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Pushing it back. Dating the CCR5–Δ32 bp deletion to the Mesolithic in Sweden and its implications for the Meso/Neo transition

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ABSTRACT – *Genetic variation in the chemokine receptor gene CCR5 has received considerable scientific interest during the last few years. Protection against HIV-infection and AIDS, together with specific geographic distribution are the major reasons for the great interest in CCR5 32bp deletion. The event for the occurrence of this mutation has been postulated by coalescence dating to the 14th century, or 5000 BP. In our prehistoric Swedish samples we show that the frequency of 32pb deletion in CCR5 in the Neolithic population does not deviate from the frequency in a modern Swedish population, and that the deletion existed in Sweden already during the Mesolithic period.*

IZVLEČEK – *Med znanstveniki je bilo v zadnjih nekaj letih precej zanimanja za genetsko variacijo na kemokinskem receptornem genu CCR5. Glavna razloga velikega zanimanja za zdrs 32bp na CCR5 sta zaščita proti okužbi z virusoma HIV in AIDS ter specifična geografska porazdelitev. S pomočjo 'datiranja zlitja' je bilo predpostavljeno, da se je ta mutacija pojavila v 14. stoletju AD ali pa 5000 BP. Na švedskih prazgodovinskih vzorcih pokažemo, da ni bistvenega odklona v frekvenci zdrsa 32pb na CCR5 med neolitsko in moderno švedsko populacijo in da je mutacija na Švedskem obstajala že v mezolitiku.*

KEY WORDS – *CCR5-Δ32; Bubonic plague; Smallpox; Mesolithic; Neolithic*

Introduction

The product of the CCR5 gene is a member of a seven-transmembrane G-protein-coupled receptor family, and is important for the cell entry of the human immunodeficiency virus HIV-1 (*Alkhatib et al. 1996; Choe et al. 1996; Deng et al. 1996; Doranz et al. 1996; Trkola et al. 1996*). In 1996 a 32bp deletion in the CCR5 gene was reported which truncates the protein and appears to provide almost complete protection against infection by HIV-1 (*Liu et al. 1996; Samson et al. 1996; Dean et al. 1996; Ometto et al. 1999*). In homozygotes for 32bp deletion the protection is well documented, but there are different opinions about the protective effect of heterozygosity against HIV-1, although it seems to delay the outbreak of AIDS (*Samson et al. 1996; Dean et al. 1996; Huang et al. 1996; Michael et al. 1997*). The CCR5 32bp deletion seems to be restricted to Caucasian populations. However, the frequency of the mutation varies widely among European populations. Whereas the frequency is quite high in St. Petersburg, Russia (0.166), and in Sweden (0.143), it is considerably lower in the southern parts of Europe, e.g., in Spain (0.050) and Greece (0.041) (*Lucotte 2001*).

However heterozygosity for the deletion was found at a frequency of 0.23 in a Swedish HIV positive group (*Bratt et al. 1998*). Since that study was cross-

sectional and not prospective, this frequency may represent a population subjected to a different selection pressure than an ordinary Scandinavian population. In cases where the deletion has been found in non-Caucasian populations, there has usually been continuous contact and a likely admixture with Caucasians (*Liu et al. 1996; Samson et al. 1996; Dean et al. 1996*). The geographic distribution of 32bp deletion in Europe has been explained in several ways, as e.g., genetic drift (*Martinson et al. 1997*) or positive selection due to resistance to a major disease (*Dean et al. 1996*). Based on coalescence, 32bp deletion has been estimated to have appeared approximately 700 years ago (*Stephens et al. 1998*). Consequently, it has been speculated that the Black Death (*Yersinia pestis*) caused a positive selection (*Stephens et al. 1998; O'Brien 1998*). The basis for this hypothesis is that CCR5 may be involved in the mechanism of Yersinia-induced macrophage apoptosis (*Stephens et al. 1998*). If the Black Death, believed to be caused by *Yersenina pestis*, resulted in a positive selection, this has to be a fairly recent process, since the outbreak of this disease was in the mid 14th century. However, according to Galvani and Slatkin (*2003*), the bubonic plague, although severe, could never have driven the frequencies to over 10% in the short time span of 700 years. In another study, it was postulated that the mutation originated about 2000 years ago, somewhere in north-eastern Europe (*Libert et al. 1998*). In later publications, smallpox is thought to be a more likely candidate, where the selective pressure from this virus would have pushed the frequency of 32bp deletion across Europe into today's figures (*Klitz et al. 2001; Galvani and Slatkin 2003*). In a recent publication Sabeti et al. (*2005*), has pushed back the estimate of the occurrence of the CCR5–Δ32 allele to over 5000 years ago. There are also several articles suggesting that the deletion was dispersed by the Vikings along its present gradient across Europe (*Lucotte 2001 and Lucotte and Dieterlen 2003*). In 2005 the CCR5–Δ32 was detected in human Bronze Age skeletons from Germany by Hummel et al., and this is the first evidence that the mutation existed before the14th century plague outburst.

Although there are disagreements about the time for the first occurrence of 32bp deletion, the cause of the event, and the distribution of the deletion all seem to agree on the fact that the deletion originated from a single historical mutation event.

Here we want to address the question regarding the deletion's first occurrence and distribution by extracting DNA from well-known and well-dated prehistoric human and animal bones and teeth. Of specific interest is a date for the occurrence of the deletion that precedes 5000 BP, i.e. approximately the Mesolithic-Neolithic transition in Scandinavia.

In Scandinavia the Mesolithic stretches from around 8300 to 4000 BC. It is a period connected to the end of the last glaciation, and marks a shift in climate towards a warmer period. During this period people began to colonise southern Sweden, both along the coasts and inland. In this study we have included samples dating to the Mesolithic from Skateholm in the south, and Huseby Klev from the west coast.

The Neolithic period began in Sweden with the introduction of farming around 4000 BC, and it ended around 1800 BC with the introduction of the Bronze Age. During the middle Neolithic in Sweden, there were three main cultures: the Funnel Beaker (TRB), the Pitted Ware (GRK) and the Battle Axe (STY) cultures. We have analysed human and animal bones and teeth from two of these cultures, the Funnel Beaker culture complex that is believed to be connected with the introduction of farming and cereal cultivation, and the GRK-complex that consists mainly of a set of hunter/gatherer dwelling sites and cemeteries along the Scandinavian Baltic coast and the major Baltic Islands. The GRK are represented by samples from the islands of Gotland and Åland, and the TRB samples are represented by two passage graves in central Sweden (Fig. 1). The individuals from Dragby represent the last phase of the Neolithic in Sweden, thus this case study encompasses more than 5000 years.

Materials and methods

Bones and teeth from two Mesolithic and six Neolithic sites were chosen for this study.

The two Mesolithic sites used in this study are Huseby Klev, radiocarbon dated to 7000–6500 BC, and Skateholm, radiocarbon dated to 5250–4900 BC (Fig. 1). Four individuals were analysed from Huseby Klev (Hk1, Hk2, Hk3 and Hk4), a Preboreal/Boreal site that was subjected to a rescue excavation in 1993–94 by Bengt Nordqvist (*Nordqvist 2000*). Next we analysed six individuals from the Skateholm site (burials 4, 5, 7, 12, 63a and 63b) that was excavated in the early 1980s by Lars Larsson (*Larsson 1988*).

The first Neolithic site, Ire on the island of Gotland (Fig. 1), is a cemetery belonging to the Pitted Ware culture complex (GRK) which was excavated in diffe-

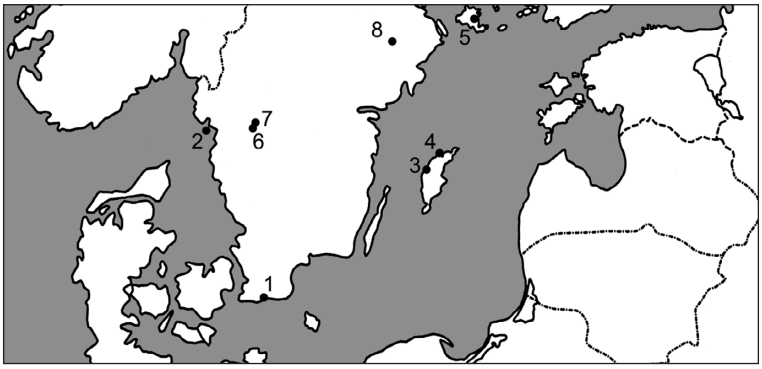

Fig. 1. Southern Scandinavia with Mesolithic and Neolithic sites 1. Skateholm, 2. Huseby Klev, 3. Visby, 4. Ire, 5. Jettböle, 6. Rössberga, 7. Hjelmars Rör and 8. Dragby. The Δ32 mutation of CCR5 was found in human material from Skateholm, Rössberga, Visby, Ire, and Dragby, indicating that the mutation is at least as old as these sites.

rent periods after its discovery in 1914 (*Janzon 1974*). Five individuals (burials 6b, 6c, 7a, 7b and 7c) were analysed from this site, which has been ^{14}C dated to 3000–2100 cal BC (*Janzon 1974*). We also extracted DNA from a horse deposited in the settlement layers connected to the burials, in order to control for contamination and bone preservation. The other GRK site on Gotland, Visby (Fig 1.), is a cemetery that was last excavated in 1960–62 by Erik Nylén (*Janzon 1974*). Here 12 individuals were selected for analysis (burials 2/09, 2/24, 2/39, 3b, 13, 19, 19/37, 23, 27, 30b, 31 and 33). They have been ^{14}C dated to 3000–2500 cal BC (*Janzon 1974*).

The next site to be analysed is Jettböle (Fig 1.), also a GRK settlement on an island in the archipelago of Åland, excavated by Björn Cederhvarf from 1905 until 1911 (*Cederhvarf 1912*). From this site, 3 individuals were analysed (J1, J2 and J3). They have been radiocarbon dated to 3370–2910 cal BC (*Lidén et al. 1995*).

The other samples are from two passage graves, Rössberga and Hjelmars Rör (Fig. 1), both from Västergötland in central Sweden and belonging to the Funnel Beaker culture complex (TRB). Five different individuals from each passage grave were analysed. Fourteen out of sixteen ^{14}C samples date Rössberga to 3506–2143 BC; the other two samples date Rössberga to the late Bronze Age (*Persson & Sjögren 1995*). Rössberga, which was excavated in 1962 by Carl Cullberg, will be treated here as a middle Neolithic passage grave (*Cullberg 1963*). Eight ^{14}C samples date Hjelmars Rör to 3350–2700 BC. The passage grave was first excavated in 1868 by Bror Emil Hildebrand, but was revisited in the 1990s by Tony Axelsson and Per Persson (*Persson & Sjögren 1995*). We added a bone from a cow deposited in this passage grave, for the same reason as stated above.

The last samples are from Dragby, a passage grave excavated by Mårten Stenberger in 1958/59 (*Gejvall 1963*). The dating of this tomb is complicated, since it was superimposed by a Bronze Age mound. However, the ^{14}C dates, 2290–1690 cal BC (*Roumelis 2002*), imply that this site was in use during the late Neolithic. Four individuals were analysed (H4/A, F15/44, F8/44 and F2f2/45).

Most publications on DNA from prehistoric material concern mitochondrial DNA (*e.g. Hagelberg & Clegg 1991; Krings et al. 1997; Krings et al. 1999; Handt et al. 1994, Torroni et al. 2000, Hofreiter et al. 2002, Forster 2004, Starikovskaya et al. 2005*). However, a number of studies have also been performed on nuclear, single copy markers from ancient tissue (*e.g. Beraud-Colomb et al. 1995; Zierdt et al. 1996; Ovchinnikov et al. 1998; Götherström et al. 1997; Greenwood et al. 1999; Noonan et al. 2005; Poinar et al. 2005*).

We designed a suitable (<150bp) primer system for the part of CCR5 that carries the 32 bp deletion, i.e. in this study we use a single copy nuclear marker applied to ancient DNA.

In all cases, except for the cow, teeth were used as the source material for DNA extraction. Samples were extracted and prepared for PCR according to Lidén et al. (*1997*) and Anderung et al. (*2005*). This study was conducted in two stages, and because of this the DNA extraction methods varied. Part one was done using a guanidium thiocyanate and silica extraction (*Lidén et al. 1997*), and the second part was done using an extraction method called fishing (*Anderung et al. 2005*).

A set of three primers was designed to give an easily detectable indication of the presence or absence of the 32bp deletion (Tab. 1), i.e., a system that amplifies fragments of different length depending on whether the 32bp deletion is present or not, and to amplify highly degraded DNA. This primer system was used in both set-ups.

DNA was amplified with Amplitaq gold™ (Perkin Elmer)/HotStarTaq (Qiagen) to receive the hot start

and time-release effect (*Götherström et al. 1997*). The 25 µl reactions contained 50 mM KCl, 10 mM Tri-HCl pH 8.3, 2.25 mM MgCl2, 0.2 mM of each dNTP, 0.5 µM of each primer, 1 U Taq-polymerase (Amplitaq gold™, Perkin Elmer, HotStarTaq, Qiagen), 10/9 µl template DNA and 2% glycerol/none. Hot start was performed automatically, due to the enzyme used. The amplification cycles were initiated by a 45 sec/5 min. denaturation step at 95° C to activate part of the enzyme and save the rest for later cycles; this was then followed by 45/30 sec. at 94° C, 1 1/2/1min. at 50° C and 1 min/30 sec. at 72° C. This cycle was repeated 55/45 times. Finally, an extension step at 72° C for 7/5 min. followed. The result was detected on a 3%/1.5% ethidium bromide stained agarose gel exposed to 40V for 1h. One primer (CCR5:1) anneals upstream the sequence, one (CCR5:2) anneals downstream on the other side of the 32bp deletion, and the third primer (CCR5:3) anneals within the 32bp deletion. Thus, an individual homozygous for the deletion will have one fragment (91bp) amplified, an individual homozygous for the wild type will have two fragments (105bp and 123bp) amplified, and a heterozygous will have three fragments (91bp, 105bp and 123bp) amplified. The result was confirmed by sequencing on a Pharmacia ALF express™ with an Amersham Pharmacia Biotech Cycle Sequencing kit.

A short mtDNA fragment, 16131–16303, according to the reference sequence from Anderson et al. (*1981*), was amplified and sequenced from a part of the material to confirm that the DNA was from different individuals in the first set-up. If authentic DNA was extracted, there should among all samples be several haplotypes present based on this short HVR1 sequence, but only one haplotype in each sample. The same protocols, with minor changes, used for CCR5 amplification and sequencing were used for the amplification and sequencing of the mtDNA fragment. However, only 45 cycles were used, and L16131 and H16303 (Tab. 1) replaced CCR5:1, CCR5:2 and CCR5:3. The primers used for the horse and cow were HBB:1 and HBB:2 (*Marklund et al. 1994*) and HTG10:1 and HTG10:2 (*Steffen et al. 1993*) respectively.

Primer name	Primer sequence
CCR5:1	5'TCCTTAGTAGAAATGGTCTAG3'
CCR5:2	5'GTCGGGGTTCTACTGATAG3'
CCR5:3	5'GAAATTACAGACCTTTAAGAAG3'
L16131	5'CACCATGAATATTGTACGGT3'
H16303	5'TGGCTTTATGTACTATGTAC3'
HBB:1	5'GATATAAAAAAGAAGACCCAGTAG3'
HBB:2	5'TACCTGAGTCATATGTAATATTCC3'
HTG10:1	5'GAATTCCCGCCCCACCCCCGGCA3'
HTG10:2	5'TTTTTATTCTGATCTGTCACATTT3'

Tab. 1. Primer name and primer sequences used in the study. The system used to detect presence of the 32bp deletion of the CCR5 gene is based on three primers CCR5:1, CCR5:2, CCR5:3. The D-loop system is a simple two-primer system L16131 & H16303 (*Anderson et al. 1981*). The primers used for the horse were HTG10:1 and HTG10:2 (*Marklund et al. 1994*) and for the cow HBB:1 and HBB:2 (*Steffen et al. 1993*).

In the second part of the study, all replications were performed in an independent laboratory, Centro Mixto UCM–ISCIII de Evoulción y Comportamiento Humanos (Madrid) in order to detect contaminations and prove the authenticity of the ancient DNA (*Hofreiter et al. 2001*).

One tooth permits one extraction, which gave enough material for ten PCRs. Whenever possible, at least two samples were taken from each individual, but only one extraction could be carried out on each individual from the passage graves due to the problem of separating individuals in a passage grave. However, thanks to high reproducibility within the extraction, we could trust the results from the ten individuals from the passage graves. Moreover, a robust protocol including extraction blanks, carrier effect blanks, PCR blanks, UV irradiation of reagents, fragment size control etc was applied (*Götherström & Lidén 1998*). To further test the state of preservation, we extracted another large bone protein, collagen, according to Brown et al. (*1988*) on both human and animal samples. Here the extracted amount of collagen, the absolute amounts of carbon and nitrogen, as well as their ratio provide information on the state of preservation (*DeNiro 1985*). We also tried to amplify DNA extracted from the horse and the cow with the primers used for the humans, to test for contamination.

Results and discussion

The collagen data were all in accordance with bones that are well preserved. In bones positive for DNA, no samples contained less than 0.66% of collagen, calculated on total bone, and the C/N ratios were all within the accepted limit of 2.9 – 3.4 (except for 3 samples), as well as the absolute values of carbon and nitrogen (Tab. 2) (*De Niro 1985*). It is therefore reasonable to believe that DNA was also well pre-

served in the samples (*Götherström et al. 2002*). There is also a correlation with previously published quantitative data (*Malmström et al. 2005*), where good preservation has been indicated on the Baltic island of Gotland. That the extracted DNA is authentic was further proved by the result of the mtDNA, where we have four different haplotypes distributed over two cemeteries, and that no products were obtained from the extracted animal DNA amplified with the human primers (Tab 2.).

We were able to extract DNA from 19 out of 50 samples, representing in total 46 individuals, i.e. a 38% success rate (Tab. 2). We were also able to extract and sequence DNA from two different teeth from the same individual in two of the burials from Ire. Of the 19 samples, now representing 17 individuals, one individual was dated to the Mesolithic and came from Skateholm, and 16 individuals were dated to the Neolithic. Of the Neolithic samples, six individuals can be attributed to the pitted ware culture, nine to the funnel beaker culture, and one to the late Neolithic.

The complete sample is in Hardy-Weinberg equilibrium ($p = 0.661$), and does not differ in frequencies (17.1%) of the CCR–Δ32 mutation from a modern Swedish population (14.3%). It could be argued that the small sample size, 17 individuals or 34 alleles, is not statistically significant. A further argument could be that the five individuals from one megalith tomb could be related i.e., providing a smaller number of alleles than we calculate. This is also obvious in the case of Ire, where the individuals were sampled from one burial, and one individual proved to be a heterozygous, while the others proved to be homozygous for the mutant allele.

Two alternative explanations for the specific distribution of the CCR5 32bp allele have been suggested, where one is a selection process related to a specific disease within a population; alternatively, the appearance of a higher gene frequency could be related to the migration of individuals already having the deletion. One such migration that has been discussed and that is of interest here is the transition from the Mesolithic to the Neolithic. Traditionally, this is connected to a change in economy from a hunter-gatherer way of life to pastoralism-farming. This change in economy is most often explained by three major themes, of which immigration is one.

Subsistence during the Mesolithic was based on hunting and gathering, as we can see in the individuals analysed from Skateholm and Huseby Klev (*Lidén et al. 2005*). This lifestyle continued during the Neolithic for the Pitted Ware culture. Their main economy seems to have been based on maritime hunting and fishing, as seen in the sites studied here: Ire, Visby and Jettböle (*Lidén 1995*). The Funnel Beaker culture is partly differentiated from the GRK by the change in subsistence towards an economy based on agro-pastoralism, and this pattern is clearly visible in the passage graves analysed here: Rössberga and Hjelmars Rör (*Lidén 1995*). In the late Neolithic, agro-pastoralism continued, as seen in the Dragby samples (*Roumelis 2002*). However, since we only have one individual from whom it was possible to extract DNA dating to the Mesolithic, we cannot draw any conclusions relating to the migration hypothesis. However, the sample allows for testing the frequencies between the two Neolithic cultures, GRK and TRB, and when using Fishers's exact test ($p = 0.005$), we do find a significant difference. This difference is in itself interesting, in that these cultures represent different economies, and where the "farming culture" has the lower frequency of the deletion. Does this mean that the hunting GRK culture could be regarded as original, and that the TRB were the newcomers? We cannot say.

The second explanation for the distribution of the CCR5–Δ32 gene was connected to a selection process due to a specific disease. It is, however, obvious that the 32bp deletion in the CCR5 gene had spread and reached a relatively high frequency in Scandinavia at least during the Neolithic, and that selective advantage due to the plague is evidently not a likely cause of the present day allele distribution. Previous studies also suggest that this is an unlikely hypothesis, as the short period during which the plague ravaged Europe would not have caused a selection pressure strong enough to alter frequency to any greater extent (*Galvani and Slatkin 2003*). Consequently, we will have to seek other explanations for the spread of the 32bp deletion in the Mesolithic and thereafter in Scandinavia. Galvani and Slatkin (*2003*) proposed that the more continuous smallpox mortality that afflicted European children since the origin of the allele could have provided the necessary selective pressure to generate the rise of CCR5–Δ32 deletion to current frequencies of 10%.

Our evidence pushes the dating of the CCR5 32 bp deletion back to around 5000 BC, which supports the suggested date for the deletions first occurrence to more than 5000 years ago (*Sabeti et al. 2005*).

Site	# extract.	Success. ampl.	WT/WT	WT/Δ32	Δ32/Δ32	^{14}C age BC	Collagen (%)	C/N	C (%)	N (%)	mtDNA haplotype
Skateholm 4	2	–	–	–	–	5250–4900	n.a.	n.a.	n.a.	n.a.	n.a
Skateholm 5	2	–	–	–	–	5250–4900	n.a.	n.a.	n.a.	n.a.	n.a
Skateholm 7	2	–	–	–	–	5250–4900	4.0	3.2	41,1	15,0	n.a
Skateholm 12	2	–	–	–	–	5250–4900	n.a.	n.a.	n.a.	n.a.	n.a
Skateholm 63a	2	–	–	–	–	5250–4900	n.a.	n.a.	n.a.	n.a.	n.a
Skateholm 63b	2	2/2			X	5250–4900	n.a.	n.a.	n.a.	n.a.	n.a
Huseby Klev 1	2	–	–	–	–	7000–6500	0.2	3.8	15.2	3.7	n.a
Huseby Klev 2	2	–	–	–	–	7000–6500	4.3	3.5	42.0	14.1	n.a
Huseby Klev 3	2	–	–	–	–	7000–6500	2.0	3.3	37.7	13.3	n.a
Huseby Klev 4	2	–	–	–	–	7000–6500	1.7	3.3	39.7	13.6	n.a
Visby 2/09	2	2/2		X		3000–2500	3.5	3.1	39.5	14.7	n.a
Visby 2/24	2	–	–	–	–	3000–2500	4.4	3.2	39.2	14.5	n.a
Visby 2/39	2	–	–	–	–	3000–2500	6.0	3.2	42.2	15.4	n.a
Visby 3b	2	–	–	–	–	3000–2500	2.8	3.5	42.4	14.2	n.a
Visby 13	2	–	–	–	–	3000–2500	1.2	3.1	31.6	11.9	n.a
Visby 19	2	–	–	–	–	3000–2500	6.2	3.1	37.5	14.1	n.a
Visby 19/37	2	–	–	–	–	3000–2500	3.6	3.1	44.6	15.3	n.a
Visby 23	2	2/2		X		3000–2500	2.2	3.4	41.9	14.2	n.a
Visby 27	2	2/2		X		3000–2500	1.3	3.5	38.2	12.6	n.a
Visby 30b	2	–	–	–	–	3000–2500	2.3	3.4	39.7	13.4	n.a
Visby 31	2	–	–	–	–	3000–2500	1.7	3.5	42.1	14.0	n.a
Visby 33	2	–	–	–	–	3000–2500	1.9	3.4	37.6	12.6	n.a
Ire 6b m_2	2	–	–	–	–	3028–2141	1.9	3.1	40.1	13.2	n.a.
Ire 6c m_2	2	–	–	–	–	3028–2141	1.1	3.6	33.2	11.1	n.a.
Ire 7a i_1	2	2/2			X	3028–2141	1.2	3.2	31.2	11.5	n.a.
Ire 7a p_3	2	–	–	–	–	3028–2141	1.8	3.1	34.7	11.3	n.a.
Ire 7a m_2	2	2/2			X	3028–2141	1.7	3.2	29.8	10.9	n.a.
Ire 7b dm_2	2	–	–	–	–	3028–2141	2.1	3.3	27.0	9.4	n.a.
Ire 7c i_2	2	–	–	–	–	3028–2141	4.3	3.0	37.9	12.7	n.a.
Ire 7c m_1	2	2/2		X		3028–2141	3.6	3.0	39.0	13.2	n.a.
Ire 7c m_2	2	2/2		X		3028–2141	4.2	3.5	28.3	9.5	n.a.
Jettböle 1	2	–	–	–	–	3370–2910		2.9	n.a.	n.a.	n.a
Jettböle 2	2	–	–	–	–	3370–2910		3.2	n.a.	n.a.	n.a
Jettböle 3	2	–	–	–	–	3370–2910		3.1	n.a.	n.a.	n.a
Rössberga 1	4	3/4		X		3506–2143	2.2	3.2	37.7	135	CRF
Rössberga 2	4	3/4	X			3506–2143	5.8	3.3	40.5	14.5	16224C
Rössberga 3	4	3/4		X		3506–2143	4.0	3.4	33.0	11.3	CRF
Rössberga 4	4	3/4	X			3506–2143	3.1	3.3	37.8	13.2	CRF
Rössberga 5	4	3/4	X			3506–2143	3.8	3.8	33.8	10.3	CRF
Rössberga 6	4	–	–	–	–	3506–2143	n.a.	n.a.	n.a.	n.a.	n.a
Hjelmars rör 17	4	–	–	–	–	3350–2700	n.a	n.a	n.a	n.a	n.a
Hjelmars rör 18	4	3/4	X			3350–2700	5.5	3.3	41.6	14.7	CRF
Hjelmars rör 19	4	3/4	X			3350–2700	5.2	3.3	42.0	14.8	16189C
Hjelmars rör 20	4	3/4	X			3350–2700	5.6	3.2	42.2	15.2	CRF
Hjelmars rör 21	4	3/4	X			3350–2700	2.9	3.2	40.1	14.4	16145A, 16231C 16261T
Hjelmars rör 22	4	3/4	X			3350–2700	3.3	3.2	40.3	14.5	16189C
Dragby H4/a	2	–	–	–		2290–1690	1.0	3.3	36.3	13.0	n.a
Dragby F15 p1	2	2/2			X	2290–1690	0.6	3.2	32.5	12.3	n.a
Dragby F8 p1	2	–	–	–	–	2290–1690	1.0	3.3	30.7	11,4	n.a
Dragby F2 p2	2	–	–	–		2290–1690	0.7	3.1	34.4	12.7	n.a
Site and animal	**# extract.**	**Success. ampl.**	**SCN**			**^{14}C age BC**	**Collagen (%)**	**C/N**	**C (%)**	**N (%)**	
Ire horse	2	2/2	X			3028–2141	6.4	3.2	39.7	14.5	
Hjelmars rör cow	2	2/2	X			3350–2700	5.2	3.2	39.5	14.3	

Tab. 2. Presence of the 32bp deletion of the (Δ32) gene in the prehistoric samples compared to the wild type (WT), (–) = no result (X) = positive indication, when the number of extractions are 2 it indicates that a reproduction has taken place in a laboratory in Madrid, i = incisor, m = molar, p = premolar, n.a. = Not analysed, SCN = Single Copy Nuclear. Collagen % is given as compared to bone weight. Mitochondrial DNA haplotypes are given as compared to Anderson et al. (1981). Samples from Ire represents in total 5 individuals 6b, 6c, 7a, 7b and 7c.

Conclusions

The CCR5–Δ32 results indicate that the frequency of 17.1% in our samples corresponds to the frequencies of CCR5–Δ32 mutation in present-day Sweden. Thus there seems to be no difference in the occurrence of the deletion from the Neolithic and onwards in Sweden.

The mutation occurs in two different Neolithic cultures, both the GRK and the TRB, despite differences in subsistence and lifestyle. This indicates that the selective pressure that caused this deletion to evolve had nothing to do with subsistence or way of life. There is also evidence for the mutation having occured in one of the Mesolithic populations, although the sample size is small and no definite conclusions can be drawn from this. The fact that the CCR5–Δ32 is present during the Mesolithic is interesting in itself, and pushes the date of the first occurrence back to around 5000 BC.

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