Association of CCR5 Δ 32 with reduced risk of childhood but not adult asthma

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CCR5 is a specific chemokine receptor expressed on a variety of cells including T helper 1 (but not T helper 2) lymphocytes.¹ A 32 base pair deletion (CCR5 Δ 32) results in a receptor that is not expressed on the cell surface. From observations of low HIV-1 prevalence in carriers of this variant² it has been postulated that the CCR5 Δ 32 polymorphism may have a wider disease modifying role related to inhibition of pathogen entry into cells.^{3–5} Further support for the role of the natural receptor in inflammation comes from the observation that significantly increased levels of the CCR5 ligand RANTES (which recruits monocytes, T cells, and eosinophils) are found in bronchial lavage samples from patients with asthma.^{6,7} Neutralisation of RANTES in mice following allergen challenge has been shown to reduce airway hyperresponsiveness,⁸ and mice lacking CCR5 exhibit diminished airway hyperresponsiveness to methacholine together with reduced levels of inflammatory cells and mediators. These observations lend further support to a role for the receptor in modulating airway inflammatory responses and associated bronchial responsiveness.

We have previously reported that children aged 5–15 years who carried the CCR5 Δ 32 polymorphism were protected against the development of asthma and that no asthma was recorded in children homozygous for the mutation.⁹ This observation suggested that the allele might determine the amount of functioning receptor and associated responses to infective agents through a "dose dependent" effect. However, Mitchell *et al* were unable to replicate the association in an older population of children, adolescents, and young adults and argued that an abnormally truncated and non-functioning receptor might be expected to direct immune responses towards a Th2 profile,

Background: A number of potential candidate genes have been implicated in the pathogenesis of asthma. A 32 base pair deletion in the CCR5 gene renders this chemokine receptor non-functioning and has been shown to be associated with a reduced prevalence of asthma in childhood. The mechanism may be related to impairment of pathogen entry into cells and modified host inflammatory response. We sought to determine the influence of the CCR5 Δ 32 mutation on asthma and allergy in the transition from childhood to adulthood.

Methods: 627 individuals first studied as part of a whole population schoolchildren cohort in 1989 when aged 8–12 years were followed up 10 years later for respiratory and allergy symptoms and laboratory markers of atopy. CCR5 Δ 32 status was also characterised and the association with childhood and adulthood symptoms determined.

Results: The follow up sample was representative of the original cohort except for a slightly greater prevalence of symptomatic individuals. As children, none who were homozygous for the CCR5 Δ 32 mutation had a current physician's diagnosis of asthma. In multivariate analysis and controlling for known confounders, the protective effect of carrying the allele in childhood was highly significant (OR 0.31, 95% CI 0.14 to 0.72, $p=0.006$). There was no protective association with "current asthma" as classified in adulthood within the same population. Subjective or laboratory markers of atopy in childhood or adulthood were not associated with the CCR5 Δ 32 mutation. Methacholine bronchial hyperresponsiveness in adulthood was also unrelated to gene carrier status.

Conclusions: In a population with a high allelic frequency for the CCR5 Δ 32 mutation, a significant protection against childhood asthma is evident which is independent of atopy. This protection is lost in the transition between childhood and early adulthood. The contribution of different genetic candidates to the expression of asthma may change with advancing maturity and confound the interpretation of association and linkage studies unless age is taken into account.

thus increasing the likelihood of atopic disease.¹⁰ They also suggested that differences in genetic background and environment could explain this failure of replication as there appears to be a north to south European gradient for prevalence of the CCR5 Δ 32 polymorphism, ranging from 16% in Scandinavia to 4% in Sardinia (mean European frequency 9%).¹¹ Iyer *et al* have also demonstrated segregation of the polymorphism according to race with frequencies of 3.57% noted in white Americans, 1.55% in the African American population, and a complete absence of the mutant allele in Asian Americans and Native Americans.¹²

From the few longitudinal studies that have followed the natural history of asthma and wheezing illness it is apparent that many children, particularly those with mild symptoms and predominantly viral associated episodes, become asymptomatic in the transition to adult life.^{13,14} Whereas atopy and non-specific bronchial hyperresponsiveness (BHR) may be risk factors for the persistence of asthma into adulthood¹⁵ and viral associated wheeze of childhood has a favourable long term outcome,^{13,14} we are unaware of any reports that have examined the association of candidate genes with asthma and wheezing illness during the transition from childhood to adult life. We therefore sought to confirm or refute our previous observations on the association of CCR5 Δ 32 with childhood asthma in our population and to test the hypothesis that the contribution of this particular candidate diminishes in the transition from childhood to early adulthood.

METHODS

If CCR5 Δ 32 is associated with the expression of childhood asthma but not with adult asthma, this could be investigated

using a number of different designs including whole population, case control or cohort studies, all spanning the age distribution from childhood to adulthood. However, in a population over an extended age range and studied at a single time point it would be difficult to account for the “epoch effect” due to the increase in incidence of asthma and atopy that has occurred over the past three decades.^{16–18} We therefore sought to address this issue by studying the association of CCR5Δ32 polymorphism in a sample drawn from a whole population followed from childhood into early adulthood.

Study population

After obtaining approval from the Grampian research ethics committee, letters were sent to all 2082 young adults who could be traced and who had taken part in a 1989 survey ($n=3406$) of Aberdeen primary schools when aged 8–12 years.¹⁶ Of those traced, attempts to contact them by telephone and letter were successful in 1407 cases in whom screening data were obtained by telephone questionnaire. Of this traced population, 711 attended the study centre during the year 1999–2000 when aged 18–22 years. From this subset of the original 1989 population, follow up data were obtained by an interviewer administered questionnaire based on the European Community Respiratory Health Survey (ECRHS)¹⁹ and the American Thoracic Society (ATS) questionnaires.²⁰ In order to maintain consistency with data obtained from the original cohort, additional questions as used in 1989 were also included. The cohort was studied as if it were two independent whole population samples of children and adults in order to assess any association of CCR5Δ32 with asthma in the two age classifications (8–12 and 18–22 years). “Current asthma in childhood” was defined from the 1989 database as a physician’s diagnosis of asthma ever plus current wheeze (wheeze within the last 3 years of the 1989 study). “Current asthma in adulthood” was defined as a physician’s diagnosis of asthma ever and current wheeze (wheeze within the last 12 months of the 1999/2000 study) – see discussion for explanation of why two different definitions of current wheeze were used and how these relate to each other. In the current study “childhood only wheeze” was defined as current wheeze reported in the 1989 survey but no longer present in the 1999/2000 study, “adult onset wheeze” was defined as current wheeze in 1999/2000 but not in 1989, and “persistent wheeze” was defined as being present on both occasions. A fourth group consisted of those who had “never wheezed” with no reported symptoms on either study occasion.

In the original 1989 study “current” atopy status was based on questionnaire responses for the presence of hayfever and/or eczema within the last 12 months.¹⁶ In order to maintain consistency, “current atopy” in 1999/2000 was defined as a history

of eczema and/or hayfever within the last 12 months of the 1999/2000 study.

Laboratory measures

In the 1999/2000 study asthma and atopy associated phenotypes were further characterised. The Cockcroft protocol was used to determine BHR to methacholine (Sigma Aldrich, Dorset, UK) and results documented as the concentration of methacholine required to reduce the forced expiratory volume in 1 second (FEV₁) by 20% (PC₂₀),²¹ with a positive result considered to be a concentration of ≤ 8 mg/ml. Results were expressed as both categorical and continuous variables for the purposes of analysis. Atopy was assessed by measurement of RAST, total serum IgE (Pharmacia and Upjohn Diagnostics, Milton Keynes, UK), and skin prick testing to a panel of 14 common inhaled allergens (ALK, Reading, UK). Positive values were taken as ≥ 1 RAST, ≥ 120 IU, and ≥ 3 mm, respectively, and a grouping variable (any one measure of atopy positive) was derived. DNA was extracted from whole blood and CCR5 genotype—WT/WT (wild type), WT/32 (heterozygous) or 32/32 (homozygous)—and determined by PCR (primers from MWG Biotech Limited, UK).⁹

Data analysis

Data analysis was carried out using SPSS version 9.0 (SPSS Inc, Chicago, IL, USA); t tests, χ^2 tests, and multivariate logistic regression models were used to assess the relationships between recorded variables.

RESULTS

Demographic data

There is no population stratification in this cohort which was in Hardy Weinberg equilibrium.

Table 1 shows the demographic breakdown at each stage of population selection. The 2082 individuals traced were representative of the original 1989 whole population sample in terms of sex, social class,²² and symptoms with no significant differences in any of these variables. The sample of individuals who consented for full assessment and who attended in 1999–2000 ($n=711$) comprised a greater proportion of symptomatic individuals as defined in the original 1989 cohort. Significant differences were noted for “ever asthma” ($p=0.04$), “ever eczema” ($p=0.008$), and “wheeze within the last 3 years” ($p=0.015$). Significantly fewer of this group were exposed to smoke during childhood ($p=0.0001$). Results from non-responders/repeated non-attenders/those declining to proceed are not shown but can be summarised as follows: there were no significant demographic differences between those declining to proceed ($n=480$) and either the 1989 population or those 927 who agreed to take part at first

Table 1 Population characteristics (based on 1989 data)

Variables categorised in 1989	1989 ($n=3406$)	Traced in 1999 ($n=2082$)	Replied in 1999 ($n=1407$)	Agreed to proceed ($n=927$)	Attended ($n=711$)	Complete phenotype and genotype data ($n=627$)
Ever asthma	10.1	11.0	11.2	11.4	13.1	12.3
Ever breathless	10.0	10.0	9.9	10.6	12.2	12.0
Ever persistent night cough	13.9	13.4	13.6	14.3	15.2	14.5
Ever eczema	12.0	13.0	13.5	14.5	15.9	16.1
Ever hayfever	11.9	12.6	13.3	12.8	13.8	14.0
Occupation*						
1–3	68.6	72.8	76.0	75.2	77.5	78.7
4–9	31.4	27.2	24.0	24.8	23.5	21.3
Male	51.7	53.3	52.1	51.7	51.6	51.2
Female	48.3	46.7	47.9	48.3	48.4	48.8
Wheeze in last 3 years	19.8	20.1	21.2	21.6	24.1	23.9
Smokers in house (1999)	57.9	55.7	54.5	53.9	50.4	49.5

Values are percentages.

*Occupation of head of household (OPCS classification²²).

Table 2 Genotype and asthma status (n=627)

Status	WT/WT	WT/32	32/32	Total
Current asthma in childhood	70 (90.9%)	7 (9.1%)	0 (0%)	77
Current asthma in adulthood	95 (81.2%)	20 (17.1%)	2 (1.7%)	117
Asymptomatic in childhood	431 (78.4%)	112 (20.4%)	7 (1.3%)	550
Asymptomatic in adulthood	406 (79.6%)	99 (19.4%)	5 (1.0%)	510
Whole population	501 (79.9%)	119 (19.0%)	7 (1.1%)	627

Table 3 Proportions of genotype by wheezing subgroups

Wheezing subgroups	WT/WT	WT/32 or 32/32	Total
Never (controls)	259 (75.3%)	82 (23.8%)	344
Childhood only	53 (93.0%)	4 (7.0%)	57
Adult onset	114 (84.4%)	21 (15.6%)	135
Persistent	75 (82.4%)	16 (17.6%)	91
Total	501	126	627

contact. Those with fewer symptoms were more likely to fail to attend at initial or rescheduled appointments (n=216) and the differences were significant for “ever asthma” (p=0.001), “ever breathless” (p=0.001), “ever eczema” (p=0.013), and “wheeze within the last 3 years” (p=0.0001) compared with the 711 individuals who attended. The likelihood of being exposed to tobacco smoke during childhood was also greater in this group (p=0.001).

Mean age at follow up was 20 years (range 18–22). Males constituted 51.2% (n=321) and females 48.8% (n=306); 52.8% (n=331) had “ever wheezed”, 27.0% (n=169) had “ever asthma” according to the combined (and thus cumulative) responses from the 1989 survey and the 1999–2000 follow up questionnaire. The overall carrier frequency of CCR5Δ32 in the whole population was 19.0% (table 2).

CCR5Δ32

Cross tabulation of frequencies (table 2) revealed that none of the CCR5Δ32 homozygous population had a current diagnosis of asthma in childhood and that the rates for heterozygotes were intermediate compared with the rates in those without the polymorphism, with a significant trend on χ^2 analysis (χ^2 for trend 6.94, p=0.009). With “current asthma in childhood” as the dependent variable in a logistic regression equation and correcting for current atopy (as reported in 1989) and sex, the protective effect of carrying the CCR5Δ32 polymorphism was highly significant (OR 0.31, 95% CI 0.14 to 0.72, p=0.006).

However, in the same population characterised as having “current asthma” 10 years later there was no significant association with the CCR5Δ32 variant on χ^2 analysis (χ^2 for trend 0.023, p=0.879) or in the same logistic regression (OR 0.88, 95% CI 0.52 to 1.48, p=0.627). Current reported atopy in childhood and adulthood showed no association with CCR5Δ32 either in univariate or multivariate analyses. Objective measures of atopy (skin prick test, RAST, and IgE) and BHR (both measured in adulthood) also showed no association with CCR5Δ32.

The frequency of the CCR5Δ32 mutation showed a significant trend across the four wheezing subgroups (table 3), with the lowest frequency in those with symptoms only in childhood and intermediate levels (compared with controls) in those with persistent symptoms or onset in adulthood (χ^2 for trend 6.47, p=0.011). When a history of smoking ever, atopy ever, CCR5Δ32 carrier status, and sex were entered into a logistic regression equation with “adult onset wheeze”, “childhood only wheeze”, and “persistent wheeze” as the dependant variables, smoking uptake appeared to be the predominant factor related to adult onset wheeze (OR 3.10, 95% CI 2.03 to 4.74, p=0.0001) and history of atopy to the persistence of wheeze (OR 3.28, 95% CI 1.93 to 5.56, p=0.0001; table 4). As in the univariate analysis, the presence of the CCR5Δ32 polymorphism was associated with a protective effect against childhood only wheeze (OR 0.23, 95% CI 0.08 to 0.66, p=0.006).

DISCUSSION

Although 2082 individuals were traced, 675 addresses were unconfirmed and thus actual contact was established in 1407 cases with 627 individuals from this group completing full assessment. Depending on which denominator population is used, the response rate could be 30.2% or 44.6%. The process of attrition is evident at all stages of the study, although we have tried to explain and describe characteristics of those lost where possible.

The 2082 individuals traced and those 1407 contacted were representative of the original 1989 whole population sample in terms of sex, social class, and symptoms (table 1). The sample of individuals (n=711) who attended for more detailed

Table 4 Significant risk factors by wheezing subgroup

Wheezing subgroup	CCR5Δ32 allele	Atopy ever	Smoking ever	Female sex
Adult onset	–	OR 1.99 95% CI 1.3 to 3.05 p=0.002	OR 3.10 95% CI 2.03 to 4.74 p=0.0001	–
Childhood only	OR 0.23 95% CI 0.08 to 0.66 p=0.006	OR 1.79 95% CI 0.99 to 3.24 p=0.053	–	OR 0.52 95% CI 0.28 to 0.94 p=0.03
Persistent	–	OR 3.28 95% CI 1.93 to 5.56 p=0.0001	OR 2.18 95% CI 1.34 to 3.54 p=0.001	–

Dependent variable = wheezing subgroup. Odds ratios (OR) are adjusted for other variables shown. All subgroups referenced to controls (“never wheezers”).

assessment had the same sex distribution as the 1989 cohort but comprised a greater proportion of symptomatic individuals whose families smoked less during childhood. There was also a trend (although not significant) towards a greater response from those in higher social classes. In 1989 the questionnaires were distributed through the schools and were therefore the responsibility of parents and teachers, whereas those attending in 1999/2000 were doing so voluntarily and, as might be anticipated, comprised a slight excess of symptomatic individuals who were only just beginning to take responsibility for their own health. The trends observed suggested that those with fewer symptoms were more likely to refuse to take part and fail to attend or reschedule appointments.

A further question arose regarding the different definitions of wheeze used in the two studies. In 1989 "wheeze within the last 3 years" constituted current wheeze. The standard definition has since changed to "wheeze within the last 12 months". In order to try and address this problem, we carried out a direct comparison of responses to the questions "wheeze within 3 years" and "within 12 months" in a different population of 908 schoolchildren of the same age as in the original 1989 survey. In this study the Aberdeen²³ and ISAAC²⁴ questionnaires were directly compared by randomising children to receive one questionnaire first, followed by the other questionnaire a month later. Despite slight differences in the phraseology of the two questionnaires, and provided they were answered by the same parent on both occasions, there was a prevalence for wheeze in the past 3 years of 20.4% which was between the 14.1% prevalence of wheeze in the past 1 year and the 23.2% prevalence of ever wheeze. From the screening telephone questionnaire used at first contact in our study, in 1285 cases where these data were recorded we also found a difference of approximately 5% between "wheeze in the last 3 years" (35.1%) and "within the last 12 months" (29.9%). Unfortunately this represents one of the many problems faced by studies carried out years apart—namely, the change in epidemiological tools. An adherence to original recording techniques would provoke questions regarding outdated methods, whereas any attempt to update them raises problems with comparison. No amount of foresight can predict these changes over time.

In the present cohort we found a similar carrier frequency for the CCR5Δ32 mutation (19.0%) to the 22.7% reported by Hall *et al*⁹ who examined a different sample drawn from the same north of Scotland population. This carrier rate is among the highest found in a white population but is consistent with the reported north/south European gradient.¹¹

The results of our study confirm the protective effect of the CCR5Δ32 polymorphism on the expression of childhood asthma in the north of Scotland population. Although some of the numbers of individuals in the subgroups—based on the transition from child to adult—were small, our data would be consistent with different subtypes of childhood and adult asthma each with their different gene/environmental interactions. Active smoking was a clear risk factor for adult onset symptoms and atopy a clear risk factor for persistence, whereas the protective effect of carrying the CCR5Δ32 polymorphism appeared to be confined to childhood. Moreover, the effect may only be significant in predominantly prepubertal children in whom the influences of atopic disease and adverse environmental exposures such as active smoking have not as yet made a significant contribution to persistence.²⁵ Our suggestion that environmental factors other than modification of host response by CCR5Δ32 effectively dilute this particular genetic contribution with advancing maturity would appear to be supported, at least in a population with a high expression of this particular polymorphism. Our observations also confirm the strength of both external (smoking uptake) and host factors (history of atopy) in influencing adult onset and persistence of childhood symptoms.

From studies examining the protective effects of the CCR5Δ32 polymorphism in the acquisition of HIV, it appears that the mechanism of action is related to the inhibition of pathogen entry into cells and hence the disruption of the host response.^{3–5} With the infection or "hygiene" hypothesis in mind,²⁶ it has been suggested that any modification of the host response to common respiratory viruses should reduce the associated Th1 driven responses, thus increasing the likelihood of atopic diseases including asthma.¹⁰ Although the infection hypothesis implies that Th1 and Th2 diseases should be mutually exclusive, we have recently reported a positive association between Th1 and Th2 modulated diseases in a large community sample.²⁷ Furthermore, identification of individuals with wheezing illness from a community child population such as that studied here may have a different pathogenesis from populations recruited from hospital clinics and may have symptoms that are more related to respiratory viral infections and less associated with atopy.²⁸ There is already strong evidence from long term follow up studies that virus associated wheeze—or what used to be labelled "wheezy bronchitis"—is more likely to resolve in the transition to adulthood.^{13–14} We therefore suggest that any protective effect of the CCR5Δ32 polymorphism on wheezing illness in mid childhood would be consistent with what is known about the different wheezing syndromes in childhood and, in particular, the significant contribution of respiratory viral infections to childhood wheezing illness.

However plausible this particular candidate polymorphism is, it needs to be remembered that the area on the long arm of chromosome 3 also contains a number of other potential candidate genes that could be in linkage disequilibrium with CCR5Δ32 and explain the association. Three other CCR genes (CCR1, CCR2, CCR3) are also clustered on human chromosome 3p21 where CCR5 is located, within about 400 kb.²⁹ The results from this study do not therefore exclude the association between other CCR genes and asthma. Further work on identifying other potential candidates for the expression of childhood asthma within this region seems justified. In support of this observation, a case-control study in Hungarian children showed that the CCR5Δ32 mutation does not confer a reduced risk to allergic inflammation in non-asthmatic children.³⁰ Our failure to find any association with the expression of the atopic diseases (eczema and or rhinitis) either in childhood or in early adulthood further substantiates the irrelevance of CCR5Δ32 to atopic disease. Finally, the hygiene hypothesis is largely focused on the Th1/Th2 switch whereas CCR5 is also expressed on dendritic cells, microglia and tissue macrophages, so a role for the dysfunctional mutation in protecting against asthma could also be argued for these cells.

It may be erroneous to assume that asthma across a wide range of ages, especially in the transition from childhood to adulthood, is caused by the same pathogenic process and this may explain the failure of replication in many reported association studies. The contribution of different candidate genes to different asthma phenotypes may therefore change with advancing maturity, and some such as CCR5Δ32 may have little to do with atopy but may affect other processes involved in intermittent airway obstruction. We suggest that the CCR5Δ32 mutation, or another gene in the same area of chromosome 3, reduces the risk of asthma like symptoms in childhood but loses its effect in adulthood and has an influence that is independent of atopy. This changing genetic contribution may confound the interpretation of association and linkage studies.

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