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沖縄県石垣市乳幼児におけるアトピー性皮膚炎（Atopic dermatitis: AD）追跡調査に関する研究

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研究要旨

乳幼児におけるADの有病率および発症率について前向き追跡調査を行った。対象は沖縄県石垣市小児(1-6才)で、2001年631例、2002年836例、2003年844例、2004年764例、計3075例を視診によりAD診断を行った。AD率は2001年6.2%、2002年6.3%、2003年11.0%、2004年3.7%であった。追跡調査により、非AD児 795 例中 44 例（5.5%）がADを発症し、発症率は3.67 %/年であった。調査開始時AD児74例の71.6 %は治癒し、そのうち58.5 %は1年後、30.2%は2年後、11.3%は3年後に治癒した。乳幼児において、AD発症率は3.67%/年であったが、ADと診断を受けてもその大部分は幼児期に治癒した。

A. 研究目的

本邦においてアトピー性皮膚炎（Atopic dermatitis: AD）の罹患率は近年増加傾向にある。しかし、その有病率や発症率について、詳細な大規模な研究は未だ少ない。今回、私共は、沖縄県石垣市における乳幼児のAD有病率および発症率について前向き追跡調査を行った。

B. 研究方法

対象は、沖縄県石垣市保育園児（0-6才）で、2001年631 例（男児342例、女児289例）、2002年836例（男児446例、女児390例）、2003年844例（男児455例、女児389例）、2004年764例（男児412例、女児352例）、合計3075例（男児1655例、女児1420例）であった。対象全例において皮膚科医による視診を行い、ADを診断した。各調査年とも、毎年6月から9月の間に行われた。なお、同時に血清IgE値（IRMA法）を測定し、230 IU/mL以上を異常高値とした。本研究は、九州大学病院倫理委員会における審査を受け、承認されている。本研究に参加した対象児は全例、その保護者により、研究目的・方法について、文書による説明を受け、同意を得ている。

C. 研究結果

AD率は2001年631例中39例、6.2%、2002年836例中53例、6.3%、2003年844例中93例、11.0%、2004年764例中28例、3.7%であった。各調査年のAD率は、男女間及び年齢別において差は認めなかった。

2001年から2004年まで1年間、2年間、3年間と追跡調査可能であった小児は、各々、466例、297例、106例で、計869例であっ

た。調査開始時に、非ADと診断された795例中ADを発症したのは44例、5.5%であり、調査年を考慮し、年発症率を人年法で解析すると3.67%/年であった。また、調査開始時に、ADと診断された74例中53例、71.6%が治癒した。その内訳は、1年後31例、2年後16例、3年後6例においてADが治癒した。

追跡調査可能869例を、AD発症例、AD持続例、AD治癒例、健常例の4群に分け、その血清IgE値の経時的推移(平均値)について検討した。AD発症例で258→449→613→575 IU/mL、AD持続例で353→611→826→2375 IU/mLと経過とともに上昇した。一方、AD治癒例で187→281→302→375 IU/mL、健常例で126→168→138→280 IU/mLと、その上昇も軽度であった。このように血清IgE値は病勢をよく反映していた。

D. 考案

本研究結果から、乳幼児におけるADの発症率は年3.67%であり、そのAD児の大部分71.6%が3年以内に治癒した。血清IgE値はADの病勢をよく反映しており、臨床現場での参考になりうる。本研究は、
hospital-based ではなく
population-based 研究であり、かつ、前向き追跡調査であり、計3075例もの大規模臨床研究である点が特長である。しかし、調査対象地区は、年平均気温が24.5度Cの亜熱帯気候であることを考えると、本邦小児におけるADのすべて代表しているとはいえず、その他の地域との比較検討が必要である。さらに、本研究では、治療や家庭での生活習慣などの介入についてもさらなる検討が必要である。本研究対象例の一部は、同意のもと、遺伝子採取を行っており、今後その解析も併せて行いたい。

E. 結論

乳幼児において、AD発症率は3.67 %/年であったが、ADと診断を受けてもその大部分は幼児期に治癒した。

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G. 知的財産権の出願・登録状況

とくになし

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ORIGINAL ARTICLE

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Linkage and association of childhood asthma with the chromosome 12 genes

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Abstract Several studies have shown linkage of chromosome region 12q13–24 to bronchial asthma and related phenotypes in ethnically diverse populations. In the Japanese population, a genome-wide study failed to show strong evidence of linkage of this region. Chromosome 12 genes that showed association with the disease in at least one report include: the signal transducer and activator of transcription 6 gene (*STAT6*), the nitrogen oxide synthetase 1 gene (*NOS1*), the interferon γ gene (*IFNG*), and the activation-induced cytidine deaminase gene (*AICDA*). To evaluate the linkage between chromosome 12 and childhood asthma in the Japanese population, we performed sib-pair linkage analysis on childhood asthma families using 18 microsatellite markers on chromosome 12. To investigate association between chromosome 12 candidate genes and asthma, distributions of alleles and genotypes of repeat polymorphisms of *STAT6*, *NOS1*, and *IFNG* were compared between controls and patients. Single nucleotide polymorphism of *AICDA* was also investigated. Chromosome region 12q24.23–q24.33 showed suggestive linkage to asthma. The *NOS1* intron 2 GT repeat and *STAT6* exon 1 GT repeat were associated with asthma. Neither the *IFNG* intron 1 CA repeat nor 465C/T of *AICDA* showed any association with asthma. Our results suggest that *NOS1* and *STAT6* are asthma-susceptibility genes and that chromosome region 12q24.23–q24.33 contains other susceptibility gene(s).

Keywords Childhood asthma · Linkage · Association · Polymorphism · NOS1 · STAT6 · IFNG · AICDA

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Introduction

Bronchial asthma is an inflammatory disease of the airways characterized by airway obstruction and increased airway responsiveness. Asthma is an etiologically complex disease and develops by the interaction of multiple genes and environmental factors. Genome-wide

linkage studies have identified a number of autosomal regions providing evidence of linkage to asthma, atopy, eosinophilia, and/or other associated phenotypes (CSGA 1997; Daniels et al. 1996; Dizier et al. 2000; Haagerup et al. 2002; Hakonarson et al. 2002; Laitinen et al. 2001; Ober et al. 2000; Wjst et al. 1999; Xu et al. 2001a; Xu et al. 2000; Xu et al. 2001b; Yokouchi et al. 2000). Some of these studies (CSGA 1997; Dizier et al. 2000; Haagerup et al. 2002; Wjst et al. 1999; Xu et al. 2001a; Xu et al. 2000; Yokouchi et al. 2000) and those focused on a single chromosome (Barnes et al. 1999; Barnes et al. 1996; Kruglyak et al. 1996; Malerba et al. 2000; Nickel et al. 1997; Wilkinson et al. 1998; Raby et al. 2003) suggested linkage of chromosome 12q regions to asthma or related phenotypes in diverse populations.

In a genome-wide linkage analysis of mite-sensitive Japanese childhood asthma, the 110–145 cM region from the pter (the telomere of the short arm) showed maximum logarithm of odds score (MLS) more than 1.0 with the highest MLS of 1.92 at 111.9–125.3 cM (Yokouchi et al. 2000). The highest MLS did not reach the value of "significant" (MLS = 3.6) or "suggestive" (MLS = 2.2) linkage to the disease (Lander and Kruglyak 1995). The region in which MLS exceeded 1.0 was roughly overlapped by those of studies on Afro-Caribbean, French, and British populations (Barnes et al. 1996; Dizier et al. 2000; Wilkinson et al. 1998). To establish the linkage between asthma and chromosome 12 region, evidence of the suggestive linkage must be replicated using a different set of samples from the same population (Lander and Kruglyak 1995).

Candidate genes of chromosome 12q15–q24 include the signal transducer and activator of transcription 6 gene (*STAT6*), interferon-γ (*IFNG*), stem cell factor (*SFC*), leukotriene A4 hydrolase (*LTA4H*), insulin-like growth factor (*IGFI*), β-subunit of nuclear factor-Y (*NFYB*), B-cell translocation gene 1 (*BTG1*), and nitrogen oxide synthetase 1 (*NOS1*) (Barnes et al. 1996; Dizier et al. 2000; Wjst et al. 1999). Of these, *STAT6*, *NOS1*, and *IFNG* were investigated with case-control studies and showed positive association with asthma in at least one study. Gao et al. (2000b) demonstrated the association of the single nucleotide polymorphism (SNP) 2964G/A of *STAT6* with adult asthma in Japanese populations. However, this association was not replicated in later studies on German/Swedish (Duetsch et al. 2002) or Japanese populations (Tamura et al. 2001). Instead, a GT repeat polymorphism in exon 1 was associated with eosinophil count in the German/Swedish study and with allergic diseases in the Japanese study. A dinucleotide repeat marker in *NOS1* was also reported to be associated with the disease in the British population (Gao et al. 2000a). An association between a SNP in *NOS1* and eosinophil count was also shown in German/Swedish patients (Immervoll et al. 2001). Hyden et al. (1997) reported that no polymorphism in the *IFNG* was associated with atopic asthma, whereas an association between the GT repeat in intron 1 of *IFNG* and childhood asthma was suggested in the Japanese population (Nakao et al. 2001). Heinzmann et al. (2000a) screened polymorphisms in *SCF*, *STAT6*, *TR2* (thyroid receptor 2), and *LTA4H* and found two polymorphisms in *SCF* and one in *TR2* in the German population. They found no evidence of linkage or association of these genes with atopy.

All of the above-mentioned studies were based on case-control design. On the other hand, using the transmission disequilibrium test (TDT), Noguchi et al. (2001) reported that the activation-induced cytidine deaminase gene (*AICDA*) was associated with childhood asthma in the Japanese population. The *AICDA* gene is located in the short arm of chromosome 12, where linkage has never been suggested. They selected this gene as a candidate gene for asthma because deficiency of *AICDA* resulted in low IgE production, thereby the variations of the gene might be responsive to atopy. More recently, Isidoro-García et al. (2003) reported a case-control study on the same SNP of *AICDA* gene in the Spanish population. They failed to show the association of this SNP with the disease.

Among candidate genes of chromosome region 12q15–q24, *STAT6*, *IFNG*, and *NOS1* have been suggested to be associated with asthma in at least one study. Although the locus was not a suspected linkage to asthma, the *AICDA* gene showed a positive result in one study. As often seen in genetic analyses of a complex disease (Ioannidis et al. 2001), inconsistencies were noticed between the studies of chromosome 12 candidate genes for asthma. Studying other sets of samples in the same population is necessary to conclude whether a particular gene is truly associated with this complex disease.

In the present study, we investigated linkage of markers on chromosome 12 to childhood asthma in the Japanese population. We also investigated association of four candidate genes, *AIDCA*, *STAT6*, *NOS1*, and *IFNG*, with Japanese childhood asthma.

Materials and methods

Families and individuals

For linkage analysis, 18 families with affected sib pairs and one family with an affected sib trio were recruited. For the association study, 184 controls and 115 patients were genotyped. One hundred control subjects were selected in the Osaka area, Japan, as previously described (Heinzmann et al. 2000b; Mao et al. 1996), and 84 controls were selected from adult staff and student volunteers from Tohoku University School of Medicine in Sendai, Japan. Individuals with a history of treatment for asthma or eczema were excluded from controls. Forty-two patients were diagnosed at hospitals in the Sendai area, which included patients from 19 families for linkage analysis. For an association study, one patient per family was selected. Other patients were recruited as described (Heinzmann et al. 2000b; Mao et al. 1996). None of the samples were previously analyzed for chromosome 12 linkage markers, *NOS1*, *IFNG*, *STAT6*, or *AICDA*. Diagnosis of asthma of probands was made by pediatricians specializing in allergic diseases.

The criteria of asthma were two or more episodes of wheezing and shortness of breath and reversibility of the wheezing and dyspnea, either spontaneously or by bronchodilator treatment. The definition of "childhood" asthma was asthma with onset before age 15 years. Diagnosis of other family members was based on the modified ATS-DLD questionnaire (Ferris 1978). Total serum IgE was regarded as high when the level was 250 IU/ml or higher. The specific IgE against house dust mite [*Dermatophagoides pteronyssinus* (Dp)] was judged positive when the RAST score against Dp was 2 (0.70 U_A/ml) or higher. "Atopy" was defined as either having high total IgE and/or positive Dp-specific IgE. Eighteen families had an affected sibpair and one family had an affected sib trio. All patients with childhood asthma were atopic. All affected sibs and their parents were genotyped.

This study was approved by the ethics committee of Tohoku University School of Medicine.

Genotyping

DNA was extracted from peripheral blood leukocytes using the Genomic DNA purification kit (Promega, Madison, WI, USA). Chromosome 12 microsatellite markers of the Human GenePairs Primers version 9 (Invitrogen, Carlsland, CA, USA) were used for the linkage analysis. The 18 markers genotyped in this study are shown in Table 1. Information on marker order and position was obtained from LDB2000: Sequence-based Integrated Maps of the Human Genome (http://cedar.genetics.soton.ac.uk/public_html/LDB2000.html) (Wilkinson et al. 1998). The location of *AICDA* was not cited in this database and was estimated using the NCBI Human Map Viewer.

In chromosome 12 microsatellite marker analyses, PCR mixtures contained 10 mM Tris/HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 μM each of deoxynucleotide triphosphate (dNTPs), 0.25 U of rTaq DNA polymerase (TAKARA, Tokyo, Japan), 5 μM of each primer, and 10 ng of template DNA in a total volume of 10 μl. The cycle conditions were 94°C for 3 min, followed by 30 cycles of 94°C for 45 s, 57°C for 45 s, and 72°C for 1 min, with a final extension of 72°C for 10 min. The size of the PCR products was estimated using a 373XL DNA sequencer (Applied Biosystems, Foster City, CA, USA). GeneScan 500XL TAMRA labeled standard (Applied Biosystems) was used for estimation of fragment lengths.

Primers for the *NOS1* intron 2 GT repeat were as described previously (Gao et al. 2000a). One of the primers, 5'-ATAGAGCCTGTGCTGAGCCTTC, was 6-FAM labeled. The PCR mixture contained 10 mM Tris/HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 250 μM each of dNTPs, 0.5 U of rTaq DNA polymerase, 200 μM of each primer, and 10 ng of template DNA in a total volume of 15 μl. The cycle conditions were 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 40 s, with a final extension of 72°C for 10 min.

The primers for the *STAT6* exon 1 GT repeat were 5'-GGAGAAGCCGGAAACAGCGG and 5'-GTTCAAGGCTGGCCCTGCTAGC (6-FAM labeled). The PCR mixture was the same as for of *NOS1*. The cycle conditions were 94°C for 3 min, followed by 30 cycles of 94°C for 45 s, 57°C for 45 s, and 72°C for 1 min, with a final extension of 72°C for 10 min.

Primers for the *IFNG* intron 2 CA repeat were as previously described (Nakao et al. 2001). The PCR mixture was the same as for *NOS1*, except that 0.25 U of rTaq and 600 μM of each primer were used. The cycle conditions were 95°C for 5 min, followed by 25–34 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 1 min, with a final extension of 72°C for 5 min.

The 467C/T (His155His) polymorphism of *AICDA* (GeneBank AB040430) was the same polymorphism reported by Noguchi et al. (2001), where they designated this polymorphism as 7888C/T. This was genotyped using a modified TaqMan PCR method employing allele-specific amplification (Fujii et al. 2000). The common forward primer was 5'-GGCCCCGAGGAAATGAGAAAAT. The reverse primers were 5'-TCCCAGGCTTTGAAAGTTCTTTAG for the C allele and 5'-TCCCAGGCTTTGAAAGTTCTTTGA for the T allele. The TaqMan probe was 5'-FAM-AGAAGACAGTTCAGGTTCCAAATCGAGG- TAMRA –3'. The PCR mixture contained 7.5 μl of 2X TaqMan Universal PCR Master Mix (Applied Biosystems), 400 μM of each PCR primer, 0.12 μM of TaqMan probe, and 5 ng of template DNA in a final volume of 15 μl. The cycle conditions were 50°C for 2 min, 95°C for 3 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min.

Table 1 Map locations for chromosome 12 markers and genes

Locus	Kb from pter	Band	Male cM	Female cM	Averaged cM
ptr	0	p13.33	0.0	0.0	0.0
D12S372	3761	p13.33	7.5	2.5	5.0
AICDA	8468	p13.31	20.0	9.3	14.7
GATA49D12	8513	p13.31	20.1	9.4	14.7
D12S391	13246	p13.2	26.1	21.4	23.8
D12S373	18347	p12.3	32.8	35.5	34.1
D12S1042	28440	p11.23	36.9	56.2	46.5
cen	39000	q11	38.7	65.0	51.9
D12S1301	46378	q12	39.7	71.3	55.5
D12S398	56808	q13.13	44.4	87.0	65.7
STAT6	61349	q13.13	45.9	92.5	69.2
D12S1294	73218	q14.2	50.4	97.7	74.0
IFNG	73860	q14.2	51.7	100.3	76.0
D12S375	74485	q14.3	52.7	103.0	77.8
D12S1052	80438	q15	57.1	106.9	82.0
D12S1064	96885	q21.33	67.2	120.5	93.8
D12S1300	105918	q23.1	69.8	133.1	101.4
PAH	111421	q23.3	73.5	143.5	108.5
D12S2070	125845	q24.22	81.8	167.6	124.7
NOS1	127541	q24.22	82.8	174.6	128.7
D12S395	130349	q24.23	84.1	184.3	134.2
D12S392	138666	q24.32	91.9	200.3	146.1
D12S2078	140437	q24.33	98.1	201.8	149.9
D12S1045	143552	q24.33	110.7	212.9	161.8
qtr	146025	qtr	119.2	218.4	168.8

Statistical analysis

Allele frequencies of microsatellite markers were estimated from the parental chromosomes. All chromosome 12 markers were assessed by PEDCHECK (version 1.0) (O'Connell et al. 1998) for pedigree inconsistencies. There was no genotype inconsistency at any loci in all the families. Multipoint linkage analysis was conducted using GENEHUNTER 2 (Kruglyak et al. 1996). For score calculations of the sib trio, we used "all independent pairs of affected/phenotyped sibs" option of the GENEHUNTER 2 program where one sib trio yielded two pairs. In all allelic and genotypic distribution analyses, Fisher's exact *P* values were calculated using SPSS for Windows version 11.0 J (SPSS Japan, Japan). For multiallelic markers, the *P* value of association with the disease of each allele or genotype was multiplied by the number of the alleles or genotypes to compensate for multiple testing and expressed as *Pc*. The level of significance for the association studies was set at $P(Pc) = 0.05$.

Results

Linkage analysis of chromosome 12

Thirty-nine sibs with childhood asthma used for the linkage analysis consisted of 20 males and 19 females. Ages of the patients ranged from 1 to 14 years with the average age of 7.3 years. All affected sibs were positive