Short communication doi: 10.1111/j.1744-313X.2009.00870.x

Frequency of the CCR5-Δ32 polymorphism in the Maltese population at birth

C. Vidal & A. Xuereb-Anastasi

Summary

In this study, the frequency of the CCR5-Δ32 polymorphism was estimated in the human population of Malta. The frequency of the CCR5-Δ32 allele was found to be 1.1% which was similar to that of other island populations, and agree with the north to south gradient observed across Europe.

Introduction

Both α- and β-chemokine receptors expressed on the surface of CD4 positive T lymphocytes and macrophages are known to serve as cofactors for the entry of the human immunodeficiency virus (HIV) into these cells, thus leading to acquired immunodefiency syndrome (AIDS). The macrophage (M)-tropic HIV-1 uses the β-chemokine receptor CCR5 to enter into macrophages although some strains can also use other β-chemokine receptors such as CCR3 and CCR2b (Rana *et al.*, 1997).

A 32 base-pair deletion (Δ32) within the CCR5 gene found on chromosome 3p21, results in a truncated protein leading to lack of integration into the cell membrane (Liu *et al.*, 1996). Individuals homozygous for this polymorphism were found to be highly resistant to HIV-1 infection with M-tropic HIV-1 strains but were not resistant to T-cell (T)-tropic viruses that use the α-chemokine receptor fusin (CXCR-4) (Samson *et al.*, 1996; Rana *et al.*, 1997). Heterozygous individuals were also observed to have partial resistance to AIDS by delaying its onset (Liu *et al.*, 1996). A marked degree of geographic variation exists in the distribution of this polymorphism, where highest frequencies are found in northern European populations which decrease southwards (allele frequency ranging from 16% to 4%) (Novembre *et al.*, 2005). Recent studies performed on ancient-DNA obtained from 2900-year-old skeletons from Italy and Germany showed that this polymorphism was already prevalent in Europe at that time (Hummel *et al.*, 2005). This polymorphism was also found in other populations of European descent (Brazilian) (Pereira *et al.*, 2000), but was absent in African and most Asian populations (Yudin *et al.*, 1998; Lu *et al.*, 1999). It is thought that strong selective pressures over long periods of time, and not the present HIV infection, structured this gradient in allele frequencies across European populations (Novembre *et al.*, 2005). In this study, the frequency of the CCR5-Δ32 polymorphism was determined in the island human population of Malta.

Institute of Health Care and Department of Pathology, University of Malta, Msida MSD2090, Malta

Received 24 February 2009; revised 24 March 2009; accepted 27 July 2009

Correspondence: Prof. Angela Xuereb-Anastasi, PhD, DNA Research Laboratory, Room 15, Institute of Healthcare, Block A, Level 1, Mater Dei Hospital, Msida MSD2090, Malta. Tel: +356 23401882; E-mail: angela.a.xuereb@um.edu.mt

Methodology

DNA was extracted from 232 anonymous cord blood samples (464 chromosomes) collected from neonates and submitted to the Laboratory of Molecular Genetics, University of Malta, Malta for routine screening. DNA was extracted from peripheral blood leucocytes using a salting out procedure as previously described (Miller *et al.*, 1988). The region harbouring the polymorphism was amplified by polymerase chain reaction (PCR) using oligonucleotide primers previously described (Pakorny *et al.*, 2005), yielding a 274-bp fragment for the wild-type (WT) allele and a 242-bp fragment for the mutated (CCR5Δ32) allele. Presence of both fragments indicates a heterozygote. PCR was performed in a 25 μL reaction, using 20 pmol of each primer, 2.5 mM $MgCl_2$, 0.2 μM dNTPs and 1 unit of Hot FIREPol® polymerase (Solis BioDyne, Tartu, Estonia). PCR was performed using an initial hot start at 95 °C for 15 min followed by 30 cycles at 95 °C for 30 s, 59 °C for 30 s and 72 °C for 45 s followed by a final extension step at 72 °C for 5 min. PCR products were analysed using 3% agarose gel electrophoresis while 10% polyacrylamide was used to re-check those carrying this variant and 20% randomly selected samples. No discrepancies were found with the initial genotyping. The chi-square test was used to compare observed genotype frequencies with those expected under Hardy–Weinberg equilibrium. Heterozygosity

was calculated using Utility Programs for Analysis of Genetic Linkage by Ott (1999) using a previously described algorithm (Nei & Roychoudhury, 1974).

Results

Genotype frequencies observed in the Maltese population ($n = 232$) were 97.8% WT/WT homozygotes, 2.2% WT/CCR5Δ32 heterozygotes, while no homozygotes (CCR5Δ32/CCR5Δ32) were identified. The minor allele frequency observed in the Maltese population was 1.1% (0.011) and allele frequencies were in Hardy–Weinberg equilibrium ($\chi^2 = 0.028$; $P = 0.87$). Calculated heterozygosity was of 0.0213 (95% confidence interval: 0.0030–0.0397).

Discussion

For the first time, the frequency of the CCR5-Δ32 polymorphism in the Maltese population is being reported. To our knowledge, it is also the first time that this frequency is being estimated in newborns, thus reflecting the frequency in the general population at birth and minimizing any possible effects that this variant might have on longevity when using older populations. Until now importance was given to the protective role of the CCR5Δ32 allele against HIV but little was said about its possible negative effects. Recently, an increased risk of symptomatic infection with the West Nile virus in CCR5-Δ32 homozygotes was reported (Glass *et al.*, 2006). The CCR5-Δ32 polymorphism results in a completely non-functional receptor and thus could lead to a weakened immune response if the host was to be attacked by other organisms where the biological role of the CCR5 receptor cannot be outweighed by that of other receptors. Also there were conflicting reports about the role of the CCR5-Δ32 polymorphism in various diseases such as juvenile idiopathic arthritis (Scheibel *et al.*, 2008), hepatitis C (Ahlenstiel *et al.*, 2004) and systemic lupus erythematosus (Mamtani *et al.*, 2008). All these factors could affect allele frequencies of this polymorphism when using an adult population, so using a random sample of newborns minimizes such influences on frequency estimation. Differences in allele frequencies between newborns and adult populations were observed for variants within the klotho and methylenetetrahydrofolate reductase genes, both thought to affect longevity (Arking *et al.*, 2002; Gueant-Rodriguez *et al.*, 2006).

The allele frequency observed in this study conforms with the European north to south gradient (Novembre *et al.*, 2005). Malta is an island located about 60 miles to the southeast of Sicily, and a frequency of 1.1% for the CCR5Δ32 allele conforms well to its geographic position. This frequency is less than that observed in other island populations, including the Croatian island of Vis (1.5%) (Smaljanovic *et al.*, 2006), Sardinia (2.1%) (Battiloro *et al.*, 2000) and the Basque region (1.8%) (Lucotte, 2002), but is slightly higher than that observed in Corsica (0.9%) (Lucotte, 2002). Isolated populations have the lowest frequency even when compared to that of other European populations which ranges from 4.2% in Greeks (Novembre *et al.*, 2005), 7.1% in mainland Croatians (Ristic *et al.*, 2005), and increasing to 16% in northern European populations (Novembre *et al.*, 2005). The only exceptions to this gradient effect are the Ashkenazi Jews, where the overall frequency of the CCR5Δ32 allele is 13.7%, probably due to a founder effect and/or genetic drift as a result of their historical background (Lucotte, 2003).

It is highly debatable as to what were the selective pressures that led to this geographical gradient across European populations. The deadly pandemics that struck Europe throughout the Middle Ages were among the reasons most usually given, although today these are highly disputed (Stephens *et al.*, 1998; Cohn & Weaver, 2006). Comparisons of frequencies between skeletal remains of victims from the 14th century plague with those from 2900 years ago showed similar results, suggesting that the Black Death was not the major selective force causing a rapid increase in CCR5-Δ32 allele frequencies (Hummel *et al.*, 2005). If the Black Death were the major selective pressure, then one could assume that mortality was much higher in northern European countries when compared to southern Europe but historical evidence about the Black Death of 1346–53 shows the opposite (Cohn & Weaver, 2006). The plague struck Malta several times between the fourteenth and nineteenth centuries with the latest epidemic being that of 1813 (Savona-Ventura, 1997). Every outbreak resulted in the death of a significant percentage of the population ranging from 9% during the 1592 outbreak to 4% in the latest outbreak of 1813, where in the latter the population was of nearly 116 000. Other sources mentioned that during the outbreak of 1675 approximately 8000–11 000 individuals perished from a population of approximately 50 000 (Blouet, 1967). The frequency of the CCR5-Δ32 allele in Malta is low, indicating that the plague hypothesis does not hold. If it did, then a higher frequency would be expected in a population that expanded following several population bottlenecks with expected high proportion of survivors being those carrying the CCR5-Δ32 allele. A reason why these several plague outbreaks did not have an effect on the frequency of the CCR5-Δ32 allele might be due to all these epidemics described as plagues, were actually caused by different etiological agents, thus exerting different selective pressures on the allele.

Another hypothesis states that it is more probable that a positive selective pressure was due to smallpox rather than the Black Death (Galvani & Slatkin, 2003; Cohn & Weaver, 2006). There are several reasons why this could be more probable including the fact that smallpox wiped out more of the population than

the Black Death but over longer periods of time, affected younger people with a reproductive potential and also affected Scandinavian countries much more than the bubonic plague (Galvani & Slatkin, 2003). Conversely, population differentiation and long-range linkage disequilibrium at the CCR5-Δ32 locus were not different from the rest of the genome, showing that probably there isn't a strong recent selection for CCR5-Δ32 as proposed by the previous hypotheses, and so its genetic variation is consistent with neutral evolution (Sabeti *et al.*, 2005).

An alternative hypothesis for the north to south gradient could be that the CCR5-Δ32 allele gives little resistance to certain organisms that are more prevalent in the south due to weakening of the immune systems as discussed above. If this happens, then there is a greater chance of survival for individuals carrying the wild-type allele than for those carrying the CCR5-Δ32 allele. This study continues to add to the existing knowledge about the geographical distribution of the CCR5-Δ32 polymorphism in Europe and the Mediterranean region.

Acknowledgements

We would like to thank Prof. Alex E. Felice and the Laboratory of Molecular Genetics, University of Malta for submitting the neonatal cord blood samples. This work was performed at the DNA Research Laboratory, Institute of Healthcare, Mater Dei Hospital, Malta and was supported by the Research Fund Committee, University of Malta.

References

Ahlenstiel, G., Woitas, R.P., Rockstroh, J. & Spengler, U. (2004) CC-chemokine receptor 5 (CCR5) in hepatitis C – at the crossroads of antiviral immune response. *Journal of Antimicrobial Chemotherapy*, **53**, 895–898.

Arking, D.E., Krebsova, A., Macek, M., Macek, M., Arking, A., Mian, I.S. *et al.* (2002) Association of human aging with a functional variant of klotho. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 856.

Battiloro, E., Andreoni, M., Parisi, S.G., Mura, M.S., Sotgiu, G., Aceti, A. *et al.* (2000) Distribution of the CCR5 delta32 allele in Italian HIV type 1 infected and normal individuals. *AIDS Research and Human Retroviruses*, **16**, 181.

Blouet, B. (1967) *The Story of Malta*. Malta Progress Press Ltd, Valletta, Malta.

Cohn, S.K. & Weaver, L.T. (2006) The Black Death and AIDS: CCR5-Δ32 in genetics and history. *The Quarterly Journal of Medicine*, **99**, 497.

Galvani, A. & Slatkin, M. (2003) Evaluating plague and smallpox as historical selective pressures for the CCR5-Δ32 HIV resistance allele. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 15276.

Glass, W.G., McDermott, D.H., Lim, J.K., Lekhong, S., Yu, S.F., Frank, W.A. *et al.* (2006) CCR5 deficiency increases risk of symptomatic West Nile virus infection. *The Journal of Experimental Medicine*, **23**, 35.

Gueant-Rodriguez, R.A., Gueant, J.L., Debard, R., Thirion, S., Hong, L.X., Bronowicki, J.P. *et al.* (2006) Prevalence of methylenetetrahydrofolate reductase 677T and 1298C alleles and folate status: a comparative study in Mexican, West African, and European populations. *American Journal of Clinical Nutrition*, **83**, 701.

Hummel, S., Schmidt, D., Kremeyer, B., Herrmann, B. & Oppermann, M. (2005) Detection of the CCR5-Δ32 HIV resistance gene in Bronze age skeletons. *Genes and Immunity*, **6**, 371.

Liu, R., Paxton, W.A., Choe, S., Ceradini, D., Martin, S.R., Horuk, R. *et al.* (1996) Homozygote defect in HIV-1 co-receptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell*, **86**, 367.

Lu, Y., Nerurkar, V.R., Dashwood, V.M., Woodward, C.L., Ablan, S., Shikuma, C.M. *et al.* (1999) Genotype and allele frequency of a 32 base pair deletion mutation in the CCR5 gene in various ethnic groups: absence of mutation among Asians and Pacific Islanders. *International Journal of Infectious Diseases*, **3**, 186.

Lucotte, G. (2002) Frequencies of 32 base pair deletion of the (delta 32) allele of the CCR5 HIV-1 co-receptor gene in Caucasians: a comparative analysis. *Infection Genetics and Evolution*, **1**, 201.

Lucotte, G. (2003) CCR5-Δ32 allele frequencies in Ashkenazi Jews. *Genetic Testing*, **7**, 333.

Mamtani, M., Rovin, B., Brey, R., Camargo, J.F., Kulkarni, H., Herrera, M. *et al.* (2008) CCL3L1 gene containing segmental duplications and polymorphisms in CCR5 affect risk of systemic lupus erythematosus. *Annals of Rheumatological Diseases*, **67**, 1076.

Miller, L.J., Dykes, D.D. & Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Research*, **16**, 1215.

Nei, M. & Roychoudhury, A.K. (1974) Sampling variances of heterozygosity and genetic distance. *Genetics*, **76**, 379.

Novembre, J., Galvani, A.P. & Slatkin, M. (2005) The geographical spread of the CCR5 Δ-32 HIV-resistance allele. *PLoS Biology*, **3**, 1954.

Ott, J. (1999) *Analysis of Human Genetic Linkage*, 3rd edn. Johns Hopkins University Press, Baltimore, MD, USA.

Pakorny, V., McQueen, F., Yeoman, S., Merriman, M., Merriman, A., Harrison, A. *et al.* (2005) Evidence for negative association of the chemokine receptor CCR5 d32 polymorphism with Rheumatoid arthritis. *Annals of Rheumatological Disease*, **64**, 487.

Pereira, R.W., Pires, E.R., Duarte, A.P.M., de Moura, R.P., Monteiro, E., Torloni, H. *et al.* (2000) Frequency of the CCR5 Δ-32 allele in Brazilians: a study of colorectal cancer and in HTLV-I infection. *Genetics and Molecular Biology*, **23**, 523.

Rana, S., Besson, G., Cook, D.G., Rucker, J., Smyth, R.J., Yi, Y. *et al.* (1997) Role of CCR5 in infection of primary macrophages and lymphocytes by macrophage tropic strains of human immunodeficiency virus: resistance to patient-derived and prototype isolates resulting from the ΔCCR5 mutation. *Journal of Virology*, **71**, 3219.

Ristic, S., Cismarevic, N.S., Brajenovic-Milic, B., Crnic-Martinovic, M. & Kapovic, M. (2005) Frequency of the CCR5 gene 32 base pair deletion in Croatian normal population. *Croatian Medical Journal*, **46**, 693.

Sabeti, P.C., Walsh, E., Schaffner, S.F., Varilly, P., Fry, B., Hutcheson, H.B. *et al.* (2005) The case for selection at CCR5-Δ32. *PLOS Biology*, **3**, 1963.

Samson, M., Libert, F., Doranz, B.J., Rucker, J., Liesnard, C., Farber, C.M. *et al.* (1996) Resistance to HIV-1 infection of Caucasian individuals bearing mutant alleles of the CCR5 chemokine receptor gene. *Nature*, **382**, 722.

Savona-Ventura, C. (1997) Medieval period. In: *Outlines of Maltese Medical History*, 19 pp. Midsea Books Ltd, St Venera, Malta.

Scheibel, I., Veit, T., Neves, A.G., Souza, L., Prezzi, S., Machado, S. *et al.* (2008) Differential CCR5-Δ32 allelic frequencies in juvenile idiopathic arthritis subtypes: evidence for different regulatory roles of CCR5 in rheumatological disease. *Scandinavian Journal of Rheumatology*, **37**, 13.

Smaljanovic, M., Ristic, S. & Hayward, C. (2006) Historic exposure to Plague and present day frequency of CCR5del32 in two isolated island communities of Dalmatia, Croatia. *Croatian Medical Journal*, **47**, 579.

Stephens, J.C., Reich, D.E., Goldstein, D.B., Shin, H.D., Smith, M.W., Carrington, M. *et al.* (1998) Dating the origin of the CCR5-*Δ*32 AIDS-resistance allele by the coalescence of haplotypes. *American Journal of Human Genetics*, **62**, 1507.

Yudin, N.S., Sergey, V.V., Potapova, T.A., Naykova, T.M., Sitnikova, V.V., Kulikov, I.V. *et al.* (1998) Distribution of the CCR5-delta 32 gene deletion across the Russian part of Eurasia. *Human Genetics*, **102**, 1432.