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Variants of Endothelin-1 and Its Receptors in Atopic Asthma

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Endothelin-1 (ET-1) is a 21 amino acid peptide released from several types of bronchial cells. It operates through two types of receptors, type A(ET-RA) and type B(ET-RB) and has various activities in the pathophysiology of atopic asthma. These genes are localised on different chromosomes where genome-wide searches have identified linkage for atopic asthma, thus supporting the candidacy of ET-1 and its receptors for atopic asthma. A genetic association study was performed with variants of these three genes in both British (n = 300) and Japanese populations (n = 200). No significant association was found between variants of EDN1 and EDNRB genes, and atopic asthma in either population. However, variants of EDNRA gene showed a marginal association with atopy [odds = 0.39(95% CI: 0.17–0.89), p = 0.022, Pc = 0.066], especially with antigen specific IgE levels [odds = 0.31 (95% CI: 0.20–0.77), p = 0.006, Pc = 0.018] in the British population. These findings suggest that *EDNRA* is a major candidate locus for atopy on chromosome 4.

Key Words: **atopy; endothelin; polymorphism; association.**

Human endothelin (ET) comprises a family of 21-amino acid peptides, ET-1, ET-2, and ET-3 to date [1]. These three genes are localised on different chromosomes, 6p24-23 (*EDN1*), 1p34 (*EDN2*) and 20q13 (*EDN3*), respectively [2]. Pharmacological studies suggest multiple ET receptor subtypes, and expression cloning has identified two types of receptors, type A (ET-RA) [3] and type B (ET-RB) [4]; the former is on chromosome 4q (*EDNRA*) [3], while the latter is on 13q22 (*EDNRB*) [4].

Of the three ETs, the focus of interest in asthma is ET-1, since ET-1 is released by several cell types in the bronchial airways; it has numerous activities in the pathophysiology of asthma, such as contraction and proliferation of bronchial muscle, and promotes mitogenesis, in co-operation with other mediators, in airways [1]. Each of these actions is mediated by two types of receptors, ET-RA and ET-RB [1]. Binding assays show that more than 80% of ET-1 receptors in the bronchial airways are ET-RB in both normal and asthmatic subjects [5]. Bronchial smooth muscle from asthmatic subjects is significantly less sensitive to the contractile effects of ET-RB receptor activation than that from normal subjects [6]. A small population of ET-RA sites has been identified and it plays a role in ET-1 induced prostanoid release and airway smooth muscle proliferation [5, 6].

The genomic structure of these genes is determined and several single nucleotide polymorphisms (SNPs) have been identified among these genes. Genome-wide searches for atopic asthma have identified linkages on 4q, 6p, 13q where the *EDNRA, EDN1* and *EDNRB* genes are located, respectively [7–9]. These findings support the candidacy of ET-1 and its receptors as atopic asthma.

The aim of this study was to test if variants of ET-1 and its receptors, ET-RA and ET-RB relate to atopic asthma. We conducted large scale genetic association study in both British (n = 300) and Japanese (n = 200) populations. No association was found with the vari-

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ants of EDN1 and EDNRB genes. However, we found significant genetic association between variants of EDNRA gene and increased IgE levels in the British population. Clinical disorder, asthma showed a marginally weaker association with this EDNRA variant. These findings are concordant with the original genome-wide search for atopic asthma in which chromosome 4 markers were linked to IgE levels as well as bronchial hyperresponsiveness, a physiological character of bronchial asthma [7]. Since mast cells produce ET-1 that operates through ET-RA and ET-RB receptors on their membranes, and play a key role in atopy characterised by high IgE responsiveness [10], we provided here a novel hypothesis of atopic asthma that variants of ET-RA on mast cells and airways might promote IgE synthesis and asthma by proliferation of bronchial muscles.

MATERIALS AND METHODS

Subjects select. In the British group [11], one hundred and fifty pregnant women were drawn as controls from the Obstetrics Clinic in Oxford. One hundred and fifty patients with atopic asthma were collected from the Chest Unit in Oxford; all were Caucasians. For the Japanese group, details on the asthmatic and control subjects have been described elsewhere [12].

All the asthmatic subjects had specialist physician diagnosed asthma with (I) recurrent breathlessness and chest tightness requiring on-going treatment, (II) physician documented wheeze, (III) documented labile airflow obstruction with variability in serial peak expiratory flow rates >30%. They showed positive skin prick test of >5 mm to one or more allergens against any antigens, or ASE titre >0.7KU/L (score 2). Marked asthma was designated as chronic rather than episodic asthma and needing multidrug therapy. Atopy was designated by IgE serology (see below). There were no heavy smokers (>20 cigarettes per day) in these subjects.

Serological analysis. ASE (Allergen specific IgE) against HDM (house dust mite) and GX (Grass mix) was detected by the CAP immunoassay system (Pharmacia, Uppsala, Sweden). The criteria for a positive titre of allergen specific IgE were as used previously [11, 12]. A high total IgE by CAP system was taken to be greater than published normal values for children and adults [11, 12]. Atopy, defined as IgE responsiveness, was diagnosed as the presence of a high concentration of total serum IgE, a positive specific IgE titre against one or more of aero-allergens (ASE), or a combination of these two features.

DNA assay. DNA samples were extracted using a commercial kit (IsoQuick, Microprobe Corporation, Garden Grove, USA). A PCR in a mixture including 1.5 mmol/L of magnesium chloride was performed in a Perkin Elmer Cetus thermal cycler using a preliminary denaturing at 95C for 10 min and then 45 cycles (95C for 30 sec, 60C for 30 sec) with ampliTaq Gold (Perkin Elmer Cetus). Primers were 5′CAATCAGGATCATTGCCATG and 5′GCTTTGGACTTCCCCAGG for EDN1, 5′CTGAAAGGGAGAAGTGAAAGTGG and 5′GTGCTAGATGAATCTTGTCTG for EDNRA, and 5′CTTACAACACAAAATCAGATC and 5′TTCCTGTAGGGTATTATT for EDNRB [13]. The underlined sequence was exchanged to incorporate the polymorphic site. PCR products were digested with BsiYI for EDN1, Afl II for EDNRA and with ApaLI for EDNRB [13], respectively. Genotypic polymorphism was defined as AA (homozygous absence of restriction sites), BB (homozygous presence of restriction sites) or AB (heterozygous).

Statistics. Contingency table analysis, odds ratio (OR), 95% confidence intervals, and significance values were estimated by computerised methods (SPSS program). We calculated adjusted p value (Pc) by numbers of phenotypes tested.

RESULTS

The genotype frequencies of EDN1, EDNRA and EDNRB were consistent with Hardy-Weinburg equilibrium in both populations; There was no significant difference in allele frequencies of EDN1 and EDNRA genes between British and Japanese controls: p(A) = 0.19, p(B) = 0.81 vs p(A) = 0.12, p(B) = 0.88 for EDN1, p(A) = 0.71, p(B) = 0.29 vs p(A) = 0.78, p(B) = 0.22 for EDNRA. However, there was a significant difference in genotype frequencies of EDNRB gene between the British and Japanese controls; p(A) = 0.39, p(B) = 0.61 vs p(A) = 0.56 p(B) = 0.44 for EDNRB [χ^2 = 15.01, df = 2, p = 0.00055].

There was no significant association between variants of EDN1 and EDNRB, and any phenotypes of atopy and asthma in either British or Japanese populations (Tables 1 and 2). Variant of EDN1 showed marginal association with atopic asthma [OR = 0.67 (95% CI: 0.42–1.05), p = 0.081]; however, further analysis did not suggest any significant association between asthma severity and this variants (data not shown). In contrast, variant of EDNRA significantly associated with ASE [OR = 0.31 (95% CI: 0.13–0.77), p = 0.006, Pc = 0.018], and weakly with atopy [OR = 0.39 (95% CI: 0.17–0.89), p = 0.022, Pc = 0.06] (Table 1). However, no significant association was found between asthma and this variant in either populations (Tables 1 and 2).

DISCUSSION

ET-1 is a highly potent bronchoconstrictor in airways with an approximately 100 times stronger potency than methacholine; asthmatics exhibit bronchial hyperreactivity to ET-1 compared with normal non-asthmatic subjects [14]. In addition, ET-1 can stimulate mucus secretion and edema formation in airways [14]. These findings support a role for ET-1 in the pathogenesis of asthma. However, the genetics of ET-1 in relation to airway inflammation in bronchial asthma has not been explored.

Human *EDN1* is a single locus spanning 12Kb on chromosome 6p24-23 and consists of 5 short exons [15]. At least two human preproendothelin-1 mRNAs are generated through the use of different promoters, and the mechanisms controlling the production of these alternative transcripts may be cell-specific [16]. In human lung, ET-1 is produced by vascular endothelial cells, bronchial epithelial cells, and pulmonary monocytes. A sequence analysis of exon 1 has identified a single A insertion/deletion polymorphism at position

TABLE 1

Association between Variants of EDN1, EDNRA, and EDNRB Genes and Atopy, Asthma, RAST, and Total Serum IgE in the British Population

		EDN1 genotype				EDNRA genotype				EDNRB genotype			
	No. of cases	AA + AB	BB	OR (95% CI)	p	AA + AB	BB	OR (95% CI)	p	AA + AB	BB	OR (95% CI)	p
Controls	150	59	91			134	16			95	55		
Atopic asthma	150	74	76	0.47 (0.19–1.14)	p = 0.081	142	8	0.47 (0.19–1.14)	p = 0.089	86	54	1.29 (0.81–2.04)	p = 0.288
Non-atopic	99	41	58			86	13			62	37		
Atopic	201	92	109	0.83 (0.51–1.36)	p = 0.475	190	11	0.39 (0.17–0.89)	p = 0.022	119	82	1.15 (0.70–1.89)	p = 0.569
Non-asthmatics	119	50	69			106	13			72	47		
Asthmatics	181	83	98	0.86 (0.53–1.36)	p = 0.512	170	11	0.52 (0.23–1.22)	p = 0.30	109	72	1.01 (0.63–1.63)	p = 0.947
Low total IgE	167	69	98			150	17			102	65		
High total IgE	133	64	69	0.76 (0.48–1.20)	p = 0.239	126	7	0.49 (0.20–1.22)	p = 0.120	79	54	1.07 (0.67–1.71)	p = 0.862
ASE negative	110	45	65			95	15			70	40		
ASE positive	190	88	102	0.80 (0.50–1.29)	p = 0.363	181	9	0.31 (0.13–0.77)	p = 0.006	111	79	1.24 (0.76–2.02)	p = 0.373

Note. OR, AA + AB vs BB genotypes.

138 in essential hypertension patients [17]. This polymorphism creates a Bsi YI restriction site. Our genetic association study in British (n = 300) and Japanese population (n = 200), shows no association between this variant and any type of atopy and asthma in the two populations (Tables 1 & 2). Since a genome-wide search has identified linkage to atopic asthma on 6p21-23 [7, 8], further studies are needed to identify new intragenic variants and test for association with atopic asthma among different ethnic groups.

ET-1 operates through two receptors, ET-RA and ET-RB [1]. Both receptors have seven transmembranous domains, a common structure of the G protein-coupled receptor family [1]. Both human cDNA's have been cloned from a placental cDNA library [18, 19]. Human ET-RA and ET-RB consist of 427 and 442 amino acids, respectively, and the overall identity between two protein is approximately 55% while the genomic structure of the genes is similar [4]. ET-RA is mainly expressed in aorta, while ET-RB in brain tissues [3, 4]. In lung tissues, both receptors are expressed, ET-RA mainly on smooth muscle cells and ET-RB in endothelium [1]. The exact roles of the two receptors in the bronchus in relation to the development of bronchial asthma remains controversial [1].

Increasing evidence implicates that *EDNRB* as the candidate locus for Hirschsprung's Disease [13]. A missense mutation has been identified in relation to Cau-

TABLE 2

Assocation between Variants of EDN1, EDNRA, and ENDRB Genes and Atopy, Asthma, RAST, and Total Serum IgE in the Japanese Population

		EDN1 genotype				EDNRA genotype				EDNRB genotype			
	No. of cases	AA + AB	BB	OR (95% CI)	p	AA + AB	BB	OR (95% CI)	p	AA + AB	BB	OR (95% CI)	p
Controls	100	23	77			91	9			79	21		
Atopic asthma	100	27	73	0.81 (0.43–1.53)	p = 0.513	93	7	0.76 (0.27–2.13)	p = 0.602	78	22	1.06 (0.54–2.08)	p = 0.863
Non-atopic	70	16	54			64	6			55	15		
Atopic	130	34	96	0.84 (0.42–1.65)	p = 0.608	120	10	0.89 (0.31–256)	p = 0.826	102	28	1.01 (0.50–2.04)	p = 0.986
Low total IgE	91	24	67			85	6			70	21		
High total IgE	109	26	83	1.14 (0.60–2.17)	p = 0.682	100	9	1.27 (0.43–3.37)	p = 0.722	89	20	0.74 (0.37–1.44)	p = 0.363
ASE negative	66	17	49			60	6			51	15		
ASE positive	134	33	101	1.06 (0.54–2.09)	p = 0.862	124	10	0.83 (0.29–2.38)	p = 0.723	105	29	0.92 (0.45–1.88)	p = 0.828

Note. OR, AA + AB vs BB genotypes.

casian cases [13]. Also, three of the SNPs have been identified in EDNRB [13], and we confirmed these SNPs by direct sequencing (data not shown). Since two of these SNPs were relatively rare in both populations, we conducted our genetic association study using the variant in intron 6. Asthma and atopy did not show any association with this variant in either British or Japanese populations. Whilst two independent genome-wide searches for atopic asthma have identified a candidate locus on chromosome 13q [7, 8] on which *EDNRB* is localised [4], *EDNRB,* therefore may not be the major locus for asthma on 13q.

Since ET-RA is the dominant type of receptor on vascular smooth muscle cells, the molecular genetic interest in this receptor has been documented on hypertension [17]. A silent SNP has been identified in codon 323His in exon 6 of the *EDNRA* among essential hypertension patients [17]. We show that this variant is weakly associated with atopy [OR = 0.39 (95% CI: 0.17–0.89), p = 0.022, Pc = 0.066], especially with allergen specific IgE levels [OR = 0.31 (95% CI: 0.13–0.77), p = 0.006, Pc = 0.018] in the British population. However, no association was found between this variant and specific IgE levels or asthma in the Japanese population; the power of study seems adequate since 100 or 150 subjects of each were available for Za = 1.96 and Zb = 0.10 to detect the 10–15% difference in allelic frequencies documented here. Since atopy has previously been linked to chromosome 4 [7], our findings support the candidacy of *EDNRA* as an atopy locus on this chromosome. The pathophysiological role of ET-RA in atopy remains unknown, but dominant expression of ET-RA on murine bone-marrow-derived mast cells is intriguing [10]. Such mast cells, co-cultured with IL-3 and IL-4, show increased release of mediators after stimulation with exogenous ET-1 and this was inhibited by an ET-RA specific antagonist in a dose dependent manner [10]. Thus IL-4 and ET-1, through the ET-RA receptor on mast cells, may co-operate in the development of atopic inflammatory responses.

In conclusion, we report that a silent SNP in *EDNRA* associated with atopy in a British population. Further genetic and functional studies are needed to clarify the role of variants of ET-RA in the development of atopy in asthmatics.

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