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An Exploration of Gene–Environment Interaction and Asthma in a Large Sample of 5-Year-Old Dutch Twins

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A consistent finding from twin studies is that the environment shared by family members does not contribute to the variation in susceptibility to asthma. At the same time, it is known that environmental risk factors that are shared by family members are associated with the liability for asthma. We hypothesize that the absence of a main effect of shared environmental factors in twin studies can be explained by gene–environment interaction, that is, that the effect of an environmental factor shared by family members depends on the genotype of the individual. We explore this hypothesis by modeling the resemblance in asthma liability in twin pairs as a function of various environmental risk factors and test for gene–environment interaction. Asthma data were obtained by parental report for nearly 12,000 5-year-old twin pairs. A series of environmental risk factors was examined: birth cohort, gestational age, time spent in incubator, breastfeeding, maternal educational level, maternal smoking during pregnancy, current smoking of parents, having older siblings, and amount of child care outside home. Results revealed that being a boy, born in the 1990s, premature birth, longer incubator time, and child care outside home increased the risk for asthma. With the exception of premature birth, however, none of these factors modified the genetic effects on asthma. In very premature children shared environmental influences were important. In children born after a gestation of 32 weeks or more only genetic factors were important to explain familial resemblance for asthma.

Findings from twin studies show that approximately 70% of the variance in asthma liability is explained by genetic factors (Duffy et al., 1990; Harris et al., 1997; Koeppen-Schomerus et al., 2001; Laitinen et al., 1998; Lichtenstein & Svaertengren, 1997; Los et al., 2001; Nystad et al., 2005; Skadhauge et al., 1999; van Beijsterveldt & Boomsma, 2007; Willemsen et al., 2008). Twin studies also find that environmental factors shared by family members do not contribute to the variance in susceptibility to asthma. These findings seem to contradict findings from epidemiological studies that suggest an important contribution from environmental risk factors, such as parental smoking, number of siblings and air pollution (Mutius, 2000).

The lack of evidence for a contribution of shared environmental factors in twin studies could be explained by gene–environment interaction. It is likely that environmental factors trigger asthma only in persons with a larger genetic susceptibility for asthma. In the classical twin design, variance due to interactions between shared environmental risk factors and genotype is included in the estimate of the genetic variance component, if not modeled explicitly (Molenaar et al., 1990; Purcell, 2002).

Several epidemiological studies have revealed evidence of gene–environment interaction in the development of asthma and related conditions. For example, Jaakkola et al. (2001) reported that children at higher genetic risk were more susceptible to environmental stressors. On the basis of parental history of asthma or hay fever, children were assigned into low and high genetic risk groups. In the low genetic risk group, there was no relation between exposition to tobacco smoke and asthma. In the high genetic risk group, however, the prevalence of asthma was higher when children were exposed to early environmental tobacco smoke.

Results from human linkage and association studies for asthma also provide evidence for gene–environment interaction (Colilla et al., 2003; Dizier et al., 2007; Meyers et al., 2005; Ramadas et al., 2007). For example, Ramadas et al. (2007) examined the interleukin-1 receptor antagonist (IL1RN) which is a potent anti-inflammatory cytokine. In a group of 921 children, no evidence was found for an association of asthma with a SNP in the Il1RN gene. However, when the analysis was restricted to a group of children with maternal smoking during pregnancy, the rs2234678 GG genotype significantly increased

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the relative risk of asthma in children, both in analyses of repeated asthma occurrences and persistent asthma. Two other genes, CARD4 and CD14, that have been associated with asthma and allergy, provide additional examples for gene–environment interaction (Eder et al., 2005, 2006; Marks, 2006; Martinez, 2007). In a study of 668 children, a strong protective effect of a farming environment on allergies was found only in children homozygous for the T allele in CARD4/-21596, but not in children carrying the minor C allele (Eder et al., 2006). Polymorphisms in CARD4 thus significantly modify the protective effect of exposure to a farming environment. In the same sample of children, an interaction effect for the CD14 gene was reported (Eder et al., 2005). The C allele of CD 14/-260 was associated with higher levels of both total and specific serum IgE to aeroallergens in children with regular contact with pets, whereas an association in the opposite direction was found in children with regular contact with stable animals.

These studies underline the importance of gene–environment interaction in the development of asthma and related conditions. The aim of the current study is to explore environmental risk factors, shared by children from the same family, which may serve as modifiers of genetic influences on asthma. First, we examine which environmental factors are related to asthma risk. Next, we explore whether these factors modify genetic effects. The approach suggested by Eaves (1982) to test for the presence of gene-environment interaction involving a measured environmental variable was followed. In this approach (Eaves, 1982; see also Boomsma et al., 1999; Boomsma & Martin, 2002; Heath et al., 1989, 1998), the relative influences of genotype (heritability) and environment on a trait are estimated conditional upon environmental exposure. When there is no interaction, the influence of genetic and environmental factors should not differ between subjects with different degrees of exposure. If genetic effects are modified by exposure, such that heritabilities differ significantly between, for example, exposure-positive and exposure-negative groups, then this constitutes evidence for genotype–environment interaction. Thus, this type of interaction is detected by testing whether the amount of variance explained by genetic factors differs between exposure-positive and exposure-negative groups.

Data on asthma and environmental risk factors were available for a large sample (12,000 twin pairs) of monozygotic (MZ) and dizygotic (DZ) Dutch 5-year-old twin pairs.

Methods

Participants and Measures

Data on asthma and environmental risk factors were collected in a longitudinal twin study, which examines the genetic and environmental influences on health, growth, and the development of behavioral and emotional problems. The twin families are volunteer members of the Netherlands Twin Register (NTR), which was established at the Department of Biological Psychology at VU University, Amsterdam (Bartels et al., 2007; Boomsma et al., 2006). From 1987 onwards, the NTR has recruited families with young twins a few months after the birth of the twins. Around 40% of all multiple births in the Netherlands are registered by the NTR. For the present study, data included were obtained from surveys mailed to parents when twins were aged 1, 2, 3, and 5 years.

After parents have registered, a first survey is send to mothers. On average, this survey is sent back within the first year after birth (mean = 8.4 months, *SD* = 13.85). In this survey, the mothers are asked to report on birth order, sex of twins, gestational age, incubator time, and smoking behavior of parents during pregnancy. Information on breastfeeding is obtained from a survey sent when the twins are 2 years of age (Orlebeke et al., 1995). Data on maternal education attainment are obtained at age 3. At age 5, the parents are asked to report (yes/no) whether a physician ever diagnosed asthma in the children. From the same survey, information on current parental smoking, the number of older sibs, the type of day care (van Beijsterveldt et al., 2005) and zygosity is obtained. At age 5, data were available for 12,009 twin pairs, from birth cohorts 1987–2000. Data from 287 twin pairs were excluded because one or both twins suffered from a severe disease or handicap. All parents who returned the survey at age 5 had also returned the first survey; 90% had returned surveys at age 2 and 87% at 3 years of age.

Determination of Zygosity

For 1253 same-sex twin pairs zygosity was based on blood group (n = 291) or DNA polymorphisms (n = 962). For the remaining same-sex twin pairs, zygosity was assessed with items about physical similarity and frequency of confusion of the twins by family and strangers (Goldsmith, 1991; Rietveld et al., 2000). There were 4007 MZ twin pairs, 3879 same-sex DZ twin pairs, and 3798 opposite-sex DZ twin pairs. Zygosity status was unknown for 38 pairs and data from these pairs were excluded from the analyses.

Data Analysis

The prevalence of asthma was estimated in children as a function of exposure to environmental risk factors. The association between risk factors and asthma was estimated by odds ratios (OR). Odds ratios and their 95% confidence intervals (CI) were estimated in Mplus using the 'complex option' (Muthén & Muthén, 1998–2007). The use of the complex option in Mplus takes into account clustering of data collected in family members. The parameter estimates are maximum likelihood estimates, and the standard errors are corrected for the dependency in the data (Rebollo et al., 2006).

Twin similarity for asthma was summarized with tetrachoric correlations. If twin correlations differed as a function of environmental exposure, a standard univariate twin model was fitted to the asthma data.

In this multiple group model, different parameters for additive genetic (A), shared environmental (C), and nonshared environmental variance components for each exposure group were estimated. Using *Mx* (Neale et al., 2003) estimates of variance components and their CIs were obtained by using a liability threshold model in which an underlying continuous distribution of liability for asthma is assumed. The underlying liability distribution has a mean of 0 and variance of 1 (Neale & Cardon, 1992). To test if the variance components were equal across the levels of environmental exposures, estimates of A, C, and E were constrained to be equal. A deterioration of the fit of the model indicates if this constraint is allowed.

Results

The association between asthma and environmental risk factors is summarized in Table 1. Sex, birth cohort, gestational age, incubator time, and child care outside home increased risk of asthma. Being a boy increased the risk of asthma by almost 30%. The strongest effect was found for gestational age (OR = 2.38). Asthma prevalence was 7.3% in twins born at term and increased to 15.8% for twins who were born very premature. For birth cohort there was an increase in asthma prevalence between 1986 and 1996. A longer incubator time also increased the risk for asthma. A small but significant effect was found for child care outside the parental home. Child care outside the home was associated with a higher prevalence of asthma. The prevalence of asthma was somewhat higher in children with mothers who smoked during pregnancy, but this effect was not significant. Maternal educational level, having older siblings, and breastfeeding did not affect asthma risk.

Twin correlations showed higher MZ than DZ similarity, indicating the importance of genetic factors on individual differences in asthma liability (Table 2). Across all environmental exposures the twin correlations were .90 or higher for MZ pairs, and between .40 and .62 for DZ pairs. The MZ and DZ correlations for asthma did not vary among the different exposure levels of the environmental risk factors. There was one exception: the DZ correlation in very premature twins (less than 32 weeks) was higher than the DZ correlation in twins after 32 weeks.

To examine the possible interaction between gestational age and genotype on asthma liability, we tested if the influence of genetic and environmental factors differed between the very premature twins and the twins born after 32 weeks. Constraining the additive genetic and common environment variance components to be equal between the very premature twins and the twins born after 32 weeks revealed a deterioration in goodness of fit ($\chi^2 = 7.166$; $df = 2$, $p = .028$). Shared environmental factors were important for the premature group, but not for the twins born after 32 weeks. For the very premature twins, the estimate of genetic effects was 45% (95% CI: 16–80), and the estimate of shared environment was 52% (CI: 16–74). For the twins born after 32 weeks, the estimate for the genetic effect was 90% (CI: 78–95) and the effect of common environment was not significant (3% and CI: 0–14). These results show that the influence of genetic factors depends on gestational age.

Table 1

Asthma Prevalence and Odds Ratio (OR) with 95% Confidence Interval (CI) of Asthma for Each Level of Environmental Risk Factors

	% asthma	OR	95% CI
Sex			
Males	10.30%	1	
Females	7.4%	0.69	(0.63–0.77)
Birth cohort			
1986–1989	5.2%	1	
1990–1992	7.8%	1.56	(1.26–1.92)
1993–1995	10.3%	2.1	(1.72–2.57)
1996–1998	10.3%	2.11	(1.74–2.57)
1999–2000	9.4%	1.9	(1.51–2.40)
Educational level			
Low	8.6%	1	
Medium	8.7%	1.01	(0.88–1.16)
High	9.2%	1.07	(0.91–1.26)
Gestational age			
> = 37 weeks	7.3%	1	
> = 32 and 37 weeks	10.7%	1.52	(1.35–1.70)
< 32 weeks	15.8%	2.38	(1.89–3.01)
Incubator time			
No	7.4%	1	
1–7 days	8.7%	1.2	(1.05–1.35)
8–14 days	11.7%	1.65	(1.39–2.09)
> 14 days	13.1%	1.88	(1.59–2.35)
Smoking pregnancy			
No	8.5%	1	
Yes	9.7%	1.14	(0.99–1.32)
Current smoking			
No	8.5%	1	
Yes	9.2%	1.08	(0.96–1.21)
Older sibs			
No	9.1%	1	
Yes	8.5%	0.93	(0.83–1.03)
Breastfeeding			
No	9.0%	1	
0.5–3 months	8.6%	0.96	(0.83–1.09)
> 3 months	8.3%	0.92	(0.79–1.08)
Child care outside home			
No	7.6%	1	
Little	8.8%	1.17	(1.01–1.34)
Medium	11.1%	1.52	(1.23–1.86)
High	9.9%	1.33	(1.04–1.69)

Discussion

We explored whether the environmental factors that are associated with asthma also modify the genetic

Table 2

Number of Concordant Affected (AA), Concordant Unaffected (UU) and Discordant (UA) Twin Pairs for Asthma

	MZ			DZ			Tetrachoric correlation			
	AA	UA	UU	AA	UA	UU	MZ	(95%CI)	DZ	(95%CI)
Sex[a]										
Boys	127	94	1516	66	250	1590	.92	(.89–.95)	.49	(.38–.60)
Girls	117	86	1796	37	190	1541	.93	(.91–.96)	.54	(.45–.63)
Birth cohort										
1986–1989	19	22	643	15	89	1036	.92	(.85–.89)	.52	(.35–.68)
1990–1992	48	41	722	28	163	1321	.92	(.87–.96)	.46	(.33–.59)
1993–1995	79	44	742	57	230	1513	.95	(.92–.98)	.52	(.43–.62)
1996–1998	67	53	827	66	282	1655	.92	(.88–.96)	.49	(.40–.58)
1999–2000	31	20	378	25	104	701	.94	(.89–.99)	.52	(.37–.66)
Educational level										
Low	63	48	932	55	254	1806	.93	(.89–.97)	.50	(.40–.59)
Medium	96	66	1243	65	315	2281	.94	(.91–.96)	.49	(.40–.57)
High	44	36	605	40	163	1117	.92	(.87–.97)	.53	(.42–.64)
Gestational age										
> = 37 weeks	109	94	1921	89	481	4062	.92	(.89–.95)	.48	(.41–.55)
> = 32 and 37 weeks	111	72	1229	76	342	1909	.94	(.91–.97)	.46	(.37–.55)
< 32 weeks	22	11	145	25	41	217	.95	(.89–.99)	.73	(.59–.87)
Incubator time[b]										
No	87	66	1374	63	347	2989	.93	(.90–.96)	.48	(.40–.57)
1–7 days	47	43	672	24	154	1044	.90	(.85–.96)	.40	(.26–.54)
8–14 days	19	12	179	12	52	219	.94	(.87–1.00)	.41	(.18–.65)
> 14 days	35	22	299	32	77	380	.93	(.88–.99)	.62	(.48–.75)
Smoking pregnancy										
No	178	151	2703	142	669	4736	.92	(.89–.94)	.49	(.43–.55)
Yes	65	29	601	49	198	1485	.96	(.94–.99)	.55	(.45–.65)
Current smoking										
No	135	105	2032	108	489	3548	.93	(.90–.95)	.51	(.44–.58)
Yes	102	68	1159	73	346	2478	.94	(.90–.96)	.49	(.41–.58)
Older sibs										
No	116	93	1630	107	467	3130	.92	(.89–.95)	.43	(.50–.58)
Yes	122	85	1605	82	376	2951	.94	(.91–.96)	.52	(.45–.60)
Breastfeeding[c]										
No	128	103	1693	88	431	2994	.92	(.89–.94)	.48	(.40–.55)
0.5–3 months	58	28	798	42	211	1418	.97	(.94–.99)	.49	(.38–.60)
> 3 months	32	31	472	35	114	966	.90	(.83–.96)	.62	(.51–.73)
Child care outside home										
No	50	40	793	36	171	1454	.93	(.88–.97)	.52	(.41–.63)
Little	136	94	1808	101	469	3423	.94	(.91–.96)	.50	(.43–.57)
Medium	24	19	247	21	87	519	.91	(.84–.98)	.50	(.37–.66)
High	15	14	215	15	62	349	.90	(.80–.99)	.48	(.29–.68)

Note: [a]same-sex twins only; [b] twins with the equal incubator times only (79% of the sample); [c] twins with the same amount of breastfeeding only (97% of the sample).

The last columns give tetrachoric correlations in MZ and DZ twins.

effects on asthma liability. We found a number of factors that increased the risk for asthma. Being a boy, born in the 1990s, a premature birth, long incubator time, and child care outside the parental home all increased risk. Almost none of these environmental factors modified the genetic effects on asthma liability. There was one exception, gestational age. If children were born before a gestation of 32 weeks, then both genetic and shared environmental factors contributed to variance in liability to asthma (43% and 52%, respectively). For children born after a gestation of 32 weeks, only genetic factors were important (heritability 90%).

Although we found a number of risk factors that were associated with asthma, most of these risk factors showed no evidence for gene–environment interaction effects on asthma liability. Although we had data from an extremely large sample of twin

pairs, the use of the classical twin design may have limited the power to detect gene–environment interaction. Studies which analyze measured genes have higher power and may be more successful. It is likely that the effect of an environmental risk factor is not the same for all genes underlying the phenotype (Eaves & Eysenck, 1976). If the influence of different genes is modified by different environmental factors then it will be difficult to detect gene–environment interaction with the classical twin method.

In our study the only factor that revealed a significant gene–environment interaction was gestational age, which also had a large main effect on the risk of developing asthma. In very premature children, the asthma rate is almost double the rate of asthma in children born after 32 weeks. Our results showed that the etiology of the variance in liability to asthma depended on the gestational age. For children with a short gestational age (very preterm), shared environmental factors were important, at the cost of genetic factors.

Refining the phenotype, optimizing the measures of the environment or measuring the genotype (instead of using latent factors) all may increase the power to detect gene-environment interactions. Asthma research with measured genotypes has provided evidence for gene-environment interaction. For example, several studies found that the effect of genotype on asthma was modified by prenatal exposure to tobacco smoke or exposure in early life (Colilla et al., 2003; Dizier et al., 2007; Meyers et al., 2005; Ramadas et al., 2007).

Although our findings revealed almost no evidence for gene-environment interaction, the risk factors that were identified could be useful in studies with measured genotypes. Our study revealed a variety of risk factors associated with asthma. Incubator time, even after correction for gestational age, was a significant risk factor. Another risk factor was birth cohort. The data from the NTR could be important, since we recruit children from birth cohort 1986–1987 onwards. Time changes should be further examined. Future research should also explore interaction effects of birth cohort with other risk factors.

Educational level, maternal smoking during pregnancy, current smoking, having older siblings, and breastfeeding did not affect the prevalence of asthma. Maybe the most remarkable negative finding was the absence of an effect of maternal smoking during pregnancy. A meta-analysis reported a pooled OR estimate of 1.37 for the risk of asthma if either parent smoked (Strachan & Cook, 1998), while we found an OR of only 1.14. A possible explanation could be our use of a dichotomous measure for smoking exposure, which may be too crude to assess exposure to tobacco smoking.

In conclusion, the present study explored environmental risk factors that modify the effect of genetic influences on asthma in 5-year-old children. We found main effects for several measured environmental factors, but only one environmental factor that modified the effect of genetic influences on asthma, namely gestational age.

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References

Bartels, M., van Beijsterveldt, C. E., Derks, E. M., Stroet, T. M., Polderman, T. J., Hudziak, J. J., & Boomsma, D. I. (2007). Young Netherlands twin register (Y-NTR): A longitudinal multiple informant study of problem behavior. *Twin Research and Human Genetics, 10*, 3–11.

Boomsma, D. I., de Geus, E. J., Vink, J. M., Stubbe, J. H., Distel, M. A., Hottenga, J. J., Posthuma, D., van Beijsterveldt, C. E. M., Hudziak, J. J., Bartels, M., & Willemsen, G. (2006). Netherlands twin register: From twins to twin families. *Twin Research and Human Genetics, 9*, 849–857.

Boomsma, D. I., de Geus, E. J. C., van Baal, G. C. M., & Koopmans, J. R. (1999). A religious upbringing reduces the influence of genetic factors on disinhibition: Evidence for interaction between genotype and environment on personality. *Twin Research, 2,* 115–125.

Boomsma, D. I., & Martin, N. G. (2002) Gene-Environment Interactions. In H. D'haenen, J. A. den Boer & P. Willner (Eds.), *Biological psychiatry* (pp. 181–187). New York: John Wiley & Sons.

Colilla, S., Nicolae, D., Pluzhnikov, A., Blumenthal, M., Beaty, T., Bleecker, E., Ober, C., Cox, N. J., & the CSGA. (2003). Collaborative study for the genetics of asthma. Evidence for gene-environment interactions in a linkage study of asthma and smoking exposure. *Journal of Allergy and Clinical Immunology, 111*, 840–846.

Dizier, M. H., Bouzigon, E., Guilloud-Bataille, M., Siroux, V., Lemainque, A., Boland, A., Lathrop, M., & Demenais, F. (2007). Evidence for gene × smoking exposure interactions in a genome-wide linkage screen of asthma and bronchial hyper-responsiveness in EGEA families. *European Journal of Human Genetics, 15*, 810–815.

Duffy, D. L., Martin, N. G., Battistutta, D., Hopper, J. L., & Mathews, J. D. (1990). Genetics of asthma and hay fever in Australian twins. *American Review of Respiratory Diseases, 142,* 1351–1358.

Eaves, L., & Eysenck, H. (1976). Genotype × age interaction for neuroticism. *Behavior Genetics, 6*, 359–362.

Eaves, L. J. (1982). The utility of twins. In V. E. Anderson, W. A. Hauser, J. K. Penry, & Sing, C.F. (Eds.), *Genetic basis of the epilepsies* (pp. 249–276). New York: Raven Press.

Eder, W., Klimecki, W., Yu, L., von Mutius, E., Riedler, J., Braun-Fahrlander, C., Nowak, D., Holst, O., Martinez, F. D.; the ALEX-Team. (2006). Association between exposure to farming, allergies and genetic variation in card4/nod1. *Allergy, 61*, 1117–1124.

Eder, W., Klimecki, W., Yu, L., von Mutius, E., Riedler, J., Braun-Fahrlander, C., Nowak, D., Martinez, F. D.; Allergy And Endotoxin Alex Study Team. (2005). Opposite effects of cd 14/-260 on serum ige levels in children raised in different environments. *Journal of Allergy and Clinical Immunology, 116*, 601–607.

Goldsmith, H. (1991). A zygosity questionnaire for young twins: A research note. *Behavior Genetics, 21*, 257–270.

Harris, J., Magnus, P., Samuelsen, S., & Tambs, K. (1997). No evidence for effects of family environment on asthma. A retrospective study of Norwegian twins. *American Journal of Respiratory and Critical Care Medicine, 156*, 43–49.

Heath, A. C., Jardine, R., & Martin, N.G. (1989). Interactive effects of genotype and social environment on alcohol consumption in female twins. *Journal of Studies on Alcohol, 50*, 38–48.

Heath, A. C., Eaves, L. J., & Martin, N.G. (1998). Interaction of marital status and genetic risk for symptoms of depression. *Twin Research*, *1,* 119–122.

Jaakkola, J. J., Nafstad, P., & Magnus, P. (2001). Environmental tobacco smoke, parental atopy, and childhood asthma. *Environmental Health Perspectives, 109*, 579–582.

Koeppen-Schomerus, G., Stevenson, J., & Plomin, R. (2001). Genes and environment in asthma: A study of 4-year-old twins. *Archives of Disease in Childhood, 85*, 398–400.

Laitinen, T., Rasanen, M., Kaprio, J., Koskenvuo, M., & Laitinen, L. (1998). Importance of genetic factors in adolescent asthma: A population-based twin-family study. *American Journal of Respiratory Critical Care Medicine, 157*, 1073–1078.

Lichtenstein, P., & Svaertengren, M. (1997). Genes, environments, and sex: Factors of importance in atopic diseases in 7–9-year-old Swedish twins. *Allergy, 52*, 1079–1086.

Los, H., Postmus, P. E., & Boomsma, D. I. (2001). Asthma genetics and intermediate phenotypes: A review from twin studies. *Twin Research, 4*, 81–93.

Neale, M., Boker, S., Xie, G., & Maes, H. (2003). *Mx: Statistical modeling*. Richmond, VA: Department of Psychiatry.

Neale, M. C., & Cardon, L. R. (1992). *Methodology for genetic studies of twins and families*. Dordrecht, the Netherlands: Kluwer Academic Publishers BV.

Marks, G. B. (2006). Environmental factors and gene–environment interactions in the aetiology of asthma. *Clinical and Experimental Pharmacology and Physiology, 33*, 285–289.

Martinez, F. D. (2007). Cd14, endotoxin, and asthma risk: Actions and interactions. *Proceedings of American Thoracic Society, 4*, 221–225.

Meyers, D. A., Postma, D., Stine, O. C., Koppelman, G. H., Ampleford, E. J., Jongepier, J., Howard, T. D., & Bleecker, E. R. (2005). Genome screen for asthma and bronchilal hyperresponsiveness: Interactions with passive smoke exposure. *Journal of Allergy and Clinical Immunology, 115*, 1169–1175.

Molenaar, P. C., Boomsma, D. I., Neeleman, D., & Dolan, C. V. (1990). Using factor scores to detect G X E interactive origin of 'pure' genetic or environmental factors obtained in genetic covariance structure analysis. *Genetic Epidemiology*, *7*, 93–100.

Muthén, L., & Muthén, B. (1998–2007). *Mplus user's guide* (4 ed.). Los Angeles: Muthén & Muthén.

Mutius, V. (2000). The environmental predictors of allergic disease. *Journal of Allergy and Clinical Immunology, 105*, 9–19.

Nystad, W., Roysamb, E., Magnus, P., Tambs, K., & Harris, J. R. (2005). A comparison of genetic and environmental variance structures for asthma, hay fever and eczema with symptoms of the same diseases: A study of Norwegian twins. *International Journal of Epidemiology, 34*, 1302–1309.

Orlebeke, J. F., Stroet, T., & Boomsma, D. I. (1995). Breastfeeding and neurological status. *Lancet, 345*, 393.

Purcell, S. (2002). Variance components models for gene-environment interaction in twin analysis. *Twin Research and Human Genetics, 5,* 554–571.

Ramadas, R. A., Sadeghnejad, A., Karmaus, W., Arshad, S. H., Matthews, S., Huebner, M., Kim, D. Y., & Ewart, S. L. (2007). Interleukin-1r antagonist gene and pre-natal smoke exposure are associated with childhood asthma. *European Respiratory Journal, 29*, 502–508.

Rebollo, I., de Moor, M. H. M., Dolan, C. V., & Boomsma, D. I. (2006). Phenotypic factor analysis of family data: Correction of the bias due to. *Twin Research and Human Genetics, 9,* 367–376.

Rietveld, M., Van der Valk, J., Bongers, I., Stroet, T., Slagboom, P., & Boomsma, D. (2000). Zygosity diagnosis in young twins by parental report. *Twin Research, 3*, 134–141.

Skadhauge, L., Christensen, K., Kyvik, K., & Sigsgaard, T. (1999). Genetic and environmental influence on asthma: A population-based study of 11,688 Danish twin pairs. *European Respiratory Journal, 13*, 8–14.

Strachan, D. P., & Cook, D. G. (1998). Health effects of passive smoking. 6. Parental smoking and childhood asthma: Longitudinal and case-control studies. *Thorax, 53*, 204–212.

van Beijsterveldt, C. E., & Boomsma, D. I. (2007). Genetics of parentally reported asthma, eczema and rhinitis in 5-yr-old twins. *European Respiratory Journal, 29*, 516–521.

van Beijsterveldt, T. C. E. M., Hudziak, J. J., & Boomsma, D. I. (2005). Short- and long-term effects of child care on problem behaviors in a Dutch sample of twins. *Twin Research and Human Genetics, 8,* 250–258.

Willemsen, G., van Beijsterveldt, T. C. E. M., van Baal, G. C. M., Postma, D., & Boomsma, D. I. (2008). Heritability of self-reported asthma and allergy: A study in adult Dutch twins, siblings and parents. *Twin Research and Human Genetics*, 132–142.