Assessing theoretical risk and benefit suggested by genetic association studies of CCR5: experience in a drug development programme for maraviroc

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The proliferation of published gene association studies of the CCR5Δ32 mutation is of relevance to drug development of a CCR5 antagonist for HIV, in highlighting potential safety concerns. We conducted an initial review of all non-HIV gene association studies of CCR5-Δ32, followed by detailed meta-analyses in the three disease areas most commonly reported. Our review indicated no consistent evidence of increased risk of susceptibility to hepatitis C virus infection or multiple sclerosis among individuals with CCR5-Δ32 mutation, and suggested treatment with a CCR5 inhibitor is unlikely to have related adverse effects. There was, however, evidence to suggest rheumatoid arthritis as a potential therapeutic target for a CCR5 antagonist. Clinical evidence would be required to confirm these findings.

Introduction

Advances in genomic technologies and the completion of the human genome sequencing effort have provided an opportunity to extend the application of genetics to drug development. The final draft of the human genome sequence has revealed approximately 3,000–5,000 potentially chemically tractable targets [1]. Candidate gene association studies are being carried out in many different disease areas to identify the specific genetic polymorphisms associated with disease (both causative and protective). The data from such studies can be used, in conjunction with other information from animal models, cell biology and pharmacology, to identify and support the 'confidence in rationale' (CIR) for pursuing new drug discovery approaches. The polymorphisms that provide the CIR for the development of a novel therapy may also impact how patients respond to that therapy, and pharmacogenomic analysis is now often included in relevant drug development programmes in order to assess the impact of genetic variation on drug response [2]. Genetic polymorphism may also impact the safety of new medicines, and the genetic association data that provides CIR for one clinical development programme may also raise questions regarding the confidence in safety in another. The pharmaceutical industry increasingly has to consider genetic association data when assessing potential benefit and risk. This article aims to illustrate this by the experience of a CCR5 antagonist (UK-427, 857, maraviroc) programme currently in development for the treatment of HIV and AIDS [3].

The identification of CCR5 as a potential therapeutic target for HIV infection was supported by a genetic study of individuals, who, despite multiple high-risk exposures, remained uninfected with HIV. A common mutation in the CCR5 gene (CCR5-Δ32) results in a non-functional protein, and individuals who were homozygous for CCR5-Δ32 were shown to be resistant to HIV infection [4,5]. Subsequent candidate gene studies have also shown that the CCR5-Δ32 mutation is associated with slower progression to AIDS [6]. The observation that there is no overt phenotype associated with being homozygous for the CCR5-Δ32 polymorphism has provided confidence in safety for the development of a CCR5 antagonist. Thus, within 7 years of the publication of genetic evidence that CCR5 would be a valid target in HIV therapy, clinical validation of this drug target was achieved with both Pfizer Inc and Schering Plough publishing data showing significant viral load drops in patients with HIV infection treated with the potent CCR5 antagonists maraviroc (UK-427,857) and Schering C, respectively [3,7]. Currently, maraviroc and Schering Plough's Vicriviroc are the only CCR5 antagonists in late stage development.

Subsequent to the original association studies between CCR5 genotype, and resistance to HIV infection and progression to AIDS, there have been numerous publications of genetic association between CCR5 and different diseases and phenotypes. Positive associations reported between CCR5-Δ32, and

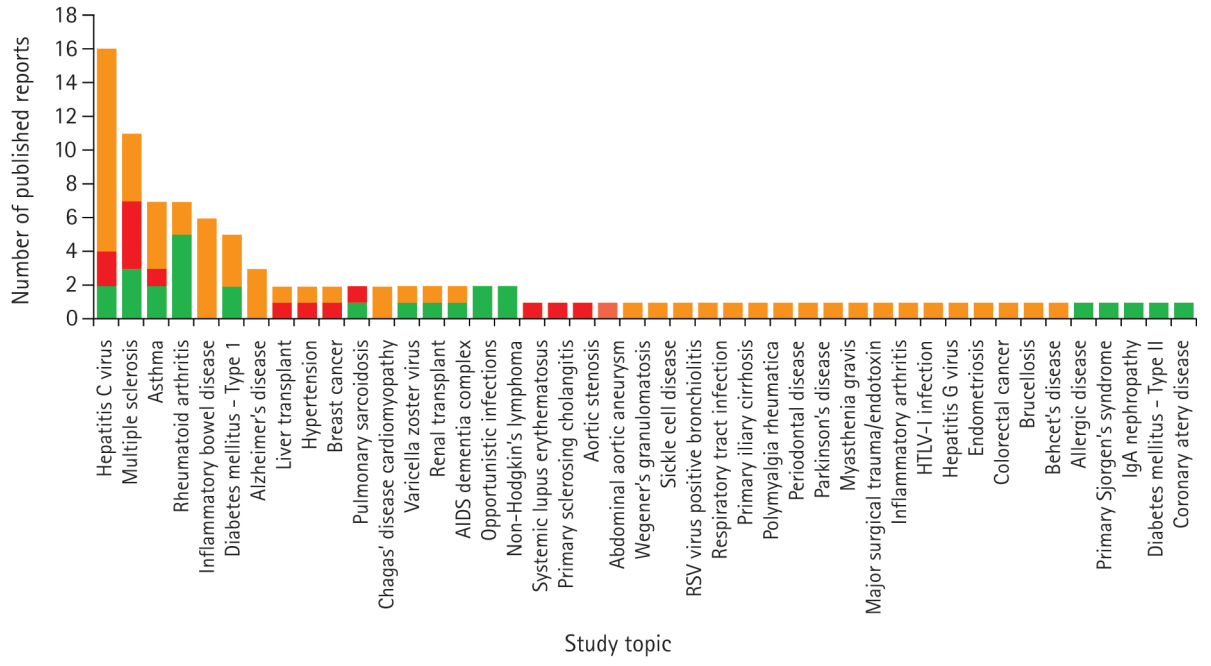
diseases and phenotypes other than HIV may have implications for the maraviroc programme by highlighting additional benefits that patients may receive on therapy, or by suggesting potential safety signals that should be considered. For example, a positive association between CCR5Δ32 and a reduced risk of AIDS dementia complex [8] may indicate an added benefit because, pharmacological blockade of the CCR5 receptor may theoretically protect against development of dementia associated with HIV infection. Other examples have been reported in conditions unrelated to HIV, such as a recent meta-analysis concluding a protective effect of CCR5-Δ32 against development of rheumatoid arthritis [9] that may indicate another therapeutic indication for a CCR5 antagonist. Association between CCR5Δ32 and more severe disease in HCV infection [10], however, may raise concerns around the safety of CCR5 antagonism in patients who are coinfected with HIV and HCV, which may need to be considered in designing a clinical trial. The objective of this study was to review the known associations between the CCR5Δ32 mutation and diseases other than susceptibility to HIV/AIDS, in order to highlight any potential safety issues or any beneficial effects that may be encountered upon antagonizing the CCR5 receptor. We conducted meta-analyses to provide a detailed quantitative assessment of the evidence in selected disease areas, focusing on disease susceptibility and treatment response, and reviewed relevant evidence concerning disease onset, progression and severity.

Methods

Initial literature review

Epidemiological genetic association studies published before June 2005 on the CCR5-Δ32 mutation and disease outcomes excluding HIV, were sought by computer-based searches, and by scanning the reference lists of all relevant studies and review articles. The initial computer searches of Medline and Embase databases used keywords relating to CCR5 (for example, CCR5 and chemokine receptor) and genes (for example, gene, genotype, polymorphism and SNP, see additional file for a full list of search terms). Literature searches used all the records since the databases began (1966 for Medline and 1988 for Embase) and were limited to English language publications. All population-based studies were selected. Studies were classified by whether individuals with CCR5-Δ32 mutations had evidence of an increased or reduced disease risk, according to the primary findings presented in the abstract. Classification was made by two authors independently (VJ, MP) and discrepancies adjudicated by a third author (JW). Study abstracts that described both increased and reduced risk, or non-significant results were classified as 'ambiguous/no effect' (Figure 1).

Figure 1. Preliminary review of gene association studies of CCR5-Δ32 in diseases other than HIV/AIDS



Coloured bars indicate number of published reports according to conclusions of the effect of CCR5 Δ32 mutation on disease: green, potential disease protection of CCR5-Δ32; red, potential disease risk of CCR5-Δ32; and orange, ambiguous conclusions/no effect of CCR5-Δ32. HTLV, human T-lymphotropic virus; RSV, respiratory syncytial virus.

Detailed review

We based our more detailed review on the three most prevalent disease associations reported in the initial review, including two conditions showing potential safety risk, hepatitis C virus (HCV) and multiple sclerosis (MS), and one condition that might be a potential therapeutic target, rheumatoid arthritis (RA). The choice of hepatitis C for detailed review is also highly relevant to an antiretroviral drug development programme in light of the high rates of hepatitis C coinfection among HIV patients. Subsequent searches reviewed manuscripts up to October 2006, and used the same search criteria of the initial review in combination with disease terms for HCV (for example, hepatitis, viral, human/hepatitis C), RA (for example, rheumatoid arthritis) and MS (for example, multiple sclerosis). To investigate disease susceptibility, all population-based case-control studies were included in the current review, with the exclusion of studies with insufficient data to derive an effect estimate, or study designs considered by us to convey a systematic bias (including, for HCV: one study using a diseased control group [11], one study in a Japanese population without CCR5-Δ32 mutations [12], and one study using a general population survey as control group [13]; and for MS: one study of post-mortem cases [14]). To investigate treatment response, population-based cohort and case-control studies were included if relevant data were reported.

Data abstraction

The following data were abstracted from published reports: study design, geographical location, ethnic group of participants, definition and numbers of cases and controls, frequency of genotypes, mean age and sex of cases and controls, details of anti-HCV treatment, and clinical severity of disease. Confirmation of genotype frequencies was sought by correspondence with investigators where numerical details were not available in the original publication [15–17], and the study was excluded if details could not be obtained (two of three replied). Relevant clinical outcomes included the following: chronic HCV without other causes of liver disease, excluding acute disease patients (although it was not possible to disaggregate data in one study with 14/257 cases of acute HCV [18]); RA defined according to the American College of Rheumatology (ACR) criteria or, previously, the American Rheumatology Association (AmRA) criteria; and clinically confirmed MS classified as relapsing/remitting, primary progressive or secondary progressive disease. Where data were reported according to subgroups of disease severity or subtype, the subgroups were combined for analysis. In one study where HCV data were reported separately according coinfection with HIV infection [10], the HIV/non-HIV subgroups were combined to avoid bias as recommended by commentators [19]. Studies of HCV treatment that provided separate analyses by treatment type were analysed as separate studies.

Statistical methods

Analyses of disease susceptibility assumed an additive genetic model of the effect of each mutated CCR5-Δ32 allele on disease risk, consistent with previously reported findings of virtual protection against HIV among CCR5-Δ32 homozygotes and a protective role against disease progression in heterozygotes [20]. The per-allele odds ratio (OR) of the CCR5-Δ32 mutation was compared between cases and controls by assigning scores of 0, 1 and 2 to homozygotes for the common allele, heterozygotes and homozygotes for the rare allele, respectively, and calculating ORs per unit score by logistic regression, with associated 95% confidence intervals (95% CIs). In subsidiary analysis, ORs were calculated under alternative assumptions of dominant or recessive genetic models, that is, assuming any effect on disease risk was conveyed in heterozygotes and Δ32 homozygotes equally, or in Δ32 homozygotes only. Analysis of treatment response was based on standard OR and 95% CIs. In the absence of strong between-study heterogeneity, fixed effect summary measures were calculated as the inverse-variance weighted average of the log OR. Heterogeneity was assessed by the I^2 statistic [21], interpreted as the percentage of total variation across studies that is due to heterogeneity rather than chance. Publication bias was assessed by visual inspection of funnel plots and Eggar's test for regression asymmetry [22]. Statistical analyses were conducted using Stata 8.0 (StataCorp, College Station, TX, USA) and SPlus 6.2 (Insightful, Reinach, Switzerland). In the Figures 2–5, areas of squares are proportional to the inverse variance, horizontal lines indicate 95% CIs, open diamonds denote weighted summaries in subgroups and shaded diamonds denote weighted summaries in total, with the diamond width indicating 95% CIs of the summary.

Results

Initial literature review

A total of 102 relevant gene association studies were published by June 2005. Of these, 27 claimed a potential protective effect on disease of the CCR5-Δ32 mutation, 15 claimed an adverse effect of CCR5-Δ32 on susceptibility to disease, and 60 reported ambiguous effects and/or no association (Figure 1).

HCV

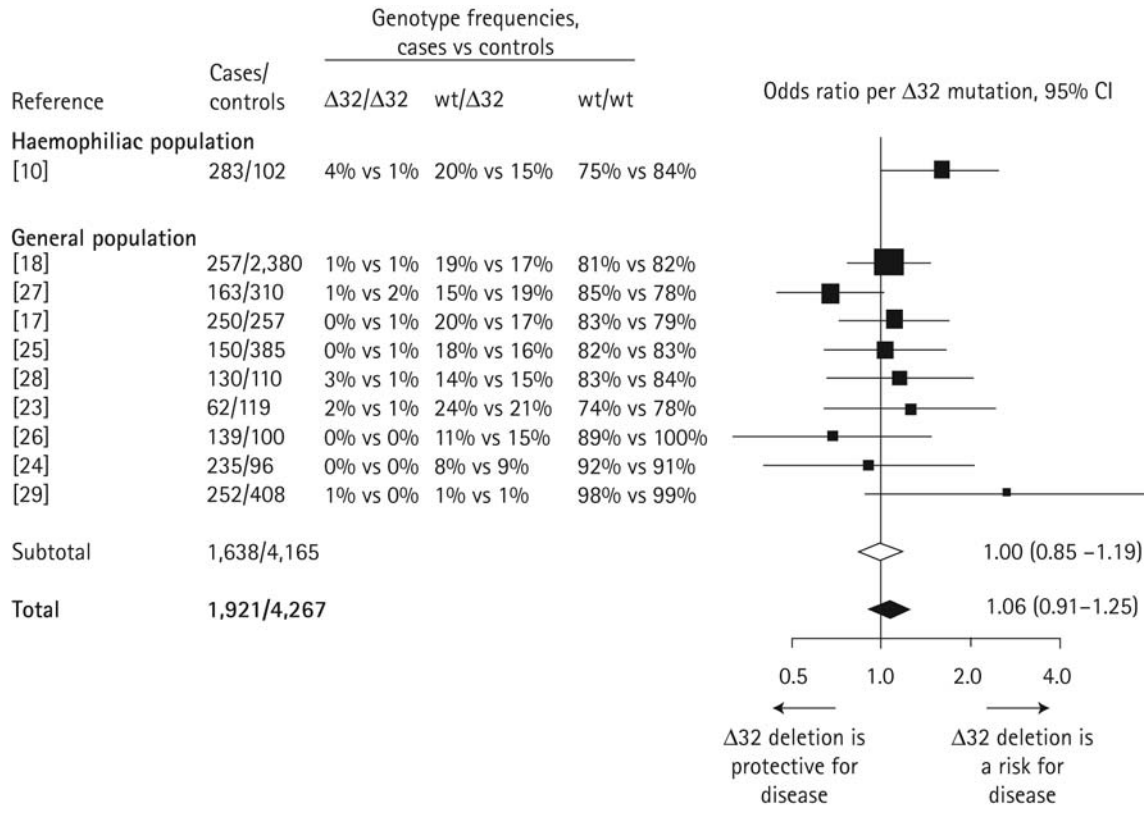
A total of 14 relevant studies of HCV were identified involving 3,044 cases of HCV. This included 10 studies

Table 1. Population studies of CCR5-Δ32 and HCV

Reference	Year	Setting	Ethnicity	Study design	Case population	Genotyped cases, *n*	Case Δ32 allele frequency	Case mean age, years*	Case % male	Control population	Genotyped controls, *n*	Control Δ32 allele frequency, *n*	Control mean age, years
[10]	2002	Germany	Caucasian	Case control	Chronic HCV/HIV transfusion clinic outpatients, 87% haemophiliac	283	16.0%	39	93%	HCV/HIV negative blood donors, randomly selected	102	8.3%	27
[31]	2002	USA	Caucasian	Cohort	Chronic HCV hepatology clinic referrals. HIV/HBV negative	250	16.8%	46 (20–73)	57%	NA	NA	NA	NA
[23]	2003	Germany	Caucasian	Case control	Chronic HCV	62	13.7%	39 ±12	55%	HIV-negative, healthy, unrelated	119	11.3% (19–72)	42 ±11
[25]	2003	Slovenia	Caucasian	Case control	Chronic HCV, 5% haemophiliacs	150	9.0%	NS	NS	Healthy volunteers from prevalence study, HCV/HIV negative	385	8.2%	NS
[24]	2003	Italy	Caucasian	Case control	Chronic HCV non-haemophiliac/intravenous transmission	235	4.7%	NS	NS	Healthy	96	4.7%	NS
[18]	2003	USA	76% Caucasian, 24% Black, Asian and other	Case control	Chronic HCV liver disease patients and HCV-positive blood donors	257	10.1%	45 (21–76)	59%	Healthy unrelated white blood donors	2,380	9.6%	45 (22–71)
[16]	2003	Europe	92% Caucasian, 8% Afro-Carribean and Asian	Cohort	Chronic HCV hepatology clinic attendees with no other liver disease	547	11.0%	NS	56%	NA	NA	NA	NA
[30]	2003	Germany	Caucasian	Cohort	Chronic HCV outpatients	156	13.5%	48 (20–73)	65%	NA	NA	NA	NA
[17]	2004	Germany	Caucasian	Case control	Chronic HCV outpatients	250	9.9%	42 (17–81)	57%	Random age-sex matched blood donors, HCV/HIV negative	257	9.6%	NS
[26]	2004	Spain	Caucasian	Case control	Chronic HCV hepatology clinic attenders, non-haemophiliacs	139	5.4%	NS	NS	Uninfected, race/age/gender/ region matched	100	7.5%	NS
[11]	2004	Germany	Caucasian	Case control	Chronic HCV non haemophiliac	333	8.9%	45	59%	Chronic disease in-patients	NA	NA	NA
[27]	2005	Belgium	Caucasian	Case control	Chronic HCV drug users and HCV positive patients	163	8.0%	NS	NS	Student volunteers	310	11.9%	NS
[28]	2006	Italy	Caucasian	Case control	Chronic HCV, HIV/HBV negative, excluding other causes liver damage	130	10.0%	46 ±15	80%	Geographically matched blood donors	110	8.6%	53 ±9
[29]	2006	India	South Asian	Case control	Chronic HCV, HIV/HBV negative, excluding other causes liver damage	252	1.4%	40 ±14	65%	Ethnically matched healthy volunteers	408	0.4%	33 ±7

*Median (range) or mean (±SD). HBV, hepatitis B virus; HCV, hepatitis C virus; NA, not applicable; NS, not stated.

Figure 2. Meta-analysis of studies of CCR5-Δ32 genotype and HCV



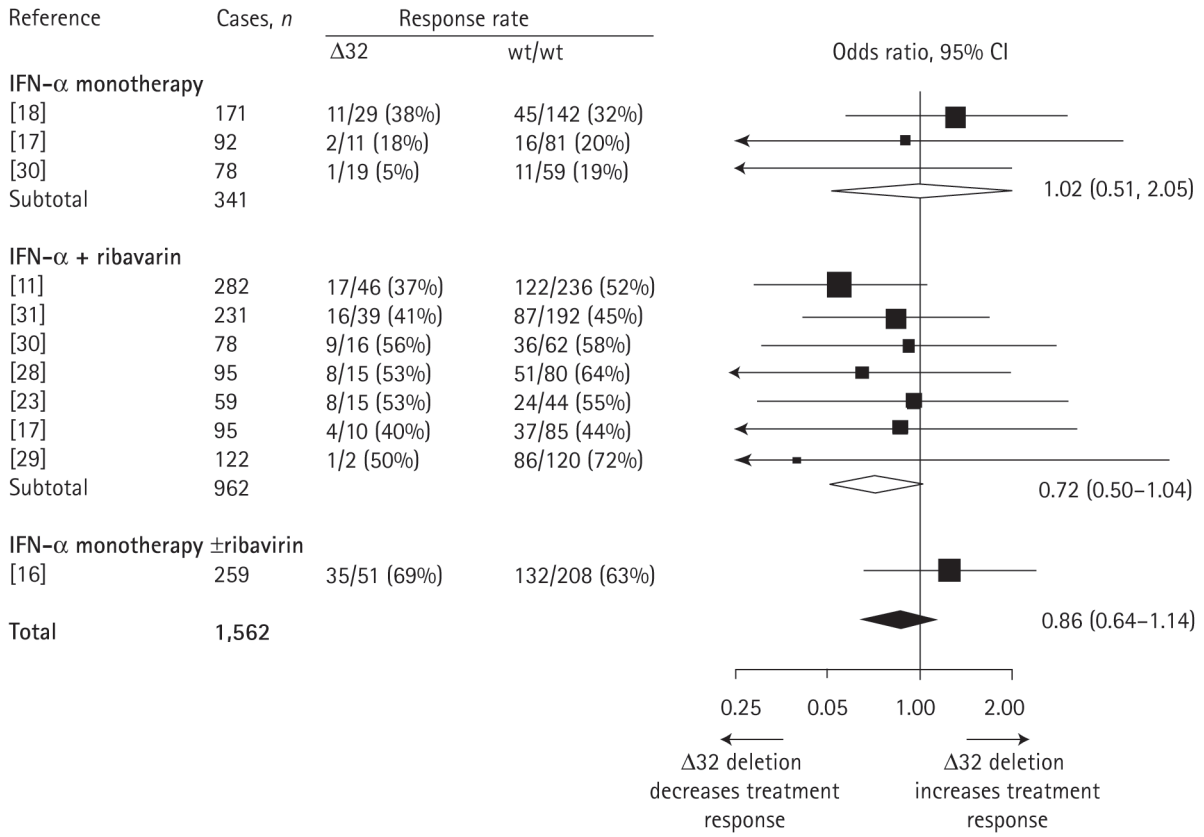
Areas of squares are proportional to the inverse variance, horizontal lines indicate 95% confidence intervals (95% CIs), open diamonds denote weighted summaries in subgroups, and shaded diamonds denote weighted summaries in total, with the diamond width indicating 95% CI of the summary. Total number of controls takes into account multiple studies reporting on a common control set where applicable. HCV, hepatitis C virus; wt, wild type.

of disease susceptibility involving 1,921 cases and 4,267 controls [10,17,18,23–29] and nine studies of treatment response involving 1,562 cases [11,16–18, 23,28–31]. A subset of studies investigated extent of disease, including portal inflammation and liver fibrosis. The weighted mean age of cases was 41.8 years, and 70% of cases were male (Table 1). The studies were conducted in Europe, North America and India, and overall 88% of cases were Caucasian. Cases were drawn from hepatology clinics, hospital outpatient departments and blood donor registries. Most studies were conducted among non-haemophiliac populations with the exception of one study of predominantly haemophiliacs [10]. Controls were blood donors, healthy individuals from other studies, or individuals of unknown source and were confirmed HCV negative in six studies. The CCR5-Δ32 allele frequency among controls was 8.6% overall, and ranged from 0.4% to 11.3% in individual studies.

In studies of susceptibility to HCV infection, the summary per-allele odds ratio of CCR5-Δ32 variant for HCV was 1.06 (95% CI: 0.91–1.25; Figure 2). The only study to demonstrate a per-allele odds ratio significantly different to the null was a study conducted among a haemophiliac population. Among the general population studies, the overall odds ratio was 1.00 (95% CI: 0.85–1.19). Although there was evidence of moderate between-study heterogeneity (I^2=27%), this was largely due to the study being conducted among a haemophiliac population, whereas the general population studies alone reflected minimal heterogeneity beyond that expected by chance (I^2=6%). There was no evidence of publication bias among the studies included. Summary estimates under alternative genetic model assumptions were: OR=1.06 (95% CI: 0.89–1.26) and I^2=0% under a dominant model; and OR=0.89 (95% CI: 0.36–2.18) and I^2=7% under a recessive model.

HCV treatment response was defined as being HCV RNA negative for 6 months after initial therapy (with or without normal liver enzymes) in all studies of this phenotype. The overall odds ratio of CCR5-Δ32 variant and response to anti-HCV treatment was 0.86 (95% CI: 0.64–1.14; Figure 3). Subgroup estimates

Figure 3. Meta-analysis of studies of CCR5-Δ32 genotype and HCV treatment response



Areas of squares are proportional to the inverse variance, horizontal lines indicate 95% confidence intervals (95% CIs), open diamonds denote weighted summaries in subgroups, and shaded diamonds denote weighted summaries in total, with the diamond width indicating 95% CI of the summary. Total number of controls takes into account multiple studies reporting on a common control set where applicable. HCV, hepatitis C virus; IFN, interferon; wt, wild type.

were 1.02 (95% CI: 0.51–2.05) for treatment with IFN-α monotherapy, 0.72 (95% CI: 0.50–1.04) for IFN-α monotherapy plus ribavirin and 1.26 (95% CI: 0.65–2.43) for a single study in which precise treatment records were not available. There was little evidence of heterogeneity either within subgroups (I^2<8%) or overall (I^2=0%), and the subgroup summary estimates were statistically compatible (χ^2_2=2.41, *P*=0.30). There was no evidence of publication bias. Studies of disease severity were reported with insufficient consistency for quantitative summary. For example, among the six studies that investigated the association between CCR5-Δ32 and liver fibrosis [11,16–18,26,30], four different scoring indices were used with varying categorization of severity.

MS

A total of seven relevant studies of MS disease susceptibility were identified [32–38] involving 1,133 cases of MS and 1,131 controls. The weighted mean age of cases was 39.3 years and, overall, 41% were male (Table 2). These studies were conducted among Caucasian populations in Europe, Asia and Australasia. Cases were drawn from generally clinically confirmed MS by the Poser criteria recruited from registries, outpatient departments, a population study or undeclared sources, and included various disease subtypes or relapsing/remitting disease only. Controls were either blood donors, healthy individuals of unknown source, hospital/laboratory staff or patient's partners. CCR5-Δ32 allele frequency among controls was 13.3% overall and ranged from 8% to 26% in individual studies.

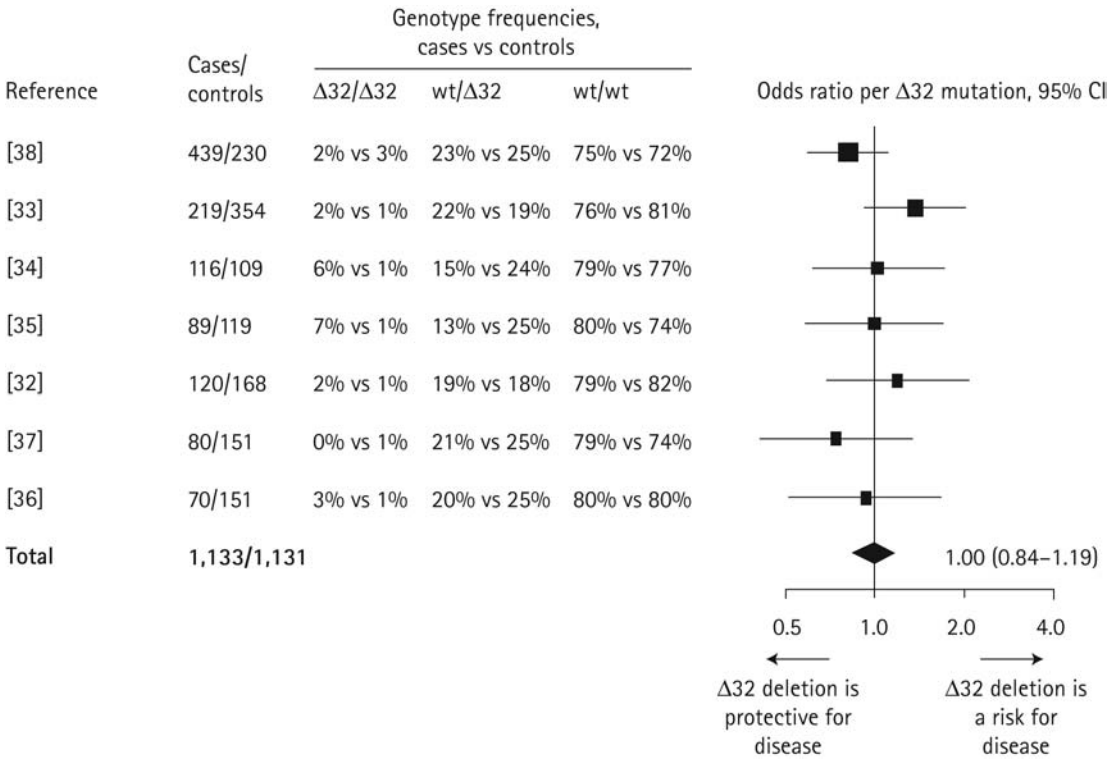
Overall, the per-allele odds ratio of CCR5-Δ32 variant for MS was 1.00 (95% CI: 0.84–1.19; Figure 4). There was no evidence of heterogeneity between the studies beyond that expected by chance (heterogeneity I^2=0%). There was no evidence of publication bias among the studies included. Summary estimates under alternative genetic model assumptions were: OR=0.95 (95% CI: 0.78–1.15) and I^2=0% under a dominant model; and OR=1.75 (95% CI: 0.88–3.45) and I^2=43% under a recessive model.

Table 2. Population studies of CCR5-Δ32 amd multiple sclerosis

Reference	Year	Setting	Ethnicity	Study design	Case population	Genotyped cases, *n*	Case Δ32 allele frequency	Case mean age*	Case % male	Control population	Genotyped controls, *n*	Control Δ32 allele frequency	Control mean age
[32]	1997	Australia	Caucasian	Case control	Clinically definite/laboratory-supported MS (Poser criteria); subtype relapsing/remitting	120	11.3%	NS	NS	Hospital staff/patient's partners	168	9.5%	NS
[37]	2000	Denmark	Caucasian	Case control + 1 year follow-up subset	Clinically definite MS (Poser criteria); subtype relapsing/remitting	80 (excluding 68 possible onset MS cases)	21.0%	36 (IQR 31–41)	32%	Blood donors + staff	151	26.0%	NS
[33]	2002	Russia	Caucasian (Russian parentage)	Case control	Clinically definite MS (Poser criteria); subtypes relapsing/remitting (70%), primary chronic, secondary chronic	219	13.0%	33 ±12	43%	Healthy unrelated	354	9.9%	28 ±10
[36]	2002	Denmark	Caucasian	Case only + external controls	Clinically stable MS randomly selected from Danish MS registry; subtypes relapsing/remitting (30%), primary progressive, secondary progressive	70	9.0%	43 (35–50)	NS	Blood donors and laboratory staff	151	8.0%	42 (36–50)
[34]	2003	Finland	Caucasian	Case control	Clinically definite MS (Poser criteria) randomly selected from neurology outpatients; subtypes relapsing/remitting (74%), primary progressive, secondary progressive	116	11.6%	46 ±10	42%	Blood donors matched for age/sex	109	10.8%	46 ±10
[35]	2004	Finland	Caucasian	Case control	Clinically definite MS (Poser criteria); subtypes relapsing/remitting (35%), primary progressive, secondary progressive	89	13.4%	46 ±10	NS	Hospital staff	119	13.4%	46 ±10
[38]	2004	Northern Ireland	Caucasian	Case control	Outpatient clinic and regional prevalence study cases clinically assessed by investigator; subtypes relapsing/remitting, primary progressive, secondary progressive (75% relapsing/remitting and secondary progressive)	439	15.6%		NS	Blood donors and patient's partners	230	17.5%	NS

*Median (range) or mean (±SD) unless otherwise stated. MS, multiple sclerosis; NS, not stated.

Figure 4. Meta-analysis of studies of CCR5-Δ32 genotype and multiple sclerosis



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RA

A total of six relevant studies of RA were identified [39–44] involving 1,932 cases of RA and 2,787 controls. The weighted mean age of cases was 56.7 years and on average 26% was male (Table 3). These studies were conducted among Caucasian populations in Europe or Australasia and a mixed race population in Central America. Controls were blood donors, laboratory staff or unrelated healthy individuals from other sources. CCR5-Δ32 allele frequency among controls was 9.0% overall, and ranged from 0% to 13.6% in individual studies.

Overall, the per-allele odds ratio of CCR5-Δ32 variant for RA was 0.68 (95% CI: 0.58–0.80; Figure 5). The only study to individually demonstrate a per-allele odds ratio above one, although not statistically significant, was a study conducted among a mixed race population in Mexico [44]. The small control population in this study had complete absence of CCR5-Δ32 mutation, in contrast to the remaining studies among Caucasians. The heterogeneity (I^2=34%) indicated moderate between-study heterogeneity that was almost entirely due to the Mexican study (I^2=0 excluding this study). This study also contributed to a positive assessment of publication bias due to its outlying effect size and small sample size (Eggar test, P=0.028). Summary estimates under alternative genetic model assumptions were: OR=0.68 (95% CI: 057–0.81) and I^2=14% under a dominant model; and OR=0.77 (95% CI: 0.21–2.92) and I^2=0% under a recessive model, although few studies contributed to this estimate.

Discussion

When the action of a therapeutic target mirrors a genetic deletion or polymorphism in man, there is an opportunity to use published gene association studies to identify potential areas of safety concern. Genetic association studies do not replace the need to test hypotheses in preclinical models or conduct well controlled clinical trials; however, there is value in designing experiments and interpreting clinical data in the context of genetic knowledge. The assumption that the effect of a CCR5 antagonist will exactly mimic the effect of the mutation is naive; pharmacologically blocking a receptor that has been functionally active may produce a distinct physiological effect to that seen in individuals where the receptor was not

Table 3. Population studies of CCR5-Δ32 and rheumatoid arthritis

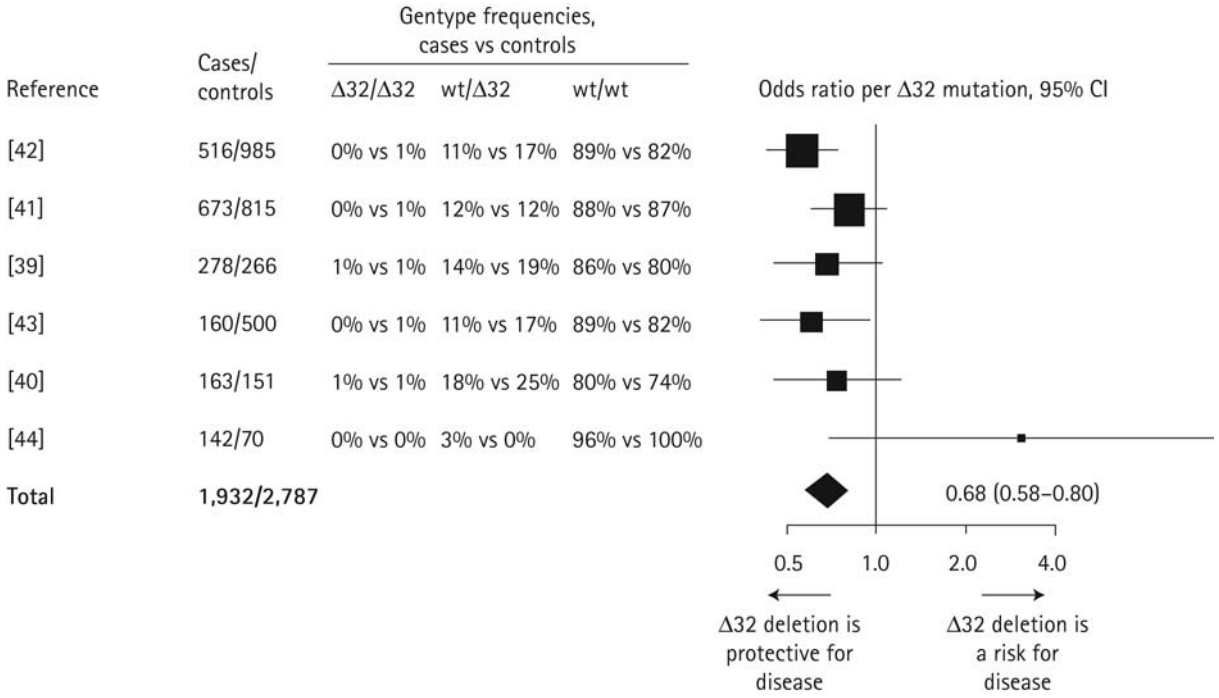
Reference	Year	Setting	Ethnicity	Study design	Case population	Genotyped cases, *n*	Case Δ32 allele frequency	Case age, year*	Case % male	Control population	Genotyped controls, *n*	Control Δ32 allele frequency	Control mean age, year
[40]	1998	Denmark	Caucasian	Case control	Hospital attendees, AmRA criteria excluding infections, malignant disease	163	10.4%	63 (24–87)	16%	Healthy blood donors/ laboratory staff	151	13.6%	NS
[39]	1998	UK	Caucasian	Case control	NS	278	7.6%	NS	NS	Healthy	266	10.7%	NS
[41]	1999	Spain	Caucasian	Case Control	Hospital attendees, ACR criteria RA	673	5.8%	NS	NS	Blood donors	815	7.1%	NS
[43]	2000	Spain	Caucasian	Case control	ACR criteria RA (44% severe disease) disease)	160	5.6%	58 (±13)	26%	Age/ethnic-matched, healthy	500	9.6%	NS
[44]	2003	Mexico	Mestizo	Case control	ACR criteria RA (refractory and non-refractory)	142	2.1%	48 (±15)	NS	Healthy unrelated, no family history of RA	70	0.0%	NS
[42]	2005	New Zealand	NS	Case control	Rheumatology clinic outpatients, ACR criteria RA	516	5.4%	NS	29%	Healthy blood donors	985	9.8%	NS

*Median (range) or mean (±SD). ACR; American College of Rheumatology; AmRA; American Rheumatology Association; NS, not stated; RA, rheumatoid arthritis.

present from birth. In addition, there may be off-target effects. Therefore, the risks or benefits identified by genetic association studies are hypothetical until conclusively demonstrated. Theoretical risks are often the most difficult to manage, because within drug development there is a higher level of confidence in interpreting and addressing findings identified from targeted studies within a controlled/ regulated environment. However, in the current risk-averse climate, the theoretical risks are being assessed in more detail and the need to evaluate theoretical risks identified by genetic association studies will become more common.

A possible association of CCR5 with risk of HCV infection, raised in a number of gene-association studies, was of high relevance to this programme due to the high coinfection rates observed with HIV infection. Whilst results from our preliminary review were conflicting, the implications of a possible effect on HCV were an important consideration for the design of the studies and for patient safety. Our meta-analysis demonstrated generally compatible findings among studies in general populations showing a lack of evidence of an association with CCR5-Δ32 and susceptibility to HCV infection (OR=1.00 [95% CI: 0.85–1.19]), despite study heterogeneity in case mix and source of controls. In addition, we found no genetic evidence to suggest a CCR5 antagonist would interfere with concurrent treatment in HCV-infected patients (OR=0.86 [95% CI: 0.64–1.14]), although our summary confidence interval is wide and compatible with both an improved and reduced response. Individual studies were generally not powered to detect or exclude moderate differences in treatment response (as evidenced by wide confidence intervals), and even the larger studies (>200 cases) reported ORs either side of the null. Although we were unable to quantify the evidence for an association with liver fibrosis due to reporting heterogeneity, studies that addressed this endpoint were conflicting. The single study to report a statistically significant deleterious effect of CCR5-Δ32 on liver fibrosis found increased prevalence among heterozygotes only and no compatible trend in homozygotes with the deletion (S Hellier, personal communication). In addition, the authors reported a conflicting association with reduced portal inflammation in CCR5-Δ32 carriers. A potential association with liver fibrosis requires further examination in large case series. Other studies have investigated spontaneous elimination of virus, including a recent study among a cohort of women infected from a common source, reporting a significant adverse effect of CCR5-Δ32, but of borderline statistical significance [45].

Figure 5. Meta-analysis of studies of CCR5-Δ32 genotype and rheumatoid arthritis



Areas of squares are proportional to the inverse variance, horizontal lines indicate 95% confidence intervals (95% CIs), open diamonds denote weighted summaries in subgroups, and shaded diamonds denote weighted summaries in total, with the diamond width indicating 95% CI of the summary. Total number of controls takes into account multiple studies reporting on a common control set where applicable. wt, wild type.

The CCR5 antagonist maraviroc is now in global Phase III trials. Our assessment that the genetic association studies do not provide evidence of an increased susceptibility or worse prognosis for HCV infection in CCR5-deleted individuals provided confidence that the continued recruitment of HCV-coinfected HIV patients into the maraviroc trials was appropriate. In some regions, up to 40% of HIV-positive individuals are also coinfected by HCV [46]. Hence if HCV-coinfected individuals were excluded, these individuals, who may have very few remaining treatment options, would be denied access to a novel therapeutic class, which may offer an effective treatment option. The inclusion of these patients allows data to be collected on the safety and efficacy of maraviroc treatment in coinfected patients within a controlled study prior to the wider availability and less controlled prescribing of a marketed drug. For all coinfected patients in the maraviroc studies, HCV viral RNA levels were collected at baseline and during the study, as were liver function tests, allowing both clinical monitoring of patients and evaluation of whether our findings in the meta-analysis were confirmed in the controlled trial setting.

Initial inspection of gene-association studies of MS suggested conflicting evidence regarding the influence of CCR5-Δ32 on disease, including potentially strong claims of adverse risk, such as one study linking CCR5-Δ32 carriage with 'early death' [14]. This was against a background of cell-based CCR5 expression data suggesting that reduced expression of CCR5 may protect against disease, and thus suggesting MS as a possible indication for a CCR5 antagonist [47–49]. Our meta-analysis of genetic evidence indicated no effect of CCR5-Δ32 on MS susceptibility (OR=1.00 [95% CI: 0.84–1.19]). Between-study heterogeneity was consistent with random scatter, possibly due to the diversity of case populations, control selection and geographical settings. Although not significant, there was some indication from the recessive model variant of a potential risk in homozygote CCR5-Δ32 only (OR=1.75 [95% CI: 0.088–3.45]), albeit with high variability between studies, which suggests vigilance for further evidence of risk is warranted. Our analysis did not consider disease subgroups separately, and whereas, some studies have highlighted associations in primary progressive disease [35] or relapsing/remitting and secondary progressive disease [38], these analyses have not been replicated. Many of the studies included in our analysis, and others not meeting our inclusion criteria, investigated MS disease severity and duration. Studies have reported CCR5-Δ32 associated with later age at onset or slower disease progression [13,15,37],

whereas others found no influence of CCR5-Δ32 [34–36,50] or reported adverse associations in subgroups [37,38]. A few studies have suggested adverse associations with other MS endpoints, including one highlighting findings in selected genotypic subgroups [33]. Another study of post-mortem cases reported OR that were strongly attenuated by adjustment for clinical subgroup, suggesting further adjustment for more detailed clinical features, if known, would reduce the association further. In summary, although a number of reported subgroup associations may need further investigation, there is currently no strong evidence of risk due to CCR5-Δ32 genotype in MS. This evidence as well as the low risk of concurrent MS and HIV meant that no formal risk management plan was required for maraviroc until clearer evidence emerges to indicate that this is a real effect.

In studies of RA, reported gene associations provided evidence suggesting therapeutic benefit from inhibition of CCR5 expression. Our summary odds ratio of 0.68 (95% CI: 0.58–0.80) demonstrated significant combined evidence from generally consistent findings among studies in Caucasians, possibly reflecting standardized case definitions and relatively homogenous control groups of predominantly blood donors. The outlying result from a Mexican study is suggestive of publication bias and can only be fully interpreted in the context of other findings from similar study populations. The finding in Caucasians is consistent with reported associations with markers of severity, including joint tenderness and swelling, and rheumatoid factor status, although these findings were not demonstrated conclusively in the largest study [42]. Evidence from three of the studies confirmed that homozygosity for CCR5-Δ32 does not preclude disease. Our analysis demonstrates the utility of combining evidence, where study characteristics are compatible, to give a clear summary estimate when the evidence from individual studies was generally non-significant. This finding suggests that further gene-association studies of this hypothesis are probably not warranted. Our conclusions are consistent with a recent meta-analysis involving the six European population studies reviewed here [9]. In summary, these data indicate that RA would be a potential therapeutic indication for a CCR5 antagonist.

The assessment of relevant emerging data, including genetic association, is an ongoing process during all stages of clinical development. In a recent example, a publication suggesting CCR5 deficiency increases risk of symptomatic West Nile Virus (WNV) infection was bought to the attention of the maraviroc team in January 2006 [51]. An initial assessment of the study suggested a strong and statistically robust association between CCR5-Δ32 homozygosity and symptomatic infection, with the implication for the programme that individuals treated with maraviroc may be at increased risk of WNV infection. As the first published genetic association data in this area, the possibility of performing a meta-analysis was precluded. However, a number of features suggested the finding requires confirmation by replication, such as limitations of the populations selected with potential for confounding due to ethnic substructure. Despite rather strong conclusions that fatal outcomes are more likely in CCR5-Δ32 homozygous individuals, the authors' suggestion to monitor individuals in clinical trials with CCR5 antagonists for WNV was warranted. The clinical programme for maraviroc already collects data on infections, and it will be possible to ascertain whether treatment with maraviroc is associated with an increase in symptomatic WNV infection.

Our experience with maraviroc highlights the care required when interpreting and extrapolating from gene-association studies. The conflicting interpretation of a few study abstracts, highlighting potential risks, and a systematic meta-analytic approach highlights the problems of selective reporting, such as emphasis on subgroup findings, varying reporting of allele/homozygote effects, and selected single nucleotide polymorphism associations among the many tested. Replication of gene associations is essential to address the challenges of inadequate sample size, population stratification and genetic heterogeneity [52]. Clearly, patients can be placed at risk if genuine safety concerns are ignored due to incorrect interpretation of data. Conversely, unwarranted emphasis on studies highlighting potential safety concerns may result in denial of new therapeutic options to patient subgroups. Heightened concerns during drug development can result in unnecessarily invasive or onerous safety monitoring that inconvenience both patients and prescribers, and may delay or halt a development programme. Given the caveats on the interpretation of these studies, it is important that any conclusions sit within a risk management strategy that includes a process to collect confirmatory data and prioritises the theoretical risks to establish which require further assessment. The latter will be governed by scientific plausibility, potential impact, the relevance of risk factors to the population being studied and the quality of the studies available.

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Conflict of interest declaration

All authors are employees of Pfizer Inc, and hold Pfizer stock. Pfizer Inc had no role in the design, drafting and interpretation of this study. None of the included studies were supported by Pfizer.

Additional files

The additional file 'Search methodology for research into the effects of CCR5-Δ32 on susceptibility/progression of various diseases (excluding HIV-1 and HIV-2)' can be accessed via the Volume 12 Issue 2 contents page, which can be found at https://www.intmedpress.com (by clicking on 'Antiviral Therapy' then 'Journal PDFs').

References

1. Drews J. Drug discovery: a historical perspective. *Science* 2000; **287:**1960–1964.
2. Penny MA, McHale D. Pharmacogenomics and the drug discovery pipeline: when should it be implemented? *Am J Pharmacogenomics* 2005; **5:**53–62.
3. Fatkenheuer G, Pozniak AL, Johnson MA, *et al.* Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. *Nat Med* 2005; **11:** 1170–1172.
4. Liu R, Paxton WA, Choe S, *et al.* Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 1996; **86:**367–377.
5. Samson M, Libert F, Doranz BJ, *et al.* Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* 1996; **382:**722–725.
6. Eugen-Olsen J, Eversen AK, Garred P, *et al.* Heterozygosity for a deletion in the CKR-5 gene leads to prolonged AIDS-free survival and slower CD4 T-cell decline in a cohort of HIV-seropositive individuals. *AIDS* 1997; **11:**305–310.
7. Feinberg J. Meeting notes from the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). New CCR5 antagonist shows antiretroviral effect. *AIDS Clin Care* 2003; 15:94–95.
8. van Rij RP, Portegies P, Hallaby T, *et al.* Reduced prevalence of the CCR5 delta32 heterozygous genotype in human immunodeficiency virus-infected individuals with AIDS dementia complex. *J Infect Dis* 1999; **180:**854–857.
9. Prahalad S. Negative association between the chemokine receptor CCR5-Delta32 polymorphism and rheumatoid arthritis: a meta-analysis. *Genes Immun* 2006; **7:**264–268.
10. Woitas RP, Ahlenstiel G, Iwan A, *et al.* Frequency of the HIV-protective CC chemokine receptor 5-Delta32/Delta32 genotype is increased in hepatitis C. *Gastroenterology* 2002; **122:**1721–1728.
11. Wasmuth HE, Werth A, Mueller T, *et al.* CC chemokine receptor 5 delta32 polymorphism in two independent cohorts of hepatitis C virus infected patients without hemophilia. *J Mol Med* 2004; **82:**64–69.
12. Konishi I, Horiike N, Hiasa Y, Michitaka K, Onji M. CCR5 promoter polymorphism influences the interferon response of patients with chronic hepatitis C in Japan. *Intervirology* 2004; **47:**114–120.
13. Kantor R, Barzilai A, Varon D, Martinowitz U, Gershoni JM. Prevalence of a CCR5 gene 32-bp deletion in an Israeli cohort of HIV-1-infected and uninfected hemophilia patients. *J Hum Virol* 1998; **1:**299–301.
14. Gade-Andavolu R, Comings DE, MacMurray J, *et al.* Association of CCR5 Delta32 deletion with early death in multiple sclerosis. *Genet Med* 2004; **6:**126–131.
15. Barcellos LF, Schito AM, Rimmler JB, *et al.* CC-chemokine receptor 5 polymorphism and age of onset in familial multiple sclerosis. *Immunogenetics* 2000; **51:**281–288.
16. Hellier S, Frodsham AJ, Hennig BJ, *et al.* Association of genetic variants of the chemokine receptor CCR5 and its ligands, RANTES and MCP-2, with outcome of HCV infection. *Hepatology* 2003; **38:**1468–1476.
17. Mascheretti S, Hinrichsen H, Ross S, *et al.* Genetic variants in the CCR gene cluster and spontaneous viral elimination in hepatitis C-infected patients. *Clin Exp Immunol* 2004; **136:**328–333.
18. Promrat K, McDermott DH, Gonzalez CM, *et al.* Associations of chemokine system polymorphisms with clinical outcomes and treatment responses of chronic hepatitis C. *Gastroenterology* 2003; **124:**352–630.
19. Klein RS. Discussion on frequency of the HIV-protective CC chemokine receptor 5-Delta 32/Delta 32 genotype is increased in hepatitis C.[comment]. *Gastroenterology* 2003; **124:**1558; author reply 1560–1561.
20. Huang Y, Paxton WA, Wolinsky SM, *et al.* The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nat Med* 1996; **2:**1240–1243.
21. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327:**557–560.
22. Egger M, Davey-Smith SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315:**629–634.
23. Glas J, Torok HP, Simperl C, *et al.* The Delta 32 mutation of the chemokine-receptor 5 gene neither is correlated with chronic hepatitis C nor does it predict response to therapy with interferon-α and ribavirin. *Clin Immunol* 2003; **108:**46–50.
24. Mangia A, Santoro R, D'agruma L, Andriulli A. HCV chronic infection and CCR5-Delta32/Delta32. *Gastroenterology* 2003; **124:**868–870.
25. Poljak M, Tomazic J, Seme K, Maticic M, Vidmar L. Prevalence of mutant CCR5 allele in Slovenian HIV-1-infected and non-infected individuals. *Acta Virol* 1998; **42:**23–26.
26. Ruiz-Ferrer M, Barroso N, Antinolo G. & Aguilar-Reina J. Analysis of CCR5-Delta 32 and CCR2-V64I polymorphisms in a cohort of Spanish HCV patients using real-time polymerase chain reaction and fluorescence resonance energy transfer technologies. *J Viral Hepat* 2004; **11:**319–323.
27. Thoelen I, Verbeeck J, Wollants E*et al.* Frequency of the CCR5-Delta32 mutant allele is not increased in Belgian hepatitis C virus-infected patients. *Viral Immunol* 2005; **18:**232–235.
28. Tommasi AM, Fabris P, Carderi I. *et al.* Lack of higher frequency of the chemokine receptor 5-Delta32 /Delta32 genotype in hepatitis C. *J Clin Gastroenterol* 2006; **40:**440–443.
29. Goyal A, Suneetha PV, Kumar GT, Shukla DK, Arora N, Sarin SK. CCR5(Delta)32 mutation does not influence the susceptibility to HCV infection, severity of liver disease and response to therapy in patients with chronic hepatitis C. *World J Gastroenterol* 2006; **12:**4721–4726.
30. Ahlenstiel G, Berg T, Woitas RP, *et al.* Effects of the CCR5-delta32 mutation on antiviral treatment in chronic hepatitis C. *J Hepatol* 2003; **39:**245–252.
31. Dorak MT, Folayan GO, Niwas S, *et al.* C-C-chemokine receptor 2 and C-C-chemokine receptor 5 genotypes in patients treated for chronic hepatitis C virus infection. *Immunol Res* 2002; **26:**167–175.
32. Bennetts BH, Teutsch SM, Buhler MM, Heard RN, Stewart GJ. The CCR5 deletion mutation fails to protect against multiple sclerosis. *Hum Immunol* 1997; **58:**52–59.

33. Favorova OO, Andreewski TV, Boiko AN, *et al.* The chemokine receptor CCR5 deletion mutation is associated with MS in HLA-DR4-positive Russians. *Neurology* 2002; **59:**1652–1655.

34. Luomala M, Lehtimaki T, Huhtala H, *et al.* Promoter polymorphism of IL-10 and severity of multiple sclerosis. *Acta Neurol Scand* 2003; **108:**396–400.

35. Pulkkinen K, Luomala M, Kuusisto H, *et al.* Increase in CCR5 Delta32/Delta32 genotype in multiple sclerosis. *Acta Neurol Scand* 2004; **109:**342–347.

36. Schreiber K, Otura AB, Ryder LP, *et al.* Disease severity in Danish multiple sclerosis patients evaluated by MRI and three genetic markers (HLA-DRB1*1501, CCR5 deletion mutation, apolipoprotein E). *Mult Scler* 2002; **8:**295–298.

37. Sellebjerg F, Madsen HO, Jensen CV, Jensen J, Garred P. CCR5 delta32, matrix metalloproteinase-9 and disease activity in multiple sclerosis. *J Neuroimmunol* 2000; **102:**98–106.

38. Silversides JA, Heggarty SV, McDonnell GV, Hawkins SA, Graham CA Influence of CCR5 delta32 polymorphism on multiple sclerosis susceptibility and disease course. *Mult Scler* 2004 **10:**149–152.

39. Cooke SP, Forrest G, Venables PJ, Hajeer A. The delta32 deletion of CCR5 receptor in rheumatoid arthritis. *Arthritis Rheum* 1998; **41:**1135–1136.

40. Garred P, Madsen HO, Petersen J, *et al.* CC chemokine receptor 5 polymorphism in rheumatoid arthritis. *J Rheumatol* 1998; **25:**1462–1465.

41. Gomez-Reino JJ, Pablos JL, Carreira PE, *et al.* Association of rheumatoid arthritis with a functional chemokine receptor, CCR5. *Arthritis Rheum* 1999; **42:**989–992.

42. Pokorny V, McQueen F, Yeoman S, *et al.* Evidence for negative association of the chemokine receptor CCR5 d32 polymorphism with rheumatoid arthritis. *Ann Rheum Dis* 2005; **64:**487–490.

43. Zapico I, Coto E, Rodriguez A, Alvarez C, Torre JC, Alvarez V. CCR5 (chemokine receptor-5) DNA-polymorphism influences the severity of rheumatoid arthritis. *Genes Immun* 2000; **1:**288–289.

44. Zuniga JA, Villarreal-Garza C, Flores E, *et al.* Biological relevance of the polymorphism in the CCR5 gene in refractory and non-refractory rheumatoid arthritis in Mexicans. *Clin Exp Rheum* 2003; 21: 351–354.

45. Goulding C, McManus R, Murphy A, *et al.* The CCR5-delta32 mutation: impact on disease outcome in individuals with hepatitis C infection from a single source. *Gut* 2005; **54:**1157–1161.

46. Amin J, Kaye M, Skidmore S, Pillay D, Cooper DA, Dore GJ. HIV and hepatitis C coinfection within the CAESAR study. *HIV Med* 2004; **5:**174–179.

47. Balashov KE, Rottman JB, Weiner HL, Hancock WW. CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. *Proc Nat Acad Sci USA* 1999; **96:**6873–6878.

48. Mahad DJ, Lawry J, Howell SJ, Woodroofe MN. Longitudinal study of chemokine receptor expression on peripheral lymphocytes in multiple sclerosis: CXCR3 upregulation is associated with relapse. *Mult Scler* 2003; **9:**189–198.

49. Sorensen TL, Tani M, Jensen J, *et al.* Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J Clin Invest* 1999; **103:**807–815.

50. Haase CG, Schmidt S, Faustmann PM. Frequencies of the G-protein beta3 subunit C825T polymorphism and the delta 32 mutation of the chemokine receptor-5 in patients with multiple sclerosis. *Neurosci Lett* 2002; **330:**293–295.

51. Glass WG, McDermott DH, Lim JK, *et al.* CCR5 deficiency increases risk of symptomatic West Nile virus infection. *J Exp Med* 2006; **203:**35–40.

52. Colhoun HM, McKeigue PM, Davey Smith G. Problems of reporting genetic associations with complex outcomes. *Lancet* 2003; **361:**865–872.

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