Genomics **58,** 1–8 (1999)
Article ID geno.1999.5806, available online at http://www.idealibrary.com on IDEAL®

A Genome-wide Search for Linkage to Asthma

Matthias Wjst,[1] Guido Fischer, Thomas Immervoll, Martin Jung, Kathrin Saar, Franz Rueschendorf, André Reis, Matthias Ulbrecht, Maria Gomolka, Elisabeth H. Weiss, Lothar Jaeger, Renate Nickel, Kai Richter, N.-I. Max Kjellman, Matthias Griese, Andrea von Berg, Monika Gappa, Frank Riedel, Martin Boehle, Silke van Koningsbruggen, Peter Schoberth, Ruediger Szczepanski, Walter Dorsch, Michael Silbermann, Sabine Loesgen, Michael Scholz, Heike Bickeböller, and Heinz-Erich Wichmann

On behalf of the German Asthma Genetics Group[2]

Received December 28, 1998; accepted February 19, 1999

Asthma is among the most frequent chronic diseases in childhood. Although numerous environmental risk factors have already been identified, the basis for familial occurrence of asthma remains unclear. Previous genome screens for atopy in British/Australian families and for asthma in different American populations showed inconsistent results. We report a sib pair study of a sample of 97 families, including 415 persons and 156 sib pairs. Following an extensive clinical evaluation, all participants were genotyped for 351 polymorphic dinucleotide markers. Linkage analysis for asthma identified four chromosomal regions that could to be linked to asthma: chromosome 2 (at marker D2S2298, $P = 0.007$), chromosome 6 (around D6S291, lowest $P = 0.008$), chromosome 9 (proximal to D9S1784, $P = 0.007$), and chromosome 12 (D12S351, $P = 0.010$). These linkage regions could be reproduced for all loci by analysis of total or specific immunoglobulin E (minimum P values at these regions were 0.003, 0.001, 0.010, and 0.015, respectively).

INTRODUCTION

Asthma is today one of the most frequent chronic diseases in childhood, with a prevalence of up to 10% (Jarvis and Burney, 1998). Many environmental risk factors have been identified in the past; however, a familial clustering of asthma has been known for a long time (Litwin, 1978; Dold *et al.*, 1992). Mendelian inheritance has not been observed. This is not astonishing, because there are well-known difficulties with the clinical definition of asthma (Marsh and Meyers, 1992), the strong influence of exogenous factors on penetrance (Sporik *et al.*, 1995), and other complexities due to locus heterogeneity and the possibility of epistatic interaction between several loci (Wjst *et al.*, 1997). The relative risk for the sib of an asthmatic subject to that of an unrelated individual from the general population is $\lambda_S = 2.58$ [unpublished own observation, Bitterfeld cohort (Dold *et al.*, 1998)]. By contrast, for cystic fibrosis $\lambda_S \approx 500$, for insulin-dependent diabetes mellitus (IDDM) $\lambda_S \approx 15$, and for schizophrenia $\lambda_S \approx 8.6$ (Barnes and Marsh, 1998).

Asthma is characterized clinically by chronic, intermittent airway obstruction with wheezing, coughing, and breathlessness. Several mechanisms contribute to flow limitation in the airways. Bronchial hyperreactivity (BHR) is the most prominent clinical characteristic. Another indicator that can be measured is the peak flow variability. The main biological process seems to be allergic inflammation with the release of mediators from activated mast cells and eosinophils in combination with elevated specific (RAST) and total serum IgE levels. Because early events in disease initiation are largely unknown, linkage analysis could be one method for discovering the genes relevant in the pathogenesis of asthma.

SUBJECTS AND METHODS

Subjects. We have collected asthma sib pair families since the beginning of 1994, mainly in pediatric university clinical centers in Germany and Sweden (Wjst and Wichmann, 1995). The families have been selected from approximately 5000 families with asthmatic children attending the participating clinics. In the choice of these families, at least two children with confirmed clinical asthma were required, and prematurity or low birth weight of the children was excluded, along with any other severe pulmonary disease. In the present analysis only the first 103 families were genotyped. All parents could be included in the analysis. Five families had to be excluded due to non-Mendelian segregation of several markers, and

Results presented in this article were partially presented at the World Asthma Meeting, 10–13 December 1998 in Barcelona, Spain [*Eur. Resp. J., 1998,* **12**(Suppl. 29): 3S]. This presentation was based on the same probands but only 68% of the genotypes. The data included erroneously two identical twin pairs and are replaced by data in this paper.

[1] To whom correspondence should be addressed at GSF–Forschungszentrum fuer Umwelt und Gesundheit, Institut fuer Epidemiologie, Ingolstaedter Landstrasse 1, D-85758 Neuherberg, Germany. Fax: +49-89-3187-3380. E-mail: m@wjst.de.

[2] See the Appendix.

0888-7543/99 $30.00

TABLE 1

Details of the 97 Families

	Families (*N*)	Percentage total
German nationality	83	85.6%
City inhabitants		
>250,000	36	37.1%
50,000 to 250,000	16	16.5%
5000 to less than 50,000	28	28.9%
<5000	17	17.5%
Pollution exposure:		
Smoking at home	54	55.7%

one family was excluded because the children were monozygotic twins. Therefore, 97 families with 415 persons, including 221 children in 156 sib pairs (200 children in 110 sib pairs affected with asthma), remained in the analysis (Table 1). Seventy-four families had 2 children, 19 had 3 children, and 4 had 4 children; 91 families contributed 2 children with asthma, 3 families contributed 3 children, and no family contributed 4 children. Participants were all Caucasians; 83 families were German, 5 were Swedish, and 9 were of other nationalities. All hospital staff were trained during a 1-day course at the GSF research center. Each study participant, including all children, signed a consent form. All study methods have been approved by the ethics commission of "Nordrhein-Westfalen."

Asthma in the children was initially defined by clinical history and validated later by interview questions. In addition to a clinical asthma diagnosis, all affected children over age 3 had a history of at least 3 years of recurrent wheezing and with no other airway diseases diagnosed. On the first home visit a complete pedigree of the family was drawn, and information about the home was collected in a questionnaire. Questionnaires were developed in accordance with previous studies (Wjst and Wichmann, 1995) with a focus on the time course of respiratory symptoms and diseases. Skin symptoms were recorded after photographs of affected skin were shown.

Phenotyping. Asthma was the primary trait examined; however, all participants were also examined for associated phenotypes such as skin prick tests with frequent allergens, serological IgE measurement, and eosinophil count. Lung function was assessed during the visit at the clinical center and during a 10-day period at home.

For this purpose patients were instructed in the use of portable pulmonary function meters (Diarycard, Calw, Germany) by doing peak flow tests (PEFR) in duplicate every morning and evening before eating or using an inhaler. Every peak flow series was manually checked before the mean of the two lowest values was divided by the period mean to determine "dips" in pulmonary function (Sierstedt *et al.,* 1994).

Lung function in the clinical center was measured with a local pulmonary function unit that was calibrated daily. Probands with present respiratory illness repeated the test on another day. Height and weight were measured with clothes on but without shoes. Pulmonary function tests were then performed by forced expiration in a sitting position using a nose clip. Forced flow volume tests were performed until three reproducible loops were achieved. Of these, the trial with the maximum sum of FVC and FEV1.0 was used for the analysis (Anonymous, 1987). Bronchial challenge with methacholine was performed with increasing doses of 0, 0.156, 0.312, 0.625, 1.25, 2.5, 5, 10, and 25 mg/ml during five consecutive breaths with 14 μg delivered from de Vilbiss 646 nebulizer chambers using a ZAN 200 breath-triggered pump (Zan, Oberthulpa, Germany) (Chai *et al.,* 1975). The provocation was stopped at the occurrence of symptoms or a decrease of 20% from the baseline FEV1.0. The variable SLOPE from the resulting dose–response curve was calculated as described earlier (Wassmer *et al.,* 1997). The dose necessary for a 20% fall of FEV1.0 is given as PD20. Probands with a PD20 $\leq$8 mg/ml were considered bronchial hyperreactive.

Skin prick tests (SPT) were performed on the upper left forearm by application of the allergen, puncturing the skin with a lancet (ALK) and reading the response as length of the largest diameter of the resulting wheal size (Dreborg, 1989). The allergens birch (*Betula verruscose*) ALK SQ108, hazel (*Corylus avellana*) ALK SQ113, ribworth (*Plantago lanceolata*) ALK N342, mugwort ALK SQ312, mixed grass ALK SQ299, dust mites (*Dermatophagoides farinae*) ALK SQ 504 and (*Dermatophagoides pteronyssimus*) ALK SQ 503, cat dander ALK SQ555, dog dander ALK SQ 553, and fungi (*Aspergillus fumigatus*) ALK N405 and (*Alternaria alternata*) ALK N402 were bought in one batch and stored at 5°C.

During patient visits at the clinic, interviews were completed for each participant. Blood was taken from a cubital vein with a closed EDTA-coated system (Sarstedt, Germany) and transferred by mail within 24 h to the respective laboratory, where either DNA was isolated or the serological analysis was performed. Total IgE was determined with an ELISA (Pharmacia, Germany), and specific IgE for the same allergens as in the SPT was determined by the CAP FEIA system from the same manufacturer (RAST). Total cell counts were measured in a Coulter counter, and eosinophil cells were counted manually as a percentage and multiplied with the total cell count.

DNA preparation and genotype analysis. DNA was isolated from peripheral white blood cells using the salt method (Miller *et al.,* 1988), and in some cases also with the Qiamp blood kit (Qiagen, Germany) according to the manufacturer's recommendation. We have developed a microsatellite mapping panel, based on microsatellites from the Généthon reference map (Dib *et al.,* 1996). Highly polymorphic markers were selected for equal distances on the genome with markers always at telomeric positions. Because there are sex differences in asthma prevalence, 18 markers on the X chromosome were also included. All markers were extensively tested for robustness and ease in scoring. Marker amplification was performed on microtiter plates on Tetrad PCR machines (MJ Research) with a similar protocol for each marker. DNA dispensing, and pooling of PCR products, was performed with separate pipetting robots with 96 disposable tips (IGEL, Opal-Jena), ensuring a fast and almost error-free liquid handling process. PCR pools were separated on ABI 377 automatic sequencers, and genotypes were scored using GENESCAN and GENOTYPER (ABI) software. Allele calling was checked by technicians, and all genotypes were subject to an automatic Mendelian check using the UNKNOWN algorithm from the LINKAGE program package. All allele sizes were standardized to known CEPH control individuals without knowledge of the disease status. All inconclusive genotypes were excluded. Of 145,665 possible genotypes, only 4.3% could not be determined; mean heterozygosity was 0.797 (range per chromosome 0.536 to 0.936), with an information content of 0.838 (range 0.591 to 0.949) and a mean marker distance of 10.7 cM (range 1.0 to 21.8 cM). Further details are available at http://cooke.gsf.de/wjst/GenomicsSuppl99.

Linkage analysis. Clinical and laboratory data were merged with SAS software and exported to the linkage format. The number of eosinophil cells and total IgE were log-transformed after addition of a constant of 0.001 before any further analysis. Information about marker order was obtained in July 1997 from the GDB (http://gdbwww.dkfz-heidelberg) and CHLC (http://www.chlc.org), and CRIMAP was run to check the order of markers. Allele frequencies were estimated from the parental chromosomes. For the qualitative traits, nonparametric multipoint linkage analyses were performed with GENEHUNTER 1.3, where exact *P* values based on the S_all score are reported (Kruglyak *et al.,* 1996). Two-point analysis was carried out with SIBPAL (SAGE 3.1) to check for consistency with the results of the multipoint analysis (data not shown). The nonparametric statistic of MAPMAKER/SIBS 2.1 was used to investigate linkages of quantitative traits (Kruglyak and Lander, 1995). Here all possible pairs of multiplex sibship were used, but weighted down to account for dependency.

TABLE 2

Type of Asthma of the 200 Children with Asthma

	Children (*N*)	Median/ percentage
Age at examination (years)	200	10
Age of symptom onset (years)	200	5
Gender male	117	58.5%
Asthma severity		
No actual symptoms	70	35.0%
One attack per month	98	49.0%
One attack per week	18	9.0%
More than one attack per week	10	5.0%
Permanent	3	1.5%
Trigger factors		
Infections	145	72.5%
Pollen, dust	143	71.5%
Exercise	109	54.5%
Weather	98	49.0%
Stress	64	32.0%
Animals	94	47.0%
Food	36	18.0%
Drugs	7	3.5%
Any RAST >0.35 kU/L	145	72.5%
Any SPT >1 mm diameter	171	85.5%
Total IgE (kU/ml)	178	261
Baseline pulmonary function (FEV1 of predicted)	197	98.4%
Lowest peak flow (mean of two by period mean)	173	80.4%
Methacholine response[a] [PD 20 (mg/ml)]	96	1.25

[a] Five children had missing values, 33 had baseline FEV1.0 values <70% or could not be tested due to respiratory symptoms during examination and 66 did not respond to a concentration of 25 mg/ml metacholine.

RESULTS

Clinical Details

Most of the families were of German nationality, and all social classes were represented. In nearly half of the households, children were exposed to passive smoking, with intermediate exposures to cat and dust mite allergens. Fifty-nine percent of the children with asthma were boys, the median age was 10 years and the onset of asthma symptoms was usually before age 5 (Table 2). Two-thirds of the children had symptoms during the month of examination, triggered primarily due to respiratory infections and exposure to pollen or house dust mites. In 86% of the affected children, at least one of the tested allergens was positive in the skin prick test, and 73% tested positive in the serum RAST assays. Median total IgE was 261 kU/L, with a highly skewed distribution (87 children with asthma had less than 250 kU/ml, 39 children had up to 500 kU/ml, 21 up to 750 kU/ml, 8 up to 1000 kU/ml, and 23 had IgE values greater than 1000 kU/ml).

Although baseline pulmonary function levels were not reduced in most of the children, a high variability of peak flow values was observed, along with a large number of pathological methacholine provocation tests.

Linkage Results

Figure 1 shows the comparative results for asthma and traits that are associated with the complex phenotype asthma. Using a screening P value of 0.01, four regions that could be linked to asthma were identified. Minimal P values for asthma were achieved at chromosome 2 (at marker D2S2298, $P = 0.0074$), chromosome 6 (around D6S291, $P = 0.0081$), chromosome 9 (proximal to D9S1784, $P = 0.0073$), and chromosome 12 (D12S351, lowest $P = 0.0103$). A more detailed comparison is given in Table 3, which shows that the analysis of total (polyclonal) IgE pointed to the same regions and gave evidence for linkage to other regions also. An analysis of RAST results, defined as at least one specific IgE from a series of 11 tested allergens, mapped also to the first three regions. Minimum P values for IgE or RAST are 0.0009, 0.0011, 0.0025, and 0.0152 for the regions on 2p, 6p, 9q, and 12q, respectively. A correlation to loci linked to eosinophil cell count could be found only on chromosome 6. For BHR regions similiar to that of asthma were seen on chromosomes 2 and 9. If bronchial hyperreactivity was defined as a quantitative trait (SLOPE) or analyzed as PEFR, there was no correlation to loci linked to asthma.

DISCUSSION

How to interpret these linkage results? From a more formal viewpoint, it is difficult to avoid false-positive linkage claims, but at the same time an overly cautious approach runs the risk of missing true linkages (Morton, 1998). We used a less conservative approach ($P < 1 \times 10^{-2}$) because it may be more appropriate during screening for new linkage regions, but we tried to confirm the result of the main phenotype with associated phenotypes. The identified linkage regions for asthma could be reproduced in the same sample by analysis of total or specific immunoglobulin E in all regions, where the linkages at chromosome 2 and 6 nearly meet the recommended criteria for "suggestive linkage" by Lander and Kruglyak (1995) defined by a critical limit of $P < 7 \times 10^{-4}$. None of the linkages met the recommended criteria for significant linkage.

For the limit of $P < 7 \times 10^{-4}$, the sample size of 110 sib pairs, and $\lambda_s = 2.6$, power estimates under a variety of models are greater than 85%. However, this power is likely to be overestimated because of the multifactorial character of asthma. Therefore, smaller values of λ_s were assumed under the same conditions as above, leading to power estimates over 74% for $\lambda_s = 1.5$ and tight linkage.

Results from other studies support the view that the reported regions are not false-positives. In a British/ Australian sample of atopic sib pairs in which only a minority of children had asthma, linkage regions for total IgE were observed on chromosomes 6, 7, 11, 13, and 16, for eosinophil cell counts on chromosomes 6

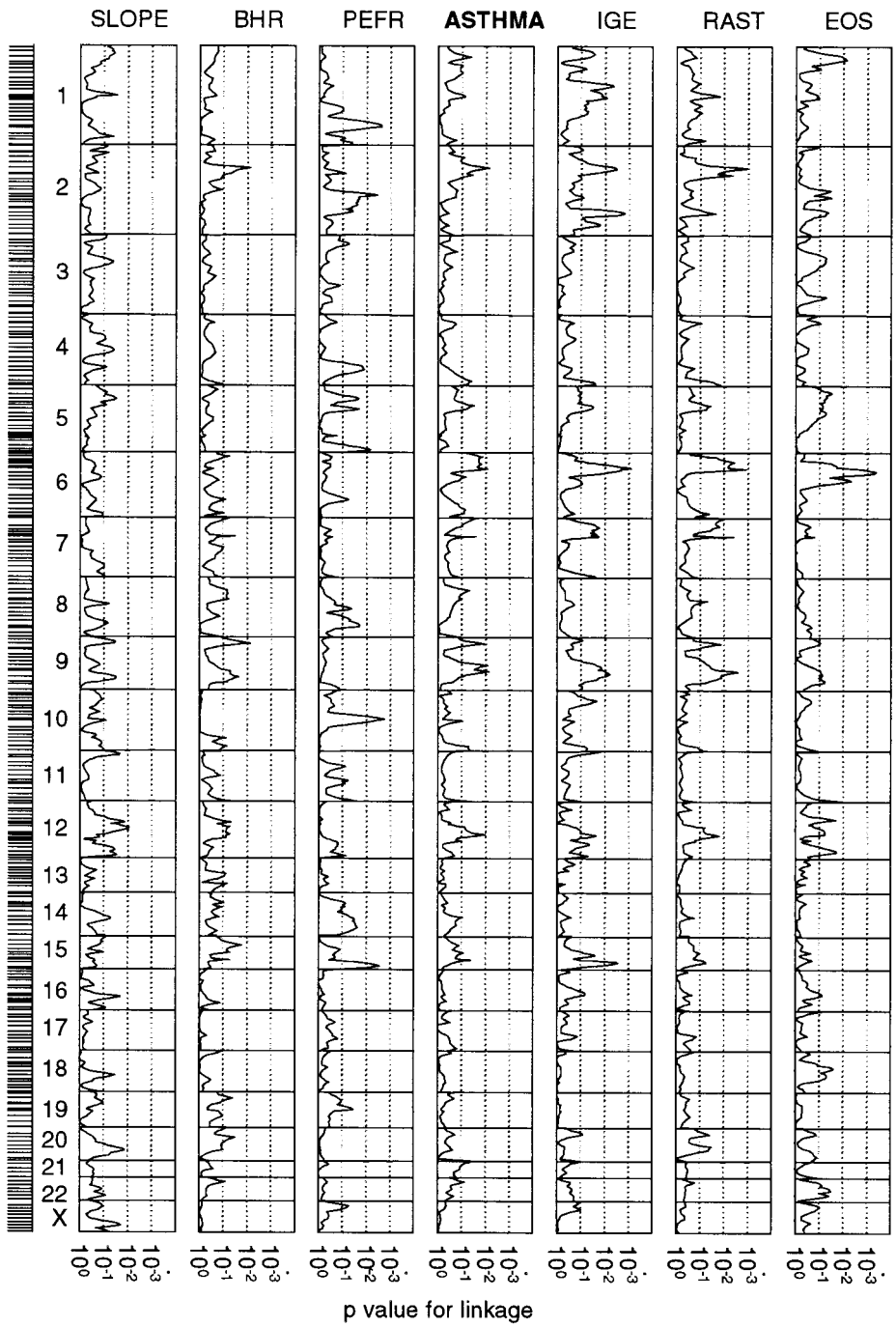

FIG. 1. Linkage results for asthma and associated traits. Chromosomes are arranged by number from p-ter to q-ter with recombination distance in centimorgans on a linear scale (0 to 3592 cM). *P* values of the corresponding statistics are shown on the *x* axis on a logarithmic scale with positions of the used markers on the left. BHR (probands with low baseline FEV1.0 or PD20 <8 mg/ml), ASTHMA, and RAST (any specific IgE >0.35 kU/L) were analyzed as qualitative, SLOPE (of methacholine response curve without low baseline FEV1.0), total IgE (IGE), and absolute number of eosinophil cells (EOS) as quantitative variables.

and 7, and for BHR on chromosomes 4, 7, and 16 (Daniels *et al.*, 1996). There may be a partial overlap with the chromosome 11 linkage data; however, linkage on chromosomes 13 and 16 could not be reproduced in our sample.

The second genome-wide screen, performed by an American Collaborative Study, examined linkage to asthma in African, Caucasian, and Hispanic groups (CSGA, 1997). Unfortunately, exact linkage locations were not given; however, estimates from the figures showed LODs between 1.0 and 2.4 on chromosomes 2, 5, 6, 11, 12–14, 17, 19, and 21. Of these, only linkages at chromosome 6 and 12 were supported by our asthma data. Another study of a founder population in Hutterites living in South Dakota identified 10 regions with possible linkage to asthma (chromosomes 2, 3, 5, 9, 12, 13, 19, and 21) with major evidence at chromosomes 5, 12, 19, and 21 (Ober *et al.*, 1998). Of these, one marker

TABLE 3

Linkage Results for Asthma and Associated Traits

Chromosome	Marker	cM	Slope	BHR	PEFR	Asthma	IGE	RAST	EOS
1p36	D1S228	33.2							0.0094
1p33	D1S207	118.1					0.0045		
1p21	D1S221	146.7					0.0135	0.0156	
1pter	D1S502	151.2					0.0098		
1pter	D1S419	237.2			0.0023				
1pter	D1S229	242.4			0.0077				
2pter	**D2S2298**	**67.8**		**0.0073**		**0.0074**	**0.0032**	**0.0009**	
2p13	D2S380	84.4						0.0187	
2p13	D2S2113	88.7						0.0045	
2	D2S368	146.9			0.0045				
2q32	D2S116	201.8					0.0016		
4	D4S393	160.9			0.0146				
4q32	D4S1597	170.8			0.0197				
4	D4S2924	208.9						0.0134	
4q35	D4S426	211.1						0.0183	
4	D4S2930	212.2					0.0160	0.0067	
5	D5S2111	189.8			0.0067				
6p24	D6S309	12.8				0.0134			
6p25	D6S470	22.5				0.0195		0.0084	
6p23	D6S260	28.2				0.0192		0.0037	
6p23	D6S422	35.0				0.0150	0.0152	0.0052	
6p22.3	D6S276	44.0					0.0012	0.0068	0.0180
6p21.3	**D6S291**	**49.8**				**0.0081**	**0.0020**	**0.0011**	**0.0015**
6p21	D6S426	60.9					0.0122		0.0005
6	D6S455	82.2							0.0046
7cen	D7S531	3.1						0.0149	
7pter	D7S517	6.1						0.0099	
7p21	D7S488	27.9						0.0145	
7p15	D7S528	58.3					0.0187	0.0039	
8	D8S529	145.2			0.0198				
9p23	D9S156	29.4		0.0073		0.0109		0.0131	
9q13	D9S283	94.2				0.0081			
9	**D9S1784**	**112.5**				**0.0073**	**0.0098**	**0.0025**	
9q32	D9S195	130.3					0.0178	0.0122	
10	D10S581	86.7			0.0017				
10	D10S537	91.9			0.0049				
11q25	D11S968	149.7							0.0084
12q13	D12S85	62.1	0.0132						
	D12S355	75.2	0.0116						
	D12S351	**97.0**				**0.0103**			
12q21	D12S327	101.1				0.0177		0.0152	
	D15S1042	29.9		0.0166					
15q22	D15S127	79.4			0.0164		0.0027		
15q26	D15S120	94.2			0.0082				
	D20S891	68.3	0.0134						
Xp11.21	DXS991	86.9	0.0190						
Xq25	DXS8081	126.1					0.0096		
Xq26	DXS8072	151.1							0.0096
Xq27	DXS998	183.8	0.0159						

Note. Shown are all markers with $P \leq 0.02$ for at least at one trait, the four asthma linkages are in bold.

on chromosome 9 and one on chromosome 12 match within an approximately 20-cM distance to our linkage regions. The chromosome 12 region has been initially described by a candidate region approach (Barnes *et al.*, 1996; Nickel *et al.*, 1997). Therefore, at least three of our linkage regions were seen in previous genome screens, while the linkage region on 2p is classified as a new candidate region [Asthma Gene Database at http://cooke.gsf.de (Wjst and Immervoll, 1998)]. Interestingly, a recent genome screen for total IgE and BHR in mouse revealed linked regions on chromsome 1, 17, and 10 that share regions syntenic to the human chromosomes 2, 6, and 12 (Zhang *et al.*, 1998). Failure to replicate other results, for example, on chromosome 5q31 (interleukin gene cluster), does not necessarily disprove these data as a large heterogeneity is expected between different populations.

Two of the four regions, chromosome 6p and chromosome 12q, contain numerous candidate genes. The major histocompatibility complex (MHC; HLA in human) is a family of highly polymorphic genes located at 6p21. HLA class II includes membrane proteins involved in

the presentation of peptides to $CD4^+$ T-helper cells. HLA class II products are associated with several autoimmune diseases (Kostyu, 1991) and with numerous allergens (Sandford *et al.*, 1996). A decreased prevalence of DBP1*0401 in asthmatic patients has been reported in two independent studies (Kemeny and Lee, 1993; Caraballo *et al.*, 1991). Caraballo and Hernandez reported linkage of class II loci with asthma in patients with skin test positivity to the mite *D. farinae*. Animal studies noted that the TH_1/TH_2 subset balance and the asthma phenotype are associated with certain HLA class II alleles (Murray *et al.*, 1992; Asherson *et al.*, 1990). Two additional potential candidate genes map within the HLA region, namely, TNF-α and TNF-β. TNF-α is a mast-cell-derived cytokine that upregulates the expression of IL-6, which is a major mediator of inflammation. TNF-β plays a role in the destruction of tumor cells and virally infected cells and is an upregulator of TNF-α expression (Shimizu *et al.*, 1990; Walker *et al.*, 1994). Some candidate gene studies report the association of TNF polymorphisms with asthma (Moffatt and Cookson, 1997; Albuquerque *et al.*, 1998; Hayden *et al.*, 1998).

Several candidate genes that show possible involvement in the development of asthma and increased IgE responsiveness map to chromosome 12q near the marker D12S351. The cytokine IFN-γ is known to promote the differentiation of type 1 helper T (TH_1) lymphocytes and inhibit differentiation and IL-4 production in TH_2 lymphocytes, which are involved in IgE synthesis and eosinophilia. The product of IGF1 promotes the differentiation of both B and T lymphocytes (Jardieu *et al.*, 1994). SCF, encoded by a gene mapped to 12q22 (Mathew *et al.*, 1992), is required for the proliferation of hematopoietic stem cells as well as mature mast cells (Anderson *et al.*, 1990), which produce IL-4 and important inflammatory mediators. LTA4H, which encodes a hydrolase involved in prostaglandin metabolism and the inflammatory response associated with asthma (Funk *et al.*, 1987), has also been mapped to 12q22 (Mancini and Evans, 1995). NFYB is an attractive candidate because of the dual role of NF-Y in upregulating the transcription of both the IL4 and the HLAD genes (Li-Weber *et al.*, 1994). Neuronal nitric oxide synthase plays an important role in pulmonary inflammatory processes (Kharitonov *et al.*, 1994). Conceivably, polymorphisms in these genes could influence bronchial inflammation, IgE production, and/or asthma.

Identifying susceptibility genes for asthma is a continuing challenge. Recruitment of additional families for fine-mapping of the identified regions is under way. Additional analyses will employ conditional linkages, the inclusion of environmental factors, and the influence of paternal or maternal inheritance. A joint analysis of all available data sets should provide the power also for subgroup analysis. The identification of susceptibility genes will be the crucial step in elucidating the pathology in the asthmatic airway, defining subtypes of asthma, and developing new treatment options for asthma.

APPENDIX: GERMAN ASTHMA GENETICS GROUP

Coordinating Center and Data Center of the German Asthma Genetics Group

M. Wjst, G. Fischer, T. Immervoll, A. Houzer, M. Hoeltzenbein, and H. E. Wichmann. *Institut fuer Epidemiologie, GSF Forschungszentrum fuer Umwelt und Gesundheit, Ingolstaedter Landstrasse 1, 85758 Neuherberg, Germany*

Participating Laboratories

M. Ulbrecht, M. Gomolka, K. Witter, and E. H. Weiss. *Institut fuer Anthropologie und Humangenetik, Richard-Wagner-Strasse 10/I, 80336 Munich, Germany*

M. Jung, K. Saar, F. Rueschendorf, T. Wienker, and A. Reis. *Mikrosatellitenzentrum, Max Delbrueck Centrum, Heubnerweg 6, 14059 Berlin, Germany*

T. Eisenhut, J. Boenisch, and E. Albert. *Labor fuer Immungenetik, Kinderpoliklinik der Ludwig-Maximilians-Universitaet, Paul-Heysestrasse 33, 80336 Munich, Germany*

G. Schlenvoigt and L. Jaeger. *Institut fuer klinische Immunologie, Am Johannisfriedhof 9, 07740 Jena, Germany*

Statistical Analysis Group

S. Loesgen. *Institut fuer Epidemiologie, GSF Forschungszentrum fuer Umwelt und Gesundheit, Ingolstaedter Landstrasse 1, 85758 Neuherberg, Germany*

M. Scholz and H. Bickeböller. *Institut fuer Medizinische Statistik und Epidemiologie der Technischen Universitaet Muenchen, Ismaninger Strasse 22, 81675 Munich, Germany*

M. Knapp, R. Kruse, and M. Baur. *Institut fuer Medizinische Statistik, Dokumentation und Datenverarbeitung, Sigmund-Freudstrasse 25, 53105 Bonn, Germany*

Clinical Centers

R. Nickel, K. Beyer, R. Kehrt, and U. Wahn. *Charité, Campus Virchow Klinikum, Kinderklinik, Augustenburger Platz 1, 12253 Berlin, Germany*

K. Richter, H. Janiki, R. Joerres, and H. Magnussen. *Krankenhaus Grosshansdorf, Zentrum fuer Pneumologie und Thoraxchirurgie, Woehrendamm 80, 22927 Hamburg, Germany*

I. M. Sandberg, L. Lindell, and N. I. M. Kjellman. *Department of Environment and Health, Pediatrics, University Hospital, S-58185 Linkoeping, Sweden*

A. Demirsoy, M. Griese, and D. Reinhardt. *Kinderpoliklinik der Universitaet Muenchen, Pettenkoferstrasse 8 a, 80336 Munich, Germany*

G. Oepen, A. Martin, A. von Berg, and D. Berdel. *Marienhospital Wesel, P.-Janssenstrasse 8, 46483 Wesel, Germany*

Y. Guesewell, M. Gappa, and H. von der Hardt. *Abteilung fuer Kinderheilkunde I, Medizinische Hochschule Hannover, Konstanty-Gutschow-Strasse 8, 30623 Hannover, Germany*

J. Tuecke and F. Riedel. *Klinik fuer Kinder und Jugendmedizin, St. Josef Hospital Bochum, Alexandrinenstrasse 5, 44791 Bochum, Germany*

M. Boehle, G. Kusenbach, and G. Heimann. *Klinik fuer Kinderheilkunde der medizinischen Fakultaet der RWTH Aachen, Pauwelstrasse, 52074 Aachen, Germany*

S. van Koningsbruggen and E. Rietschel. *Kinderklinik der Universitaet zu Koeln, Joseph-Stelzmann-Strasse 9, 50927 Cologne, Germany*

P. Schoberth. *Kinderkrankenhaus, Amsterdamer Strasse 59, 51058 Cologne, Germany*

G. Damm, R. Szczepanski, and T. Lob-Corzilius. *Kinderhospital Osnabrueck, Iburger Strasse 187, 49082 Osnabrueck, Germany*

L. Schmid and W. Dorsch. *Kinderarztpraxis, Aidenbachstrasse 118, 81379 Munich, Germany*

M. Skiba and M. Silbermann. *Kinderarztpraxis, Kreuznacher Strasse 9, 14197 Berlin, Germany*

ACKNOWLEDGMENTS

We thank all participating families for their help and all members of the clinical centers for their work. We thank Sophie Eichenbaum-Voline for the power estimations; M. Mueller, S. Mueller, G. Wachter, G. Kruse, M. Saemeier, H. Bergmann, M. Poesch, S. Albmeier, T. Mandl, B. Knoll, B. Huber, and M. Neher for participating in this study; and M. Emfinger for proofreading the manuscript. We also thank the Bundesministerium fuer Forschung, Bonn (Grant 07ALE087), and the GSF Forschungzentrum, Neuherberg (Grant FE77264). The MDC Mikrosatellitenzentrum is supported by the German Genome Project (BMBF). Parts of the analysis were obtained using the program package SAGE, which is supported by a U.S. Public Health Service Resource Grant (1P41RR03655) from the National Center for Research Resources.

REFERENCES

Albuquerque, R. V., Hayden, C. M., Palmer, L. J., Laing, I. A., Rye, P. J., Gibson, N. A., *et al.* (1998). Association of polymorphisms within the tumour necrosis factor (TNF) genes and childhood asthma. *Clin. Exp. Allergy* **28:** 578–84.

Anderson, D. M., Lyman, S. D., Baird, A., Wignall, J. M., Eisenman, J., Rauch C., *et al.* (1990). Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. *Cell* **63:** 235–243.

Anonymous (1987). Standardization of spirometry—1987 update. *Am. J. Respir. Crit. Care Med.* **153:** 1285–1298.

Asherson, G. L., Dieli, F., Gautam, Y., Siew, L. K., and Zembala, M. (1990). Major histocompatibility complex regulation of the class of the immune response: The H-2d haplotype determines poor interferon-gamma response to several antigens. *Eur. J. Immunol.* **20:** 1305–1310.

Barnes, C. B., and Marsh, D. G. (1998). The genetics and complexity of allergy and asthma. *Immunol. Today* **19:** 325–332.

Barnes, K. C., Needly, J. D., Duffy, D. L., Freidhoff, L. R., Breazeale, D. R., and Schou, C. *et al.* (1996). Linkage of asthma and total serum IgE concentration to markers on chromosome 12q: Evidence from Afro-Caribbean and Caucasian populations. *Genomics* **37:** 41–50.

Caraballo, L., Marrugo, J., Jimenez, S., Angelini, G., and Ferrara, G. B. (1991). Frequency of DPB1*0401 is significantly decreased in patients with allergic asthma in a Mulatto population. *Hum. Immunol.* **32:** 157–161.

Chai, H., Farr, R. S., Froehlich, L. A., Mathison, D. A., McLean, J. A., Rosenthal, R., Sheffer, A. L., *et al.* (1975). Standardization of bronchial inhalation challenge procedures. *J. Allergy Clin. Immunol.* **56:** 323–327.

Collaborative Study on the Genetics of Asthma (CSGA) (1997). A genome-wide search for asthma susceptibility loci in ethnically diverse populations. *Nat. Genet.* **15:** 389–392.

Daniels, S. E., Bhattacharrya, S., James, A., Leaves, N. I., Young, A., Hill, M. R., Faux, J. A., *et al.* (1996). A genome-wide search for quantitative trait loci underlying asthma. *Nature* **383:** 247–250.

Dib, C., Faure, S., Fizames, C., Samson, D., Drouot, N., Vignal, Millasseau, P., *et al.* (1996). A comprehensive genetic map of the human genome based on 5, 264 microsatellites. *Nature* **380:** 152–154.

Dold, S., Wjst, M., von Mutius, E., Reitmeir, P., and Stiepel, E. (1992). Genetic risk for asthma, allergic rhinitis and atopic dermatitis. *Arch. Dis. Childhood* **67:** 1018–1022.

Dold, S., Heinrich, J., Wichmann, H.-E., and Wjst, M. (1998). *Ascaris* specific IgE and allergic sensitization in a cohort of East German school children. *J. Allergy Clin. Immunol.* **102:** 414–420.

Dreborg, S. (1989). Pathophysiology of skin tests. *Allergy* **44**(Suppl. 10): 13–21.

Funk, C. D., Radmark, O., Fu, J. Y., Matsumoto, T., Jornvall, H., Shimizu, T., and Samuelsson, B. (1987). Molecular cloning and amino acid sequence of leukotriene A4 hydrolase. *Proc. Natl. Acad. Sci. USA* **84:** 6677–6681

Hayden, C., Albuquerque, R., Rye, P., Palmer, L., Burton, P., Goldblatt, J., and LeSouef, P. (1998). Polymorphisms in the TNF gene cluster and asthma. *Am. Rev. Resp. Crit. Care Med.* **3:** A856

Jardieu, P., Clark, R., Mortensen, D., and Dorshkind, K. (1994). In vivo administration of insulin-like growth factor-I stimulates primary B lymphopoiesis and enhances lymphocyte recovery after bone marrow transplantation. *J. Immunol.* **152:** 4320–4327.

Jarvis, D., and Burney, P. (1998). The epidemiology of allergic diseases. *Br. Med. J.* **316:** 607–609.

Kemeny, D. M., and Lee, T. H. (1993). An analysis with sequence-specific oligonucleotide probes of the association between aspirin-induced asthma and antigens of the HLA system. *J. Allergy Clin. Immunol.* **92:** 114–123.

Kharitonov, S. A., Yates, D., Robbins, R. A., Logan-Sinclair, R., Shinebourne, E. A., and Barnes, P. J. (1994). Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* **343:** 133–135.

Kostyu, D. D. (1991). The HLA gene complex and genetic susceptibility to disease. *Curr. Opin. Genet. Dev.* **1:** 40–47.

Kruglyak, L., and Lander, E. S. (1995). Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am. J. Hum. Genet.* **57:** 439–454.

Kruglyak, L., Daly, M. J., Reeve-Daly, M. P., and Lander, E. S. (1996). Parametric and nonparamentric linkage analysis: A unified multipoint approach. *Am. J. Hum. Genet.* **58:** 1347–1363.

Lander, E. S., and Kruglyak, L. (1995). Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. *Nat. Genet.* **11:** 241–247.

Li-Weber, M., Davydov, I. V., Krafft, H., and Krammer, P. H. (1994). The role of NF-Y and IRF-2 in the regulation of human IL-4 gene expression. *J. Immunol.* **153:** 4122–4133.

Litwin, S. D. (Ed.) (1978). "Genetic Determinants of Pulmonary Disease," Dekker, New York.

Mancini, J. A., and Evans, J. F. (1995). Cloning and characterization of the human leukotriene A4 hydrolase gene. *Eur. J. Biochem.* **231:** 65–71.

Marsh, D. G., and Meyers, D. A. (1992). A major gene for allergy—fact or fancy? *Nat. Genet.* **2:** 252–254.

Mathew, S., Murty, V. V., Hunziker, W., and Chaganti, R. S. (1992). Subregional mapping of 13 single-copy genes on the long arm of chromosome 12 by fluorescence *in situ* hybridization. *Genomics* **14:** 775–779.

Miller, S. A., Dykes, D. D., and Polesky, F. H. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16:** 1215.

Moffatt, M. F., and Cookson, W. O. C. M. (1997). Tumor necrosis factor haplotypes and asthma. *Hum. Mol. Genet.* **6:** 551–554.

Morton, N. (1998). Significance levels in complex inheritance. *Am. J. Hum. Genet.* **62:** 690–6797.

Murray, J. S., Pfeiffer, C., Madri, J., and Bottomly, K. (1992). Major histocompatibility complex (MHC) control of CD4 T cell subset activation. II. A single peptide induces either humoral or cell-mediated responses in mice of distinct MHC genotype. *Eur. J. Immunol.* **22:** 559–65.

Nickel, R., Wahn, U., Hizawa, N., Meastri, N., Duffy, D. L., Barnes, K. C., *et al.* (1997). Evidence for linkage of chromosome 12q15–q24.1 markers to high total serum IgE concentrations in children of the German Multicenter allergy study. *Genomics* **46:** 159–162.

Ober, C., Cox, N. J., Abney, M., Di Rienzo, A., Lander, E. S., Changyaleket, B., and Gidely, H., *et al.* (1998). Genomewide search for asthma susceptibility loci in a founder population. *Hum. Mol. Genet.* **7:** 1393–1398.

Sandford, A., Weir, T., and Paré, P. (1996). The genetics of asthma. *Am. J. Respir. Crit. Care Med.* **153:** 1749–1765.

Shimizu, H., Mitomo, K., Watanabe, T., Okamoto, S., and Yamamoto, K. (1990). Involvement of a NF-kappa B-like transcription factor in the activation of the interleukin-6 gene by inflammatory lymphokines. *Mol. Cell. Biol.* **10:** 561–568.

Siersted, H. C., Hansen, H. S., Hansen, N. C. G., Hylderbrandt, N., Mostgaard, G., and Oxho, H. (1994). Evaluation of peak expiratory flow variability in an adolescent population sample: The Odense Schoolchild Study. *Am. J. Respir. Crit. Care Med.* **149:** 598–603.

Sporik, R., Ingram, J. M., Price, W., Sussman, J. H., Honsinger, R. W., and Platts-Mills, T. A. E. (1995). Association of asthma with serum IgE and skin test reactivity to allergens among children living at high altitude. *Am. J. Respir. Crit. Care Med.* **151:** 1388–1392.

Walker, C., Bauer, W., Braun, R. K., Menz, G., Braun, P., Schwarz, F., Hansel, T. T., and Villiger, B. (1994). Activated T cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia. *Am. J. Respir. Crit. Care Med.* **150:** 1038–1048.

Wassmer, G., Heinrich, J., Wjst, M., Reitmeir, P., and Wichmann, H.-E. (1997). The association between baseline lung function and bronchial responsiveness to methacholine. *Eur. J. Med. Res.* **2:** 47–54.

Wjst, M., and Wichmann, H.-E. (1995). Collaborative study on the genetics of asthma in Germany. *Clin. Exp. Allergy* **25**(Suppl. 2): 23–25.

Wjst, M., Wassmer, G., and Seuchter, S., For the ECRHS group (1997). Genes for asthma? A pooled analysis of the European Community Respiratory Health Survey. *Am. Rev. Resp. Crit. Care Med.* **156:** 1773–1780.

Wjst, M., and Immervoll, T. (1998). An internet linkage and mutation database for the complex phenotype asthma. *Bioinformatics* **14:** 827–828.

Zhang, Y., Lefort, J., Hyne, V., and Vargafting, B. B., and Cookson, W. O. C. M. (1998). A genome wide search for quantitative trait loci using the bps2 mouse model of asthma. Paper presented at the Human Genome Meeting, Turin, 1998; Book of abstracts, p. 73.