Oxford Genome Screen for Asthma-Associated Traits

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A genome screen for linkage of quantitative traits underlying asthma has been carried out previously by our group on 80 families sub-selected for discordant phenotypes from a general population sample. The families contained a total of 203 offspring forming 172 sib-pairs. Genotypic data for at a total of 296 markers were available. This paper describes the ascertainment, phenotypic data, and genotypic data made available for Genetic Analysis Workshop 12.

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INTRODUCTION

Asthma is the most common chronic childhood disease in the developed nations. It is due to a combination of genetic and environmental influences. It is associated with intermediate quantitative phenotypes, such as the total serum IgE concentration. The population prevalence of asthma is of the order of 10% in many English-speaking countries [ISAAC, 1998], and the sibling recurrence risk is consequently less than two. For this reason, the study of quantitative traits in families discordant for the traits may represent the most powerful means to detect linkage.

Busselton is a rural coastal town in Southwestern Australia that has been serially studied for the prevalence of asthma since 1996. A sample of 230 families from the Busselton population has been the basis for segregation analyses of the contribution of quantitative traits to asthma [Dziri et al., 1995; Palmer et al., 2000]. A subset of 80 families from this sample was the basis for a genome screen carried out by our group [Daniels et al., 1996], and the data used in this screen were released to the Genetic Analysis Workshop (GAW) 12 workshop.

The selection of families from the full population sample and their subsequent sub-selection are described below, together with the methods used for their phenotyping and genotyping.

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STUDY SUBJECTS

The subjects were all from Busseston. The aim was to recruit young nuclear families. Children under five were excluded because they could not complete respiratory testing. Families were identified through adults aged 55 years or older, from an electoral roll of approximately 9,000. Families were serially recruited until a pre-determined target of 1,000 individuals was reached. The final sample consisted of 1,004 subjects in 230 nuclear families.

Of the 1,867 individuals approached, 177 refused a part in the study, 140 could not be contacted, and 434, although contacted, had not been tested by the end of the study. Another 708 subjects were excluded because their spouses were older than 55 years, or because they were not married with two or more natural children over the age of 5. The remaining 408 individuals and their spouses and children not on the electoral roll completed the study. Subjects knew the respiratory interest of the investigation before agreeing to participate. It was emphasized that normal people were important to the study.

Clinical protocol. Testing took place in May, June, and July 1992. A respiratory questionnaire, based on the American Thoracic Society (ATS) questionnaire but including questions on rhinitis and allergies, was administered. Skin prick testing to Dermatophagoides pteronyssinus and Phleum pratense (Timothy grass) was determined (Pharmacia FEIA CAP system, Lund, Sweden). A specific IgE RAST class 1 (≥ 0.35 KU/L) was considered positive. Eosinophils and white cells were counted automatically (Western Diagnostic Laboratories, Western Australia).

A skin test index (STI) was calculated as the sum of the prick skin test results to HDN and grass mix (95% of individuals in this population who were atopic reacted either to HDN, or to grass pollen or both). A RAST index was calculated as the sum of RAST scores for the same two allergens.

“Asthma” was defined as a positive answer to the questions “Have you ever had an attack of asthma?” and “If yes, has this happened on more than one occasion?”

Selection of families for genome screen. The primary genome screen was carried out on 364 subjects in 80 nuclear families sub-selected from the population sample of 230 families. Families in the genome screen included both atopic and non-atopic members, and all those of three or greater were not exclusively atopic or non-atopic.

The 80 families contained a total of 203 offspring forming 172 sib-pairs. The mean age of the children was 12.6 years (± SE 1.3), their geometric mean IgE was 55.7 IU/L ± 1.1, and their mean STI was 4.0 mm ± 0.41. Twelve percent of the children were asthmatic and 25% admitted to wheezing; 52% were atopic, 36% were non-atopic, and 12%
had an intermediate phenotype as defined; the latter were classified as unknown. The sib pairs subdivided into 44 atopic affected pairs, 87 in which one sibling was affected, and 41 in which both were unaffected or of intermediate phenotype.

MARKERS
Genomic DNA was isolated from peripheral blood leukocytes by phenol/chloroform extraction. Twenty-two dinucleotide repeat markers were typed by radioactive techniques [Weissenbach et al., 1992]. For 274 markers, 50 ng of each DNA sample was genotyped by fluorescence-based semi-automatic methods [Reed et al., 1994]. Allele sizes were determined using the GENOTyper software [Reed et al., 1994]. Data was converted into numbered alleles using the GAS (version 2.0) program [http:// linkage.rockefeller.edu/soft/list.html]. Allele size differences within families rather than exact alleles were determined. Non-Mendelian marker data were flagged by GAS, and by UNKOWN. Potentially incorrect genotypes were re-examined and if necessary re-tested. Two-point lod scores between markers and marker order were compared with published maps [Weissenbach et al., 1992; Reed et al., 1994].

A list of markers and the results of our analyses of linkage [Daniels et al., 1996] are available on www.well.ox.ac.uk/asthma. The families were initially screened with 269 markers (253 autosomal and 16 X-linked). A genome-wide map was constructed from these data, and the order and distances between the markers were compatible with previously published values [Weissenbach et al., 1992; Reed et al., 1994]. Based on these families, the markers had an average heterozygosity of 75% and spanned approximately 3,100 cM.

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REFERENCES