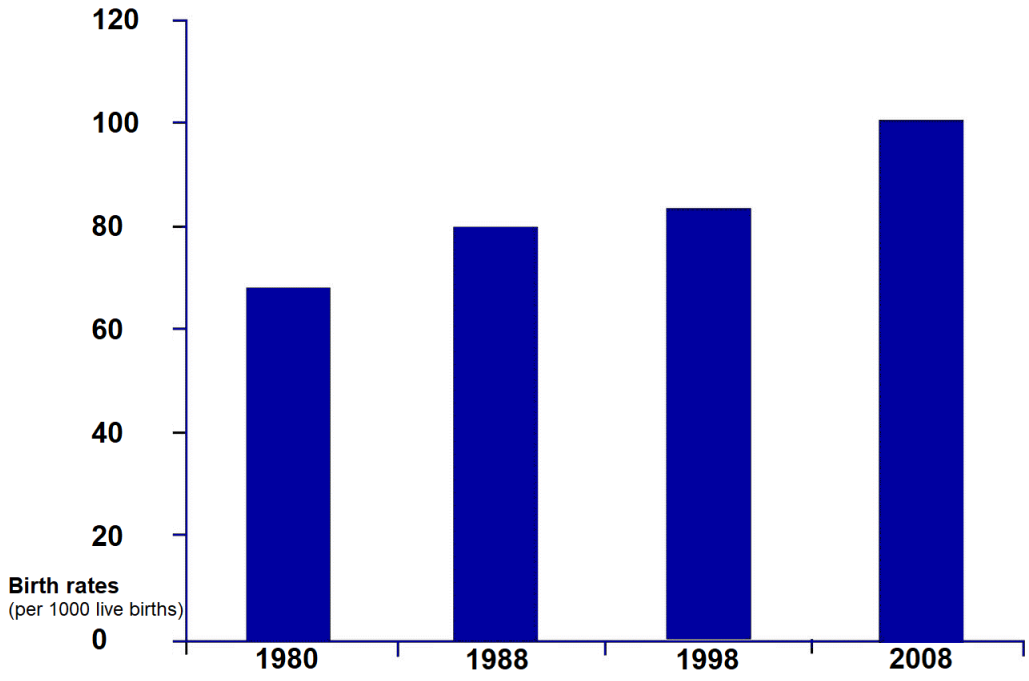

Figure 1) Increased birth rates among older fathers. (Momand et al., 2013) (15)

Wylde et al. (2019) not only confirmed that both, maternal and paternal age at breeding have negative effects on offspring longevity. They also suggest that parental age variations at

breeding could have an impact on intra-population divergence regarding mortality and longevity, although our knowledge about parental age effects is poor up to now. Many different organisms, such as plants, insects, birds and mammals, have shown a maternal age effect, mostly regarding offspring lifespan. Two other factors, which have been described to be influenced by parental age, are juvenile viability and adult reproductive performance in offspring. (11) Carslake et al. (2017) revealed a maternal age effect that resulted in a higher BMI, blood pressure, height, intelligence, non-cognitive ability and socioeconomic position in offspring. As well the children of older mothers tended to smoke or be left-handed less likely. Their findings also suggested that older mothers and their child's intra-uterine and early life environmental exposures contribute to a higher risk of cardiovascular health issues in offspring. On the other hand their children might benefit from their greater life experience and social position, hence intellectual and social advantages are suspected. (1)

Gao et al. (2019) found evidence that an accumulation of damage in oocytes, as well as postzygotic mutations in the embryo might have an impact on the female germline mutation rate and therefore give an explanation for a maternal age effect. (22)

Janecka et al. (2017) added, that primarily animal studies revealed the role of de novo mutations in the mediation of advanced parental age effects. So, the interpretation should be inspected critical as the effect of familial factors has to be taken into consideration as well, because an elimination of these could distort the importance of the novo factors. If familial factors and de novo mutations aren't separated and investigated apart from each other, the importance of either of it to the parental age effect remains equivocal. Whether it's familial or sporadic forms of disorder, genetic liability likely interacts with age at conception and both by themselves increase the risk of psychiatric disorder. (Table 1) They suggested an interaction of both effects, referred to an APA effect in neurodevelopmental disorders. (18)

Hercus et al. (2000) postulated the possibility of an interaction of APA effects and environmental factors like diet and stress. Although an accumulation and interaction of such effects over multiple generations might be the most important gap to be taken into account. (23)

	High genetic liability	Normal genetic liability
Average age at reproduction	A 1. High familial risk 2. Normal de novo burden Result: Familial cases	B Normal development
Old age at conception	C 1. High familial risk 2. High de novo burden Result: Familial cases	D 1. Normal familial risk 2. High de novo burden Result: Sporadic cases

Table 1) Different disorder aetiologies among sporadic and familial cases. (Janecka et al., 2017) (18)

Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory autoimmune disorder of the central nervous system (CNS) with demyelination. The destruction of the protective myelin sheath of neurons results in macroscopic lesions in the brain and imply progressive disability. (4) It's suspected that inflammation and degeneration occur parallel, although the inflammatory process seems to be more important in the beginning of the disease, whereas neurodegeneration weighs more towards disease progression. (10)

Over two million people worldwide are affected by multiple sclerosis. (24) MS is usually first diagnosed between the third and fourth decade of life and is the most disabling neurologic disease in young adults. (25, 26) Women are affected much more often by MS than men. The MS prevalence ratio of women to men is about 2.3-3.5 to 1. (27) Life expectancy in MS patients is approximately reduced by seven to eight years. (28) A late onset of MS and a higher number of relapses in the first few years after first manifestation are accompanied by a poor prognosis. (29)

The disease is most common in North America and Europe, with a prevalence of 0.1% to 0.2% of the population. The prevalence of MS differs a lot around the world. MS is observed more often in northern regions compared to the southern hemisphere. Comparatively, Asian countries and native populations across the Americas and Oceania show low prevalence rates of MS and the disease is almost non-existent in black Africans. This distribution might be due to different genetic, environmental, cultural and behavioural factors. (27, 26, 25) (Figure 2) Approximately over 400.000 people suffer from MS in the United States, about 700.000 Europeans and 23.000 Australians are affected. (30)

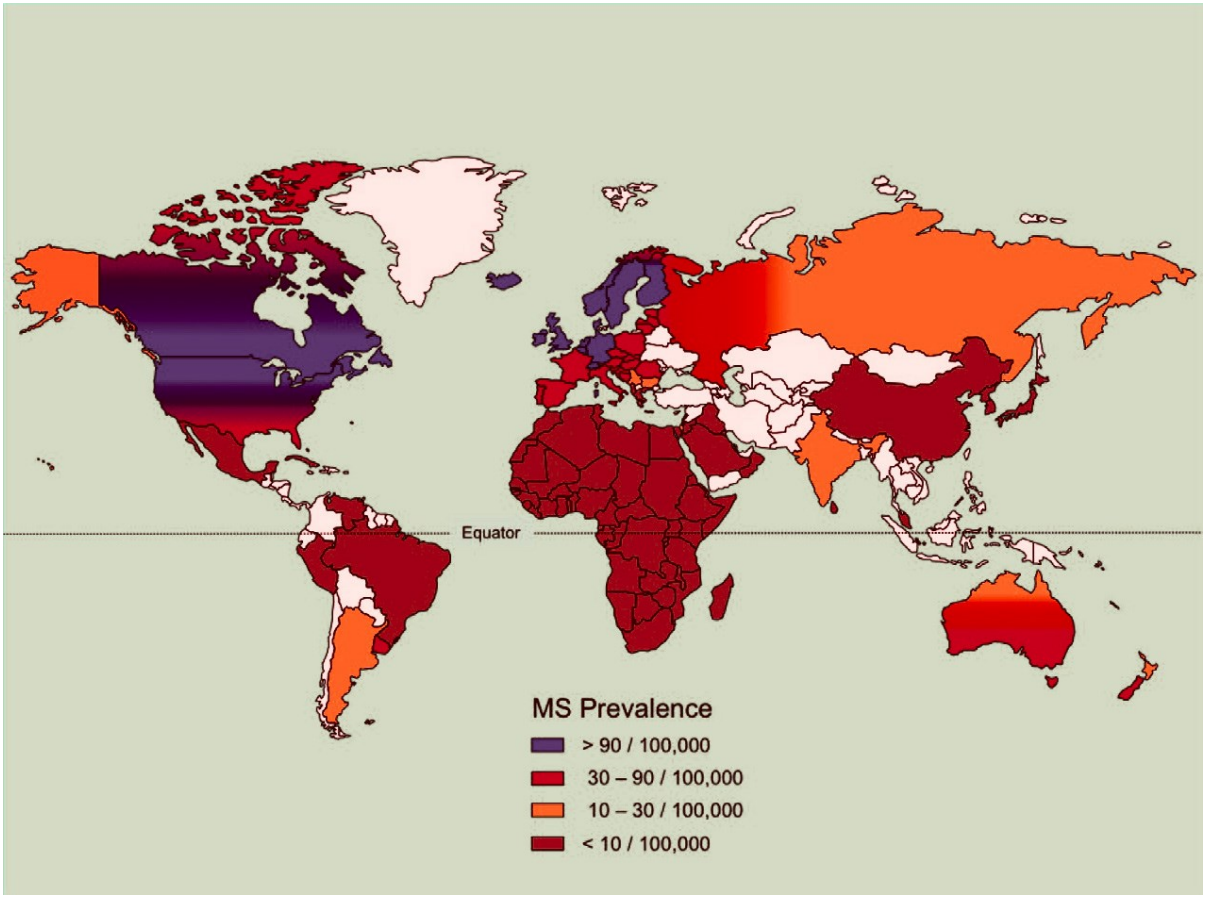

Figure 2) Global distribution of MS prevalence (Goodin, 2016)

For the clinical diagnosis of MS several criteria, known as the McDonald diagnostic criteria for MS, must be fulfilled, containing a dissemination in time and space. (Table 2) Therefore, at least one lesion in at least 2 of 4 locations within the brain (juxtacortical, periventricular, infratentorial and spinal cord) must be detected by imaging, separated in time. (31) The state of the first clinical presentation of MS is called clinically isolated syndrome (CIS). CIS describes the appearance of characteristics of inflammatory demyelination, which last at least 24 hours, without fulfilling the McDonald diagnostic criteria. It's the first episode of neurologic symptoms such as visual blurring or double vision (optic neuritis), incoordination (brain stem/cerebellum), weakness/ incontinence/ muscle spasms (spinal cord), cognitive impairment or hemiparesis (cerebral hemispheres), depending on the location of plaques within the CNS. (31, 32) This means, that the exact neuroanatomical location of the plaque determines the clinical MS symptoms. The manifestation of neurological symptoms is as essential for the diagnosis of MS as lesions of white matter and the exclusion of other possible diagnosis. (29) An acute MS lesion is accompanied by pathological features, such as inflammation, a disturbed blood-brain barrier, demyelination and axonal damage. (28) An additional diagnostic tool are CSF markers. CSF examination helps to predict the possibility of a conversion to MS by finding an elevated leukocyte count, by the detection of activated B cells or plasma cells, elevated IgG synthesis,

oligoclonal IgG bands (OCBs) and an increased level of intrathecally produced immunoglobulins against particular viruses. (32) Secondary, analysis of CSF helps to rule out differential diagnosis, such as infection or vasculitis. (29)

The 2017 McDonald criteria for diagnosis of MS		
Clinical presentation	**Number of lesions with objective clinical evidence**	**Additional data needed for MS diagnosis**
≥ 2 clinical attacks	≥ 2 lesions	None
≥ 2 clinical attacks	1 lesion (as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location)	None
≥ 2 clinical attacks	1 lesion	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI
1 clinical attack	≥ 2 lesions	Dissemination in time (DIT) demonstrated by an additional clinical attack or by MRI OR demonstration of CSF-specific oligoclonal bands
1 clinical attack	1 lesion	Dissemination in space (DIS) demonstrated by an additional clinical attack implicating a different CNS site or by MRI and Dissemination in time demonstrated by an additional clinical attack or By MRI or demonstration of CSF-specific oligoclonal bands

Table 2) McDonald criteria for diagnosis of MS (Thompson, 2017) (33)
(An attack is defined as an event of symptoms typical of an acute inflammatory demyelinating event in the CNS lasting 24 hours at least, without being accompanied by fever or infection. Abbreviations: MS: multiple sclerosis; CNS: central nervous system; MRI: magnetic resonance imaging; DIS: dissemination in space; DIT: dissemination in time; CSF: cerebrospinal fluid) (32)

There are four different main forms of multiple sclerosis. The first is called relapsing and remitting multiple sclerosis (RRMS), which describes changing episodes of neurologic dysfunction and stable phases. It's the most common form, with about 85% of MS patients, who are initially diagnosed with RRMS. The second form is primary progressive multiple sclerosis (PPMS), with an incidence of about 15%. In this MS form the neurological symptoms progress constantly from the onset of the disease. Secondary progressive multiple sclerosis (SPMS) is the third and second most common form of MS (about 40%), which is characterized by a delayed appearance of progressive neurologic disability. The last and rarest subtype of MS is the progressive relapsing MS (PRMS) with an incidence of 5%. (8, 30)

The aetiology of this disease is not completely explored yet. So far, the risks for the disease seem to include both, external factors such as environmental exposures and genetic susceptibility. (12) Strong environmental risk factors include Vitamin D deficiency, smoking and viral infections. An impact of month of birth, associated with maternal ultraviolet exposure or viral infections during the foetal period, has also been postulated. (27) A multifactorial interaction of genetic, epigenetic, infectious, nutritional, climatic factors and other environmental influences such as EBV-infection, sunlight exposure and smoking result in a disturbed immune homeostasis and the development of inflammatory reactions against the CNS. Neurological dysfunction is due to myelin loss, gliosis and axonal pathology in a more advanced stage. (25)

EBV is a virus of the herpes family and represents a very common infection in in humans, with an infection rate of over 90% of the worldwide population. Most infected individuals are asymptomatic, except for people with a delayed initial infection in adolescence. In young adulthood the first contact with EBV results in the syndrome of infectious mononucleosis in 35-50% of cases. Produced antibodies of the IgG class persist for a lifetime. MS susceptibility has been linked to an EBV infection, as nearly 100% of MS patients showed a prior EBV infection, before the first symptoms of MS occurred. (26) EBV encodes microRNAs (miRNAs), which interfere with antigen presentation and immune cell activation, subsequently miRNAs suppress the antiviral immunity. (10) Therefore, it's suspected that EBV plays a role in the causal pathway leading to MS. (26)

Vitamin D deficiency is also suggested to be linked to the pathogenesis of MS. The two human populations that show a very low risk of MS are Inuit, who eat a lot of oily fish, and Sami, who eat reindeer meat regularly. Both populations have natural dietary sources with a sufficient quantity of Vitamin D. Comparing the worldwide distribution of MS and the regions level of UVB radiation, an accordance of the MS prevalence with regions of reduced UVB availability is striking. Since Vitamin D plays a role in cellular processes, including diverse immune functions and immunomodulation, the deficiency of Vitamin D seems to be related to the pathogenesis

of several autoimmune diseases. (26) Waubant et al. (2019) confirmed a possible beneficial effect of UV radiation and vitamin D for MS by influencing the immune function. (10)
Another environmental factor associated with the susceptibility of MS is tobacco. Smoking has been taken into account for some autoimmune diseases, like systemic lupus erythematosus, Crohn's disease, rheumatoid arthritis, and thyroid disease. This linkage might be due to an effect of nicotine on the CNS. Another study gave a different explanation by showing an interaction between nicotine abuse and the presence of HLADRB1 susceptibility alleles. Secondary, MS progression seems to be associated with smoking behaviour. (26)
The last environmental factor of greater interest is obesity. Multiple studies showed consistent evidence that childhood obesity and obesity in adolescence are linked to a higher risk for MS. Obesity is associated with a chronic inflammatory process, including higher levels of proinflammatory cytokines and lower serum levels of vitamin D. Therefore, an increased susceptibility for MS in obese individuals might be due to a state of chronic inflammation.
Other environmental factors mentioned in table 3) lack biologic plausibility in the pathogenesis of MS. (26)

Potential environmental triggers of MS	
Strong association with MS	**Low association with MS**
• EBV infection • Vitamin D deficiency • Tobacco • Obesity	• Other infections (typhoid, smallpox, human herpesvirus (HHV)-6, chickenpox, Chlamydia) • Trauma • Psychological stress • Vaccinations, Cosmic rays, Occupational hazards • Living with domesticated animals • Dietary, habits, and toxic exposures

Table 3) Potential environmental triggers of MS (Goodin, 2016) (26)

Multiple sclerosis is a neurodegenerative disease of multifactorial aetiology that is not inherited, but a greater susceptibility to acquiring MS can be transmitted. (28) A genetic susceptibility of MS was observed in family studies showing a recurrence rate of approximately 15% in relatives and a decreasing risk with genetic distance. (27) Siblings risk of MS is about seven times higher than the risk of the general population. Twin studies have revealed a higher concordance rate in monozygotic twins (20-30%), compared to dizygotic twins (2-5%). (25) The influence of human leukocyte antigen (HLA) class II on the susceptibility of multiple sclerosis has been known for a long time. (10) The human leukocyte antigen (HLA) gene cluster appears to be a major genetic risk factor and the strongest risk is conferred by HLA-DRB1. (4)

It's known, that approximately 25-30% of the northern European and of the population of the United States are carrier of this gene variant. HLA-DRB1 is responsible for the attack of the CNS by T cells. (10)
CNS infestation is mediated by autoreactive lymphocytes. By crossing the blood-brain barrier these lymphocytes cause local inflammation. As a result, demyelination, gliotic scarring and axonal loss emerge. MS is a T-cell driven autoimmune disease showing a predominance of $CD8^+$ cells. The initial inflammation is suspected to be induced by $CD8^+$ T cells, $CD4^+$ T cells and active microglia/ macrophages. Then, MHC-I expression is required for a specific interaction of $CD8^+$ T cells and target cells. Since the MHC-I expression is regulated in neurons and MHC-I molecules a response to strong danger signals like proinflammatory cytokines (INF-γ or TNF-α) follows. (29)

Several viruses are suggested to cause diseases involving demyelination, including measles (in case of delayed infection), human immunodeficiency virus (HIV), and the human T-cell lymphotropic virus type I. Epstein-Barr virus (EBV) shows a very strong association with MS and EBV-induced infectious mononucleosis may increase the risk of MS similarly high as the strongest genetic risk factor. (12, 4) Multiple sclerosis is a complex disease that indicates a substantial genetic influence. (34) DNA methylation represents an important epigenetic modification and plays a major role in the pathology of multiple sclerosis. Therefore, it is part of current research as potential therapeutic targets. (35) Methylation changes have been found at HLA-DRB1 in $CD4^+$ in MS patients but not in $CD8^+$. Therefore, it remains unclear if these changes are responses to MS or can be considered as drivers to MS. (10)

Since 2007 more than 200 gene loci have been discovered with the advent of genome-wide association studies (GWAS), that contribute to the pathogenesis of multiple sclerosis. Many of these detected gene variants are not only associated with MS susceptibility, but with immunological processes and other autoimmune diseases as well. (9)
So far, functional consequences have been described for only a few out of 200 MS susceptibility loci and pathway analysis should be conducted to enable further genetic interpretations. Baranzini & Oksenberg (2017) are in the opinion, that their genetic approach prospectively provides the possibility of computing a personalized risk profile. (25) Gene and environment interactions are in the focus of current research, as their combined effects might reveal a lead to a better understanding of the aetiology of multiple sclerosis. (9)
Current therapeutic approaches of multiple sclerosis include monoclonal antibodies, immunotherapy/vaccines, potassium channel blockers and protein growth factors. (30) The form of the disease, as well as the clinical course of MS is essential to the indication of drugs. (29) The goals of MS medication include reducing MS symptoms or enabling a better handling of neurological symptoms, aiming a fast recovery from relapses, slowing down disease

progression, resulting in an improved quality of life for the patients. Unfortunately, MS progression can only be prolonged by medical treatment. Up to now, a stop of disease progression is not possible yet. (30)

Genetic approach

Generally, mutations are important and necessary to provide sequence diversity hence, to create substrate for selection. DNMs play a major role in human evolution, besides genetic drift, gene flow and natural selection. (13)
DNMs are new mutations that are only found in the offspring and not observed in the genomes of the biological parents. Errors during DNA replication or errors in recombination can lead to DNMs, which can occur anywhere in the genome. These Mutations, neither parent possessed nor transmitted, are unique to the child when compared to the parent. DNMs appear to have a stronger effect on an organism than carrying a transmitted copy number variation (CNV), which play important roles in human evolution and disease as well. (3, 36) De novo single nucleotide variants (SNVs) are implicated as well in the aetiology of schizophrenia and autism spectrum disorder. (37) The types of DNMs across the genome are described in table 4). (36)

Summary of the types of DNMs across the genome			
DNM class	Size	Description	Average number of DNMs per genome
Copy number variant (CNV)	> 50 bp	Genomic deletions or duplications that can span both gene regions and noncoding, regulatory regions	0.05-0.16
Insertion/Deletion (indel)	< 50 bp	Insertions or deletions of a small number of nucleotides that alter the reading frame of a protein are called frameshift mutations and typically result in a truncated peptide	2.6-9
Single-nucleotide variant (SNV)	1 bp	Single base-pair change in the genome	45-89

Table 4) Summary of the types of DNMs across the genome (Wilfert et al., 2017) (36)

Mostly the sperm and not the egg of one parent carries DNMs and transmits the germinal mutations to the embryo. Hence, these transmitted mutations are present in all cells within the offspring. Most of DNMs originate in the father, because of the higher number of germ-cell divisions with age in males. The number of DNMs is positively correlated with paternal age and increases at a rate of about two mutations per year. In contrast, the number of DNMs passed maternally remains comparatively constant, because female eggs don't actively divide during the reproductive years. (3) An icelandic study estimated an exponential effect of DNMs in male sperm doubling every 16.5 years. (13) Ova don't divide postnatally, so the increase of DNMs per year in female is estimated 0,37 – 0,43 per year of mother's age, as each oocyte is limited to 23 divisions, which take place before birth. (36, 2, 18) Male and female mutation rate is approximated at the ratio of 4-6:1. The identification of de novo mutations is technically challenging, therefore DNMs are poorly explored in common diseases. (38) Maternal mutations are associated with failure of DNA double-strand break repair more likely than with replication errors. (18)

The percentage of sperm with damaged DNA is significantly higher in men aged 36-57 years than in men aged 20-35 years. (6) Which means, that delayed parenthood is accompanied by a detrimental impact on the offspring. Gao et al. (2019) revealed a maternal age effect on the paternal genome mutation rate. Their findings support the hypothesis, that mother's age at conception has an impact on the mutagenesis of the child's early embryonic development. (22)

Investigating the genomes of multi-sibling families, Rahbari et al. (2016) discovered an average number of 76.9 mutations per child, fitting an average mutation rate of a mean paternal age of 29.8 years. With each additional year in parent's age the number of DNMs in the child rises linearly by 2.9. Their study revealed a probability of 1.3% for a shared DNM between siblings. An estimation suggests that a 20-year-old man's germline shows approximately 160 genome replications, whereas the mutation rate in a 40-year-old man rises up to a number of 610. In their opinion different study results regarding the paternal age effect are attributed to a limited sample size. (39) Gao et al. (2019) added that an increased number of germline mutations can not only be attributed to DNA replication in spermatogonial stem cells but to other metabolic activities or unrepaired damage that accumulates over time as well. They indicate that the so-called "male mutation bias", which says that the origin of germline mutations is primarily replication-driven, can't be seen as an evidence for the paternal age effect, since the male-to-female mutation ratio is already high in young parents and seems to be stable with age. Although the number of male cell divisions increases rapidly with age. (22)

Despite a lower number of DNMs per year of maternal age, recent findings support that mutation rates are higher in some regions of the genome, regardless of an individual's sex. (36)

Data and Methods

We used the Wisconsin Longitudinal data set to investigate, if advanced parental age at conception has an impact on the risk of multiple sclerosis in offspring. We explored if age of father, respectively age of mother of birth is significantly associated with i) if a person ever had been diagnosed having multiple sclerosis (MS) and ii) the age of the onset of multiple sclerosis.

> The Wisconsin Longitudinal Study (WLS) is a long-term study of a random sample of 10,317 men and women who graduated from Wisconsin high schools in 1957. The WLS provides an opportunity to study the life course, intergenerational transfers and relationships, family functioning, physical and mental health and well-being, and morbidity and mortality from late adolescence through 2011. WLS data also cover social background, youthful aspirations, schooling, military service, labour market experiences, family characteristics and events, social participation, psychological characteristics and retirement. Survey data were collected from the original respondents or their parents in 1957, 1964, 1975, 1992, 2004, and 2011; from a selected sibling in 1977, 1994, 2005, and 2011; from the spouse of the original respondent in 2004; and from the spouse of the selected sibling in 2006. (Herd et al., 2014) (40)

The prevalence and the onset of MS were sampled during the years 1992 and 1993, per mail. In table 5) frequencies of diagnosed multiple sclerosis are shown (in total 0.5% of the individuals had been diagnosed with MS). Table 6) shows the age of onset of MS. If someone has ever been diagnosed with MS was encoded as 1: MS diagnosed on basis of symptoms, 0: no MS.
Additionally we included the following variables in our analysis: sex (encoded as 1= male and 2= female), years of education after high school (as continuous variable), yearly wages in 100$ in the year 1993 and age of the mother as well as age of the father at birth of the person.

	Male	**Female**	**Total**
Symptoms of MS	12 (0.76%)	22 (0.6%)	34 (0.49%)
No symptom of MS	3180	3661	6841
SUM	3192	3683	6875

Table 5) Frequencies of multiple sclerosis sampled 1992/ 1993 for the WLS sample, separately for men and women.

Age of Onset	Males	Females	Total
23 - 26	2	4	6
30 - 37	3	3	6
42 - 48	2	4	6
49 - 52	1	5	6
55 - 66	3	5	8
Sum	**11**	**21**	**32**

Table 6) Age of onset of MS, separately for men and women.

We calculated the following general linear models: i) if someone ever has been diagnosed with MS regressing on sex, years of education after high school and age of father, respectively age of mother on basis of a binomial error structure and ii) age of onset of MS regressing on sex, years of education after high school and age of father, respectively age of mother on basis of a Gaussian error structure.

Results

We found that age of father at birth of an individual is significantly positive associated with the diagnosis of MS, but age of mother is significantly negative associated. This means the effects go in the opposite direction for age of fathers and age of mothers at birth: the older a father the higher is the probability to develop MS, whereas the older a mother is, the lower is the probability of MS. However, the absolute magnitude of the mother's age estimate is higher than the effect of father's age (Table 7). Concerning the age of onset effect, we found no significant results (Table 8).

	Estimate	Std. Error	z Value	P
(Intercept)	-3.6660	1.0540	-3.4770	0.0005
Sex women (ref. men)	0.2815	0.3095	0.9100	0.3630
Years of education	0.0232	0.0609	0.3820	0.7027
Wages in 100$	0.0000	0.0000	-0.6930	0.4884
Age Father at Birth	0.0618	0.0260	2.3790	**0.0174**
Age Mother at Birth	-0.1067	0.0330	-3.2300	**0.0012**
Residual deviance: 574.09 on 3890 degrees of freedom				

Table 7) Generalized Linear Models if someone ever has been diagnosed with MS regressing on sex, years of education after high school and age of father, respectively age of mother on basis of a binomial error structure.

	Estimate	Std. Error	z Value	P
(Intercept)	6.9663	36.9789	0.1880	0.8530
Sex women (ref. men)	8.9286	9.7470	0.9160	0.3760
Years of education	-0.4729	1.8739	-0.2520	0.8050
Wages in 100$	0.0004	0.0003	1.5890	0.1360
Age Father at Birth	0.5740	0.5853	0.9810	0.3450
Age Mother at Birth	0.4879	0.9614	0.5070	0.6200
Residual deviance: 2881.7 on 13 degrees of freedom				

Table 8) Age of onset of MS regressing on sex, years of education after high school and age of father, respectively age of mother on basis of a Gaussian error structure

Discussion

Multiple Sclerosis is a chronic inflammatory disease of the central nervous system, with a complex and so far, mostly unknown aetiology.

Some studies indicate a so-called “maternal effect” in MS, which describes an influence of maternal factors on the risk of multiple sclerosis. Ebers et al. (2004) were able to show evidence for a maternal parent-of-origin effect in multiple sclerosis susceptibility. (41) Ramagopalan et al. (2009) were able to confirm this theory when they discovered, that a maternally transmitted HLA-DRB1 gene variant is accompanied by a higher susceptibility for MS compared to a paternal transmission. (42) Therefore a parent-of-origin effect is described that suggests that MS, due to epigenetic influence, is generationally transmitted by mothers more often than by fathers. (43) In contrast to the “maternal effect” some studies suggest the “Carter effect” for MS. The Carter effect in MS says that fathers with MS transmit the disease to their children more often than affected mothers, probably due to a greater genetic loading in men. (44)

In this population-based study, we observed an association between the risk of developing MS and parental age at birth. Our study revealed an effect of both, maternal and paternal age, in the opposite direction. The results of this study show, that an advanced paternal age at birth is accompanied by a higher risk of MS in offspring, whereas an advanced maternal age reduces the susceptibility of MS in offspring. The maternal age effect we found even showed a higher statistical significance than the effect of paternal age on MS.
Montgomery et al. (2004) observed an association of increasing paternal age with MS risk as well, but in contrast to our study they couldn’t find an influence of maternal age on risk of MS. (12) In contrary to our findings, a Canadian study of 2009 could not find an association between advanced parental age and a susceptibility to MS. (45)

Montgomery et al. (2004) think, that de novo mutations, which are associated with older paternal age, are more likely among patients without a family history of MS. Their study also revealed that the presence of siblings (both younger and older) is negatively associated with MS risk. Further they found a minor increase in risk of MS for the offspring of fathers working in nonmanual occupations compared with manual occupations. (12) Microenvironmental influences, like birth order, adopted individuals and siblings or halfsiblings raised together or apart, don’t seem to affect the risk of MS. (41, 46) In our study additionally analysed variables such as sex, years of education after high school and yearly wages did not influence the risk of MS. Our results also revealed that parental age at conception doesn’t have an impact on the age of onset of MS.

The paternal age effect on MS susceptibility we found could be attributed to the spermatocytes of aging men, which are associated with genetic mutations, and therefore may play a key role in the development of MS. So far, we don't have any explanation for the maternal age effect on MS, resulting in a minor risk of MS in offspring of mothers with advanced age at birth. Although Wilfert et al. support the assumption of female protective effects found in some DNMs, that have been observed in certain neurodevelopmental disorders. (36)

Wong et al. (2016) are in the opinion that mothers age is of importance regarding the DNM rate. Most studies only considered chromosomal anomalies concerning advanced maternal age. But investigations on mice confirmed a greater number of cell divisions in the oocyte with advanced maternal age. Their analysis also indicates that the mutation rate in female germline accelerates, while spermatocytes mutate in a constant rate with paternal age. It's not clarified yet if female DNMs are due to spontaneous mutations (more likely than male), either zygotes have higher mutation rates when women age, or cell divisions in older oocytes increase. But Wong et al. (2016) revealed that DNMs are also common in female germline. (47) Although women show a higher mutation rate and older mothers transmit more maternal recombinations to their offspring than young mothers, according to Kong et al. (2012) the number of mutations transmitted to the offspring is much higher in men than women. (13)

Janecka et al. (2017) also support the hypothesis that DNMs in female germline are correlated with an increased risk of neurodevelopmental disorders, based on a contribution of maternal age effects to inherited and de novo risk factors. (18) Gao et al. even indicate a potential maternal age effect on the mutation rate of maternal and paternal genomes. (22)

Since germline mutations represent the source of evolution, it's likely that the maternal age effect in multiple sclerosis is due to germline mutations, as DNMs contribute substantially to many health-related processes as well as pathological processes. (47) McGrath et al. (2014) postulated, that in contrast to an APA effect, their study revealed an increased risk for disorders of substance use, hyperkinetic disorders and mental retardation in offspring of young mothers. (48) Their findings of a higher risk for mental health disorders in younger mother's offspring, may support our results of a decreased risk for MS in offspring of an older mother. In their opinion young mother's offspring's risk for mental disorders is almost corresponding to the risk accompanied by advanced paternal age. Although they suggest that the cause of the shown maternal age effect is most likely attributed to shared parent-offspring risk factors, including genetics and environmental exposures, or maternal mental health before and after birth. (48)

Waterhouse (2013) suggested that maternal immune system dysfunction and less competent placenta formation seen in older women, or accumulation of environmental exposures might contribute to an advanced maternal age effect in autism. (49)

The aetiology of MS has been and still is under active investigation. Oxidative injury, autophagy, and inflammation events represent the core issues of current research, since these are pathophysiological mechanisms that are involved in the development and pathogenesis of MS. (50) According to our findings a parental age effect on MS exists and should be part of further investigations as well.

Parental age at conception is increasing continuously and has an impact on birth outcomes and public health, therefore it's important to do further research on the parental age effect. Plenty of effects regarding advanced maternal age are already well known, but there still must be accomplished additional studies on the impact of the paternal age effect on the health of offspring. The cumulative risk of advanced paternal age shouldn't be underestimated and a possible impact on economy and public health should be taken into consideration.

Since the importance of APA regarding the reproductive outcome has been recognized for years, the American Society of Reproductive Medicine and the British Andrology Society both, restricted the age of sperm donors and set an upper age limit at 40 years. (51)

Nybo Andersen and Urhoj (2017) assume severe adverse health effects in children caused by rising paternal age. Nevertheless, quantitatively they might be of minor importance. So they take the position, that the priority should be set on an improved understanding of the pathomechanism of morbidities due to an increased age of fathers at conception. (52) Further investigations on public health implications of increasing paternal age need to be carried out, with large human data sets and detailed epidemiological background information.

Abbreviations

MS	Multiple Sclerosis
RRMS	relapsing and remitting multiple sclerosis
PRMS	progressive relapsing multiple sclerosis
PPMS	primary progressive multiple sclerosis
SPMS	secondary progressive multiple sclerosis
CIS	clinically isolated syndrome
APA	advanced paternal age
CNS	central nervous system
MRI	magnetic resonance imaging
DIS	dissemination in space
DIT	dissemination in time
CSF	cerebrospinal fluid
OCB	oligoclonal bands
IgG	Immunoglobulin G
DNM(s)	de novo mutation(s)
CNV	copy number variation (= major cause of structural variation in the genome, involving both duplications and deletions of sequences)
SNV	single nucleotide variants
GWAS	genome-wide association studies
WLS	Wisconsin Longitudinal Study
HLA	human leukocyte antigen
HIV	human immunodeficiency virus
EBV	Epstein-Barr virus
HHV	human herpesvirus
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
INF- γ	Interferon gamma
TNF-α	tumor necrosis factor alpha
MHC-I	major histocompatibility complex
miRNAs	microRNAs
HLA	human leukocyte antigen
IQ	intelligence quotient
US	United States

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Ich habe mich bemüht, sämtliche Inhaber*innen der Bildrechte ausfindig zu machen und ihre Zustimmung zur Verwendung der Bilder in dieser Arbeit eingeholt. Sollte dennoch eine Urheberrechtsverletzung bekannt werden, ersuche ich um Meldung bei mir

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