Allele frequencies of *AVPR1A* and *MAOA* in the Afrikaner population

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The Afrikaner population was founded mainly by European immigrants that arrived in South Africa from 1652. However, female slaves from Asia and Africa and local KhoeSan women may have contributed as much as 7% to this population's genes. We quantified variation at two tandem repeats to see if this historical founder effect and/or admixture could be detected. The two loci were chosen because they are in the promoters of genes of neurotransmitters that are known to be correlated with social behaviour. Specifically, arginine vasopressin receptor 1A's (*AVPR1A*) RS3 locus has been shown to correlate with age of sexual onset and happiness in monogamous relationships while the tandem repeat in the promoter of the monoamine oxidase A (*MAOA*) gene correlates with reactive aggression. The Afrikaner population contained more *AVPR1A* RS3 alleles than other Caucasoid populations, potentially reflecting a history of admixture. Even though Afrikaners have one of the lowest recorded non-paternity rates in the world, the population did not differ at *AVPR1A* RS3 locus form other European populations, suggesting a non-genetic explanation, presumably religion, for the low non-paternity rate. By comparing population allele-frequency spectra it was found that different studies have confused *AVPR1A* RS3 alleles and we make some suggestions to rectify these mistakes in future studies. While *MAOA* allele frequencies differed between racial groups, the Afrikaner population showed no evidence of admixture. In fact, Afrikaners had more 4-repeat alleles than other populations of European origin, not fewer. The 4-repeat allele may have been selected for during colonisation.

Introduction

The Afrikaner population of South Africa derives from about the same proportion of German, French and Dutch immigrants that came to the Cape from 1652 to 1806.[1] Because the number of immigrants was finite, the Afrikaner population is considered a textbook example of a founder effect[2], with many genetic diseases in overabundance compared with European populations[3-5]. However, as many as 5000 European men settled at the Cape between 1657 and 1866.[6] As most of the immigrants were men, they occasionally married non-European women[6,7] who were either slaves from Africa and India (including Indonesia and East Asia) or local Khoe and San (KhoeSan) women[6-9]. This practice is reflected genetically by the presence of non-European alleles in the Afrikaner population.[10]

The Afrikaner population fought several local wars against the KhoeSan, Xhosa, Zulus and British. It could be argued that these aggressive encounters may have been frequent and severe enough to have left traces of selection on the population. One gene that may have played an important role in this regard is monoamine oxidase A (*MAOA*) which breaks down serotonin and dopamine and which has been linked to increased reactive aggression.[11-14]

Reactive aggression in humans may be affected by the alleles they carry at the variable number of tandem repeats (VNTR) in the promoter region of the *MAOA* gene, which is located on the X chromosome at Xp11.23.[11-15] These VNTRs occur in 2, 3, 3.5, 4 or 5 repeats of 30 base pairs (bp).[13,16] These repeats can be classified as either high or low activity alleles, with the 2, 3 and 5 repeats constituting the low activity (*MAOA*-L) alleles and the 3.5 and 4 repeats the high activity (*MAOA*-H) alleles.[12] The *MAOA*-H allele has a 2–10 times more effective transcription than the *MAOA*-L alleles.[13,16,17] Others[11,12,18] have determined that carriers of *MAOA*-L react more aggressively in provocational circumstances than their *MAOA*-H counterparts. The *MAOA*-H carriers better tolerated maltreatment and were also less likely to develop antisocial traits.[18]

Recent studies revealed a very low non-paternity rate of less than 1% in the Afrikaner population[19,20] (see Greeff and Erasmus[21] for an exception to the rule). Strong religious convictions, as was the case for Afrikaners[22], have been suggested as an important determinant of marital fidelity[23]. However, the low rate of non-paternity in Afrikaners may have a genetic component: two studies point to the potential importance of arginine vasopressin receptor 1a (*AVPR1A*) in this context. Prichard et al.[24] have shown that age of first sexual encounter is correlated to repeat length of alleles of *AVPR1A*. Similarly, Walum et al.[25] have shown that certain alleles of *AVPR1A* seem to predispose their carriers to a less fulfilling monogamous life. Given Afrikaners' low non-paternity rates, it is of interest to quantify *AVPR1A* for this population.

AVPR1A is located at 12q14-15 and there are three polymorphic repeat regions in its 5' flanking region.[26] One of these, a complex$(CT)_4$-TT-$(CT)_8$-$(GT)_{24}$ repeat known as RS3, is 3625 bp upstream from transcription initiation[26] and has been linked to human social behaviour in a number of studies. Given the early lead from voles in which longer microsatellite length results in higher levels of transcription[27], functional magnetic resonance imaging showed that carriers of longer repeats had significantly stronger activation of their amygdala upon an emotional test[28]. Similarly, longer *AVPR1A* RS3 alleles were found to be significantly more transcribed in post-mortem examination of the hippocampal area of humans.[29] From the behavioural side, male individuals with two long alleles are significantly more likely to have sexual intercourse before the age of 15 than male individuals with a short/long genotype.[24] Individuals with longer alleles are also more likely to be altruistic in the dictator game.[29] Other studies have linked specific alleles with altruism in pre-schoolers[30], happiness in monogamous relationships[25], social behaviour and autism[31-34], musicality[35-37], creative dance[38] and eating attitudes[39].

Both of these genes, *AVPR1A* and *MAOA*, could have unusual frequencies in the Afrikaner population because of the founder effect and/or admixture and may have affected the population's average behaviour. The aim of this study was to characterise the frequencies of *AVPR1A*'s RS3 microsatellite and the *MAOA* VNTR alleles in the Afrikaner population and to compare them to other populations. However, comparing allele frequencies across populations highlighted a problem with standardised allele calls at the RS3 locus.

Materials and methods

Sample collection

Ethical clearance was obtained from the Ethics Committee of the Faculty of Natural and Agricultural Sciences, University of Pretoria (no. EC11912-065). A total of 200 male volunteers from the Afrikaner population were confirmed not to be fourth-degree relatives through self-supplied ancestries and the majority were clustered into 23 groups that are very distantly related by paternal ancestry (at least 14 degrees). Note that this is no more than can be expected of random individuals.[9] These men considered themselves Afrikaners and have typical Afrikaner surnames. All volunteers completed an informed consent form and signed an agreement which stated that they understood that their DNA was going to be used for analysis and that they donated it willingly. The study adhered to the principles of the Declaration of Helsinki. Saliva samples were collected from participants using the Oragene-DNA self-collection kit supplied by DNA Genotek (Kanata, Ontario, Canada) and genomic DNA was isolated according to the manufacturer's instructions.

Genotyping

For both loci, the polymerase chain reaction (PCR) set-up consisted of 50 ng DNA, 1X AmpliTaq® 360 Buffer (Applied Biosystems, Foster City, CA, USA), 20 μM dNTPs, 250 μM $MgCl_2$, 0.4 μM forward and reverse primers, 2% AmpliTaq® 360 GC Enhancer and 1.25 units AmpliTaq® 360, in a final reaction volume of 10 μL, and the PCR reactions were run in the 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The basic PCR cycle was repeated 35 times and included an initial denaturation step of 5 min at 95 °C and a final elongation step of 5 min at 72 °C.

MAOA

The genomic DNA of the promoter region in the *MAOA* gene was PCR amplified for subjects with the primer MAOaPT1 5'-ACAGCCTGACCGTGGAGAAG-3' and MAOaPB1 5'-GAACGGACGCTCCATTCGGA-3'.[16] The cycling conditions were as follows: 95 °C denaturation step for 1 min followed by primer annealing at 62 °C for 1 min and elongation at 72 °C for 1 min. The PCR products were separated on 3% agarose gels, with a 20-bp ladder (Promega, Madison, WI, USA). Six bands were excised from the gel and purified with the High Pure PCR purification kit (Roche Diagnostics, Germany) and were successfully sequenced with BigDye (Applied Biosystems, Foster City, CA, USA). Cycle sequencing products were purified with ethanol precipitation and ran on a 3130xl genetic analyser (Applied Biosystems, Foster City, CA, USA). These sequences were aligned to the reference sequence from the National Centre for Biotechnology Information (GenBank: M89636.1) with BioEdit version 7.2.2[40] and three unique sequences were deposited in the European Nucleotide Archive (accession numbers LN813020 – LN813022). Based on size differences, cases with 3, 4 and 5 repeats were selected to serve as size markers for the identification of the remaining samples on the gel.

AVPR1A

The RS3 microsatellite of *AVPR1A* was amplified with a labelled forward primer 5'-6-FAM-TCCTGTAGAGATGTAAGTGC-3' and the reverse 5'-GTTTCTTTCTGGAAGAGACTTAGATGG-3'.[32] Cycling conditions were as follows: denaturation at 94 °C for 1 min, primer annealing at 54.7 °C for 45 s and elongation at 72 °C for 1 min. Amplicons were run on an ABI 3500 XL genetic analyser (Applied Biosystems, Foster City, USA). The final allele lengths were scored with GeneMapper software version 4.1.1 (Applied Biosystems, Foster City, USA). Individuals homozygous for alleles 332, 334, 336, 338, 340, 342 and 346 were sequenced with the PCR primers to determine the actual number of CT and GT repeats and the sequences were deposited in the European Nucleotide Archive (accession numbers LN812321 to LN812340).

Comparable allele frequencies

Like other microsatellites, *AVPR1A* RS3 allele calling can easily vary among studies because of different Taq, dye, polymers, size standards and machines, which prevents comparisons among studies. To complicate matters further, different studies have used primers that result in different sized amplicons. Working from the sequence published by Thibonnier et al.[26] (AF208541), primer sets can result in an amplicon of 260 bp[41], 324 bp[25,28,32,37] (and the present study), 316 bp[29-31,33,38,39] or 317 bp[34], while others do not report primers used[36]. Fortunately, the allele spectrum for Caucasoids has a very characteristic profile that can be used to slide the alleles along so that they align well (compare Figure 1a and 1b). It can be seen that typically there are two alleles that are much more frequent than the others, and that there is another frequent allele that is 5 repeat units larger than the biggest common allele (Figure 1). We used this allele profile to make data sets comparable.

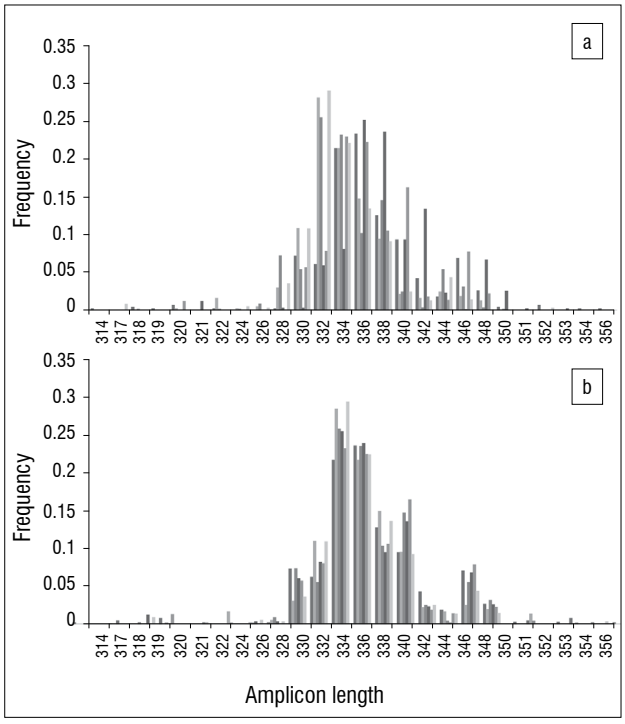

Figure 1: The population frequencies for *AVPR1A* alleles when (a) expected amplicon differences caused by primer differences are taken into account and (b) allele sizes are adjusted so that allele-frequency spectra of populations correspond best with each other. The data are drawn from Table 2.

Statistical analysis

For both loci, pairwise F_{ST}s were calculated between the populations using Arlequin version 3.5.1.2.[42] For *AVPR1A* we also compared the Afrikaner's frequency of allele 334 to that of the other populations by first testing if the allele's frequency varied significantly over all five population samples (prop.test as implemented in R[43]). Then the two European populations[32,37] and the two Israeli samples[30,33] were combined and compared to each other and the Afrikaner population in a pairwise proportion test as implemented in R (pairwise.prop.test).[43]

Results

MAOA

Sequencing confirmed that we were amplifying the correct DNA and we used these confirmed allele sizes as standards for electrophoresis. The frequencies of the observed alleles in the Afrikaner population and other populations are summarised in Table 1. The 3 and 4 repeat alleles were most frequent and varied across populations (Table 1; Figure 2). Pairwise F_{ST} values split the populations into two groups (Supplementary table 1 online). One group consisted of those of European descent for whom the 4-repeat allele was more common (Figure 2, clear symbols), and the other group consisted of those of African and Asian descent for whom the 3-repeat allele was more frequent (Figure 2, filled circles). Within each group, F_{ST} values were generally smaller than 0.01, and F_{ST} values between groups were mostly greater than 0.1 and significantly different (all $p<0.001$). A sample from Italy was significantly different from the Afrikaner population but not significantly different from the African American sample. Interestingly, two of the admixed populations – Hispanics and Afrikaners – did not fall in between European and non-European populations but had a higher frequency of the 4-repeat allele.

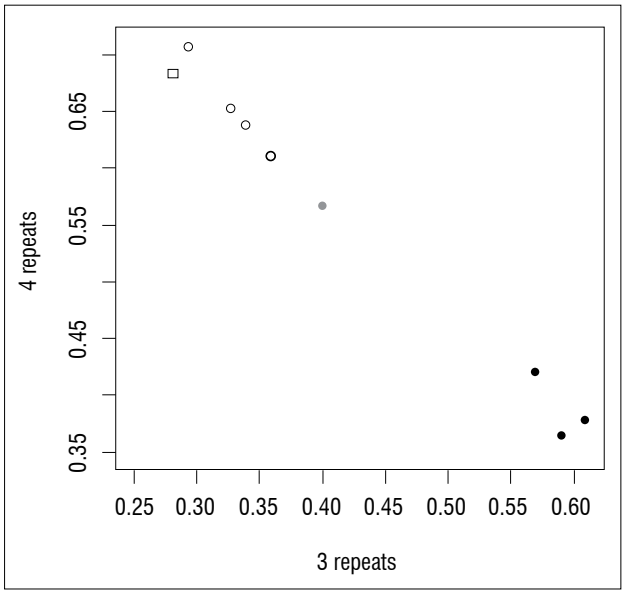

Figure 2: The frequencies of the two common *MAOA* alleles for the 10 populations included in Table 1. Open symbols indicate the six populations of a mostly European background that are not significantly different from one another and the filled symbols indicate the Asian and African populations. The grey circle represents the Italian population which differs significantly from the Afrikaner population (indicated by the square symbol), but not from the African American population.

AVPR1A

Alleles that contained a combined number of 32 CT and GT repeats ran to a length of 334 on our machine (Table 2). We identified 20 alleles in the Afrikaner population whereas the other studies identified 15 or 16 alleles (Table 2). Afrikaners had a higher expected heterozygosity of 0.86 compared to values ranging from 0.83 to 0.84 (Supplementary table 2). For population comparisons it was impossible to align the allele frequencies of one Asian study[34] with those of the Caucasoid populations because the allele frequency spectrum did not have the characteristic hallmarks of the locus in Caucasoid populations (Figure 1). As a result, no African or Asian populations could be compared to the Caucasoid populations. The frequencies of the adjusted allele sizes in other studies and ours are given in Table 2. With the allele sizes used as published and corrected for primer differences, pairwise F_{ST}s were as high as 0.07 and all populations differed significantly from one another (Supplementary table 3). With the corrected sizes as given in Table 2, the F_{ST} values were all lower than 0.006 and mostly considerably lower (Supplementary table 4). The two Israeli samples[30,33] were not significantly different from one another ($p=0.775$) but were significantly different from the other populations (all $p<0.045$), which in turn were not significantly different from one another (all $p>0.11$). The frequency of allele 334 differed among the population samples ($\chi^2 = 16.723$, df = 4, $p=0.0022$) as follows: Afrikaners = 0.21; Israeli 1[30] = 0.28; Israeli 2[33] = 0.29; British[37] = 0.25; and American, mainly Caucasoid[32] = 0.25. The pairwise test suggests that the Israeli sample had a significantly higher frequency of allele 334 than the Afrikaner ($p=0.006$) and European populations ($p=0.010$), but that the latter two did not differ significantly from each other ($p=0.143$).

Table 1: The observed number of the *MAOA* alleles in the Afrikaner population and in nine other populations

	Alleles					
Population group	**2**	**3**	**3.5**	**4**	**5**	***N***
Hispanic/Latino[a]	0	27	0	65	0	92
Afrikaner	0	55	0	134	7	196
White/non-Hispanic[a]	0	529	8	1056	26	1629
New Zealand, European origin[b]	3	658	9	1238	32	1940
German, European origin[c]	0	47	1	80	3	131
German, European origin[d]	3	140	3	238	6	390
Italian, European origin[c]	3	72	0	102	3	180
Chinese[e]	1	122	0	90	1	214
Asian/Pacific Islander[a]	0	50	1	31	0	82
African American[a]	0	52	2	32	2	88

Sources: [a]Sabol et al.[15]; [b]Caspi et al.[18]; [c]Deckert et al.[44]; [d]Kuepper et al.[13]; [e]Lu et al.[45]
Alleles 2–5 refer to the number of times the repeat element is repeated and N *is the total sample size. Populations are arranged in decreasing frequency of the allele with 4 repeats.*

Discussion

The two loci we considered provided two very different depictions. *MAOA* did not reveal any traces of admixture in the Afrikaner population as its allele frequency was displaced away from the African (as gauged from African American frequencies) and Asian populations rather than towards them (Figure 2). On the other hand, the *AVPR1A* showed an increased number of alleles in the Afrikaner population compared to other European populations, which could indicate the influence of admixture with older African and KhoeSan populations.[6] Neither locus suggested a strong deviation from European frequencies caused by a founder effect.

The unexpected high frequency of the 4-repeat allele of *MAOA* in the Afrikaner population (Figure 2) requires an explanation. We need to take into account that the founder effect was more severe for female individuals in the population[9]; despite an influx of male individuals, there was no such influx of female individuals.[6,9] In addition, because male individuals contribute only a single X chromosome, X chromosomes may have experienced a more severe bottleneck than other autosomal chromosomes. If we also consider that all non-European genetic contribution to this population was female derived,[6,7,9] it seems questionable that the frequency could be skewed away from African and Asian frequencies. It also is interesting that one of the other populations with an admixed heritage, the Latinos from America, also had a higher frequency of the 4-repeat allele (Figure 2).

Table 2: Allele frequencies at the *AVPR1A* RS3 locus for the Afrikaner and seven other populations

x=y[a]	Allele	Afrikaners	Israeli[b]	Israeli[c]	Israeli[d]	European origin			
						American[e]	British[f]	Swedish[g]	American[h]
	Correction[i]	0	+9	+9	+9	+2	-1	0	+2
	314	1							
	317			7					
	318	2							
	319	1		62			13		
	320	3				1		21	1.3
	322	1	1						
	324		6			1			
	326		1	39		1	4		
	328	1	2	26	0.6	4	4		0.2
	330	27	11	249	4.3	32	61	92	6.1
30	332	23	39	746	13.1	24	83	128	9.3
32	334	80	101	2000	24.4	112	257	**371**	22.2
33	336	87	**77**	1528	**19.2**	102	241	359	21.3
34	338	47	53	929	13.6	45	96	170	12.0
35	340	35	34	630	9.8	64	137	263	10.2
36	342	16	8	173	**2.4**	11	24	30	1.7
	344	7	6	95	2.4	2	2	23	2.8
38	346	26	9	304	6.1	24	69	126	8.3
	348	10	7	101		14	27	37	3.5
	349						3		
	350	2	5		2				0.2
	351						3		
	352	3			1				0.2
	354	1		26					
	356	1							0.2
	Total	374	360	6915		440	1024	1620	

Alleles in bold indicate alleles that were significantly linked to behavioural traits, whereas underlined alleles showed non-significant linkage with traits.
[a]The combined number of CT and GT repeats confirmed by sequencing for the Afrikaner population. Thibonnier et al.'s[26] $(CT)_8(GT)_{24}$ corresponds with allele 334.
[b]Avinum et al.[30] The authors kindly provided us with the allele frequencies of the parent population. Allele 336 was significantly linked to reduced altruistic behaviour.
[c]Yirmiya et al.[33] frequencies from control samples. While RS3 locus did not show a significant link with autism, it did so in the proband when combined in haplotypes with other SSR loci of AVPR1A*.*
[d]Bachner-Melman et al.[39] relative frequencies based on 280 families. Not used for population frequency estimates.
[e]Kim et al.[32] 82% of the sample were Caucasoid, the remainder were African American, Asian American and Hispanic. Samples were from parents of autistic children. Allele 342 was marginally over-transmitted.
[f]Morley et al.[37] British choral singers and controls combined as RS3 had no significant effect.
[g]Walum et al.[25] This is not a random sample as twins were genotyped and so should not be used for population genetic comparisons. However, the allele spectrum should show similar hallmarks. Allele 334 was linked to reduced happiness in monogamous relationships.
[h]Meyer-Lindenberg et al.[28] relative frequencies based on 258 healthy people with an European ancestry. Allele 336 had the highest activation of amygdala, and overall, longer alleles had significantly stronger activation than shorter ones.
[i]Number of base pairs added to reported allele sizes to match allele frequency spectra best (compare to Figure 1).

This finding may suggest that this allele may integrate more easily into a heterogeneous genetic background or that this small deviation simply stems from a small founder effect. Another explanation may be selection on the social phenotype of this allele. If *MAOA*-H (4-repeat allele) carriers are less likely to react aggressively in provocational circumstances[12,13] and by extension be less likely to be berserkers in war scenarios, cope better with maltreatment[18], and be less likely to develop antisocial behaviour[11], then the allele may have been selected for in this founding population. It is, however, important to note that this allele's frequency is not significantly higher in the Afrikaner population and we should caution against over-interpreting this result as such.

For *AVPR1A* RS3, it is firstly important to make sure that alleles compared among studies are indeed the same. Because of the complex nature of *AVPR1A* RS, the various repeat alleles that run to the same length may differ in their proportions of GT and CT repeats.[41] However, for a number of studies in which microsatellites in the promoter regions affect expression of the allele it seems to be the length rather than the content that is important.[41] In addition to this complication, it is easy to systematically call alleles a number of base pairs shorter or longer when their identity is inferred from the rates of amplicon migration in different genetic analysers and/or when labelled with different dyes etc.; for this reason, it is important to standardise allele size in some way. We followed two approaches that were both effective. Comparable lengths can be obtained either by sequencing amplicons to confirm their length or by comparing allele-frequency spectra between populations. In this light, it is important to note that this study only considered Caucasoid populations, and the same characteristic peaks may not be observed in other populations. In fact, it was impossible to align the Asian study[34] with the Caucasoid ones.

Confusion over which alleles are which is not trivial. The Israeli group has correctly linked their allele 327 to allele 334 from the Meyer-Lindenberg et al.[28] and Kim et al.[32] studies, but Walum et al.'s[25] 334 is in fact one base pair repeat longer than those in these other studies. As several researchers are comparing the effects of this locus on many behavioural patterns, it is important to have a gold standard to avoid confusion. We have compiled a ladder that can be used for such standardisation that is available on request. The confusion is not limited to comparisons of specific alleles; binning of alleles into groups of short, medium and long alleles can also lead to confusion. For instance, Prichard et al.[24] classified short alleles as those that have 12–19 repeats, medium alleles as those with 20–21 repeats and long alleles as those with 22–29 repeats; however, these numbers of repeats are substantially lower than those observed in this and other[26] studies (Table 2).

While the Afrikaner population had a lower frequency of the 334 allele, the frequency was not significantly lower and it would be premature to link their low levels of non-paternity with their low frequency of this allele. It is more likely that the Afrikaners' strong religious convictions[24] could explain their low non-paternity rate[19,20]. The fact that the Israeli samples had a significantly higher frequency of allele 334 suggests that an investigation into marital happiness may be interesting for this population.

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Authors' contributions

A.K. performed the work under the mentorship of J.C.E. and J.M.G. J.C.E. and J.M.G. performed the analyses. A.K. wrote the initial draft of the manuscript, J.M.G. made major editorial adjustments and A.K., C.J.E. and J.M.G. made further editorial adjustments; all authors approved the final version.

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