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Birth Order and Narcolepsy Risk Among Genetically Susceptible Individuals: a Population-based Case-control Study

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Abstract

Background—Birth order may play a role in autoimmune diseases and early childhood infections, both factors implicated in the etiology of narcolepsy. We investigated the association between birth order and narcolepsy risk in a population-based case-control study in which all study subjects were HLA-DQB1*0602 positive.

Methods—Subjects were 18-50 years old, residents of King County, Washington, and positive for HLA-DQB1*0602. Birth order was obtained from administered interviews. We used logistic regression to generate odds ratios adjusted for income and African American race.

Results—Analyses included 67 cases (mean age 34.3 [SD=9.1], 70.2% female) and 95 controls (mean age 35.1 [SD=8.8], 58.1% female). Associations for birth order were as follows: First born (cases 38.8% vs. controls 50.2%, OR=1.0; Reference), second born (cases 29.9% vs. controls 32.9%, OR=1.6; 95% CI 0.7, 3.7), third born or higher (cases 31.3% vs. controls 16.8%, OR=2.5; 95% CI 1.0, 6.0). A linear trend was significant ($p<0.05$). Sibling number, sibling gender, having children, and number of children did not differ significantly between narcolepsy cases and controls.

Conclusions—Narcolepsy risk was significantly associated with higher birth order in this population-based study of genetically susceptible individuals. This finding supports an environmental influence on narcolepsy risk through an autoimmune mechanism, early childhood infections, or both.

Keywords

Narcolepsy; Birth order; HLA-DQB1*0602; genetics; autoimmune

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Introduction

Birth order is associated with altered immunity [1], autoimmune diseases [2], and infections early in life [3], all factors implicated in the etiology of narcolepsy. An autoimmune process triggered by select environmental exposures in genetically susceptible individuals has been hypothesized to cause narcolepsy [4]. Genetically, narcolepsy has strong associations with HLA-DQB1*0602 [5] and polymorphisms in the T-cell receptor alpha locus [6]. Environmentally, narcolepsy has been associated with passive smoking [7], streptococcal infection [8,9], and season of birth [10]. The combination of these, and likely other factors, is thought to initiate an immune response resulting in selective loss of hypocretin-producing cell in the dorsolateral hypothalamus and reduced cerebrospinal hypocretin levels [11]. Findings of elevated anti-trib2 antibodies in these patients further supports this hypothesis [12]. Thus, we sought to explore if birth order was associated with increased risk of narcolepsy in a population-based case-control study of narcolepsy where all subjects were positive for HLA-DQB1*0602.

Methods

Recruitment methods for cases and controls have been detailed elsewhere [13]. Briefly, we attempted to identify all prevalent cases of physician-diagnosed narcolepsy 18 years of age and older and residing in King County, Washington, between July 2001 and June 2005. Patients were identified using multiple overlapping methods, some of which were directed at clinicians and some at patients. Controls were 18-50 year old residents of King County identified through random digit dialing. Potential controls within households were sampled using the randomized recruitment method in which age and gender-specific sampling fractions were applied to create a distribution of controls similar to that of the projected distribution of cases [7,9]. All study procedures were reviewed and approved by the University of Washington Institutional Review Board.

Trained interviewers questioned narcolepsy patients about clinical manifestations, onset of disease, and diagnosis. Medical records were requested and abstracted by the study neurologist (WL). Narcolepsy was defined according to the criteria of the International Classification of Sleep Disorders-2 [14]. Ninety-three percent of 67 narcolepsy patients were either diagnosed by a sleep medicine specialist, were cataplexy positive, underwent formal diagnostic sleep studies, or had some combination of these criteria. Diagnostic testing status was either unknown or absent in the remaining 7% of physician-diagnosed narcolepsy patients.

Cases and controls were asked, "What was your order among your siblings?" The presence or absence of siblings was assessed by the following questions: "How many brothers (not including half-brothers) do you have, living and deceased?" and "How many sisters (not including half-sisters) do you have, living and deceased?" Number of children was obtained from the following questions: "How many sons do you have, living and deceased?" and "How many daughters do you have, living and deceased?"

During the interview, cases and controls provided a buccal smear for DNA. Genotyping of HLA-DQB1 entailed quantitative DNA amplification and fluorescence detection with sequence-specific probes [15]. To be considered study eligible, both cases and controls were required to carry at least one HLA-DQB1*0602 allele.

We calculated several dichotomous variables to describe family characteristics, including whether subjects have brothers, sisters, sons, or daughters. In addition, we classified subjects according to their birth order: first, second, and third or higher. The first-born category served as the reference group. For linear trend of birth order on narcolepsy prevalence, we

used a score test for trend. For multivariable models, we used unconditional logistic regression to obtain estimated odds ratios (ORs) and 95 percent confidence intervals (CIs), adapted to account for the sampling mechanism for controls [16], as described elsewhere [7]. Because household income was missing for five cases and seven controls, we multiplied imputed household income using age, African American race, interview year, and case status as predictors, as detailed previously [7,9]. Imputation was performed using the *mice* package in R statistical language, version 2.5. All other analyses were conducted in Stata version 10.0 (StataCorp LP, College Station, TX, USA).

Results

Analyses included 67 cases (mean age 34.3 [SD=9.1], 70.1% female) and 95 controls (mean age 35.1 [SD=8.8], 58.1% female). As expected under the sampling scheme for controls, cases and controls did not differ significantly in regards to age and gender. Cases were slightly less likely than controls to have had a graduate school education, were significantly more likely to be African American, and significantly less likely to have higher levels of household income (Table 1). Consequently, African American race and income were included in all multivariable models.

Adjusted associations for birth order were as follows: First born (cases 38.8% vs. controls 50.2%, OR=1.0; Reference), second born (cases 29.9% vs. controls 32.9%, OR=1.6; 95% CI 0.7, 3.7), third born or higher (cases 31.3% vs. controls 16.8%, OR=2.5; 95% CI 1.0, 6.0). A linear trend was significant ($p<0.05$; Figure 1). An analysis unadjusted for African American race and income revealed similar findings (first born OR=1.0 ref; second born OR=1.2, 95% CI 0.6, 2.5; third born OR=2.6, 95% CI 1.1, 6.0; linear trend $p<0.05$). Sibling number was not significant (linear trend $p=0.4$). Sibling gender also did not differ significantly between narcolepsy cases and controls (Brothers: cases 55.2% vs. controls 52.8%, OR=1.0; 95% CI 0.5, 2.0; Sisters: cases 59.7% vs. controls 59.2%, OR=1.5; 95% CI 0.7, 3.2). Among women, having children (OR=0.9; 95% CI: 0.4, 2.1) or having two or more children (OR=1.1; 95% CI: 0.4, 3.0) was not associated with narcolepsy. Similarly, having sons or daughters did not differ between cases and controls (Sons: cases 34.3% vs. controls 40.8%, OR=1.1; 95% CI 0.5, 2.3; Daughters: cases 25.4% vs. controls 31.3%, OR=0.8; 95% CI 0.3, 1.7).

Discussion

We found a significant association between birth order and narcolepsy, with higher birth order more prevalent in cases than in controls. The association of disease with birth order usually reflects the effects of environmental factors, specifically exposure status to early life infections, and has been observed in atopic and some autoimmune diseases [1,2]. Added to associations with anti-streptolysin O and anti-trib2 antibodies, T-cell receptor alpha locus polymorphisms, and select destruction of hypocretin producing cells in the dorsolateral hypothalamus, our findings support the narcolepsy autoimmune hypothesis. Narcolepsy has been found to be associated with season of birth, with increased risk with March birth and reduced risk with September birth [10]. This finding suggests that not only the presence, but the timing, of environmental triggers may be crucial to disease initiation. Our birth order finding suggests that along with seasonal timing, infection related to developmental timing is also important to disease expression.

Previous group A streptococcal infection [9] and anti-streptolysin O antibody seropositivity [8] are associated with narcolepsy and autoimmunity. Group B streptococcus is part of normal gut and genital tract flora present in 20-40% of women. Colonization is positively associated with the number of previous pregnancies, and neonatal exposure and infection can occur during parturition from colonized mothers [17]. Group A (pyogenes) and group B

(agalactiae) streptococci are both beta-hemolytic organisms from the same bacterial genus. Although group B streptococcal infections are not known to be associated with autoimmunity, our birth order results taken in this context suggests that group B streptococcal exposure during parturition may play a role in narcolepsy development in genetically susceptible individuals. This notion deserves further research.

Microchimerism is the persistence of one individual's cells in another individual and is another potential explanation for our findings. Natural microchimerism involves cell transfer between mother and fetus during pregnancy and results in the presence of small populations of genetically distinct cells in each individual. Typically the cells exchanged are immune cells such as T and B lymphocytes, monocytes, macrophages, and natural killer cells. Male microchimerism has been demonstrated in women who have never given birth to a son, suggesting that microchimerism could derive from an older sibling transferred via the maternal circulation to the fetus of a later pregnancy [18]. Decades-long persistence of these retained foreign cells has been proposed as a contributing factor in autoimmune disease pathogenesis [19,20]. The pathophysiology is not well understood, but proposed mechanisms include microchimeric cells directly effecting autoimmunity or amplifying latent immune responses by indirect antigen presentation. Higher birth order, with exposure to chimeric cells from previous pregnancies with resultant autoimmune response, is a potential explanation for our finding of higher birth order in narcolepsy patients.

Some limitations related to our study design are notable [7,9]. Narcolepsy diagnostic precision in our sample was compromised by lack of objective diagnostic testing in some subjects. However, 93% of our narcolepsy sample were either cataplexy positive, underwent diagnostic testing, or were diagnosed by sleep specialists, and all subjects were HLA-DQB1*0602 positive. Medical record extraction, when available, confirmed the diagnosis. Also, the majority of cases (67.2%) were positive for cataplexy, a pathognomonic symptom of narcolepsy virtually unheard of outside the context of narcolepsy. The International Classification of Sleep Disorders-2 states that sleepiness with cataplexy is adequate to diagnose narcolepsy, with confirmatory objective testing encouraged but not required [14]. Regardless, misspecification from lack of objective testing would have included controls among our case group, attenuating our results. We also lacked data regarding miscarriages, and, therefore, do not have an accurate count of total pregnancies in our case group, which compromises our conclusions regarding the impact of total pregnancies on narcolepsy development. Lastly, we desired to investigate potential interactions between narcolepsy associated variables, but sample size limitations obviated this possibility. Future epidemiological studies of narcolepsy with larger sample sizes are needed to assess for the potential presence of multiplicative interactions between narcolepsy associated variables.

In summary, we found that higher birth order was associated with an increased risk of developing narcolepsy in people positive for HLA-DQB1*0602. Sibling number, sibling gender, number of children, and children's gender were not related to narcolepsy risk. This work suggests that immune responses to early childhood infections, or potentially microchimerism, predisposes to disease development and supports the autoimmune hypothesis of narcolepsy etiology.

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Figure 1.

Odds ratio (OR), 95% confidence interval (CI), and linear trend estimated from logistic regression model for narcolepsy as a function of birth order controlling for African American race and income.

TABLE 1

Sociodemographic Characteristics of Cases and Controls Ages 18-50 Who Are Also Positive for HLA DQB1*0602, King County, Washington, 2001-2005

Characteristic	Weighted Percentages		OR	95% CI	Test of Trend P-value
	Cases (n=67)	Controls (n=95)			
Age group (years)					0.4
18-25	22.4	18.0	1.0	Reference	
26-30	13.4	18.2	0.5	0.2 - 1.5	
31-35	22.4	17.7	0.8	0.3 - 2.2	
36-40	7.5	15.8	0.2	0.1 - 0.8	
41-45	16.4	17.2	0.7	0.2 - 1.9	
46-50	14.9	13.0	0.7	0.2 - 2.2	
Gender					
Male	29.9	41.9	1.0	Reference	
Female	70.1	58.1	1.1	0.5 - 2.4	
Race					
White	80.6	92.1	1.0	Reference	
African-American	14.9	1.4	8.2	2.1 - 31.1	
Asian	1.5	0.9	1.2	0.1 - 13.8	
Hispanic	1.5	0.5	2.5	0.2 - 40.2	
Other	1.5	5.1	0.3	0.0 - 2.6	
Annual income (imputed)					<0.001
Under \$20,000	39.7	18.2	1.0	Reference	
\$20,000-\$39,999	18.8	23.3	0.3	0.1 - 0.8	
\$40,000 - \$59,999	17.6	11.7	0.5	0.2 - 1.6	
\$60,000+	23.9	46.8	0.2	0.1 - 0.4	
Education					0.15
High school or less	28.4	24.3	1.0	Reference	
College	59.7	54.8	0.9	0.4 - 1.9	
Graduate School	11.9	20.9	0.4	0.1 - 1.2	

Characteristic	Weighted percentages		OR	95% CI	Test of Trend P-value
	Cases (n=67)	Controls (n=95)			
Confidence interval (CI), odds ratios (OR)					