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Aggression and polymorphisms in *AR*, *DAT1*, *DRD2*, and *COMT* genes in Datoga pastoralists of Tanzania

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The aim of this study was to analyse the relationships between polymorphisms in four candidate genes (*AR*, *DAT1*, *DRD2*, and *COMT*) and aggression in men from a traditional society of East African pastoralists, the Datoga. Buss and Perry's Aggression Questionnaire was used to measure aggression. The number of CAG repeats in the *AR* gene was negatively correlated with physical aggression, anger, and hostility. Among the genes of the dopaminergic system, a significant single-gene effect was detected only for *DRD2* with regard to anger. At the level of a two-gene model, a significant effect for *DRD2* and a tendency for *DAT1* were observed for the *DAT1-DRD2* gene pair regarding hostility, and two tendencies were observed for the interaction effect of the *DAT1-COMT* pair regarding anger and hostility. These data suggest a probable link between physical aggression and direct fitness caused by strong sexual selection in Datoga men.

Aggressive behaviour in humans is highly sexually dimorphic, with men exhibiting more violent (physical) same-sex aggression than women in almost all cultures[1–4], which is most likely attributable to the greater impulsiveness of men and a stronger fear of physical danger in women[5,6]. A meta-analysis of 24 genetic studies on aggression demonstrated that heritability accounts for approximately 50% of the variance in aggression[7,8], with the heritability of aggressive behaviour being higher in men than in women[9]. Aggressive behaviour (physical and verbal) and its negative emotional bases (anger and hostility) are the products of complex interactions between many genes associated with the secretion of steroid hormones and the sensitivity of tissues to these hormones. These genes include those associated with the dopaminergic and serotonergic systems (both receptors and transmitters) and likely many others[10–12].

The results of some studies suggest a relationship between exposure to testosterone in utero and subsequent aggression in adulthood as a result of masculinisation of the brain[13,14]. Individual behavioural reactions to testosterone have also shown a close dependence on the sensitivity of brain tissue to this steroid. Androgen receptor genes are located on the X chromosome and are therefore only inherited by men from their mothers. Earlier studies, predominantly conducted among representatives of industrial populations, appear to support the hypothesis of a negative correlation between an increasing the number of CAG repeats in the androgen receptor gene (*AR*) and physical aggression and/or the 2D : 4D ratio (the ratio between the lengths of the second and fourth digits, an indicator of the masculinisation of the foetus in utero)[15]. Another study reported that fewer CAG repeats in *AR* were more frequently found in male rapists and murderers than in control subjects[16]. Indeed, an increased number of these repeats in *AR* results in receptors with lower androgen sensitivity[17,18]. Manning and colleagues demonstrated in a male sample from an industrial population that masculine finger ratios are associated with androgen receptor alleles with fewer CAG microsatellite repeats in the terminal domain[15]. In contrast, a recent study performed by our group in traditional hunter–gatherers, the Hadza of Tanzania, revealed no correlation between the number of CAG repeats and aggression or the right-hand 2D : 4D ratio in Hadza men[19]. Thus, the

recent data on the associations between *AR* polymorphisms and aggression are somewhat contradictory and demand a more thorough investigation in other small-scale societies.

Many recent molecular genetic studies on aggression have focused on the genes involved in dopamine neurotransmission. The distributions of polymorphic loci in the dopamine receptor genes *DRD4* and *DRD2* and the dopamine transporter gene *DAT1* were analysed to identify an association between specific alleles and aggressive behaviour (particularly obsessive–compulsive disorder, hostility, and physical aggression), some mental disorders, and alcohol addiction[20–27]. Another gene associated with aggression is the gene encoding catechol-O-methyltransferase (*COMT*), which plays a key role in dopamine catabolism in the prefrontal cortex: the V158M polymorphism in the fourth exon of the *COMT* gene leads to a 40% reduction of enzymatic activity in carriers of the M allele[28].

Dopamine plays a critical role in reward processing and is a potent neuromodulator of ventral striatum (VS) reactivity, which is known to be highly involved in reward processing. VS reward reactivity may be a key neurobiological pathway through which *DAT1* and *DRD2* polymorphisms contribute to the variability of behavioural impulsivity[29].

DAT1 and *DRD2* polymorphisms were examined in adolescents with pathological aggression, and it was found that carriers of the *DAT1* 10/10 genotype were overrepresented in a sample of aggressors compared to the control group and that *DRD2* A1/A1 and A1/A2 were also overrepresented in aggressors[30]. Vaughn and colleagues[31] suggested *DAT1* as one of the main genetic markers of antisocial behaviour in adolescent criminals. According to another study, the *DAT1* 10/10 and 10/9 genotypes are present in violently delinquent young adults twice as often as in controls[26,32–34]. Guo and co-authors[26] demonstrated that heterozygotes for the *DRD2* A1/A2 variant presented higher aggression scores than A1/A1 or A2/A2 homozygotes; these authors further suggested that there is no correlation or epistasis between *DRD2* and *DAT1*.

Remarkably, the data from a nationally representative sample of American youth revealed that genetic risk factors measured based on two dopamine polymorphisms (*DAT1* and *DRD2*) confound the association between paternal incarceration and a child's arrest[35].

A serious problem with the current studies on the molecular genetics of aggression and antisocial behaviour is that such research frequently cannot be replicated[36]. Moreover, a recently conducted meta-analysis of genetic association studies on violence and aggression provided a rather pessimistic outlook, indicating that the candidate gene approach has not succeeded in identifying genes associated with these outcomes[37]. One of the general explanations for this difficulty is that "there are few, if any, loci with large effect size, and it is becoming increasingly obvious that it will be necessary to consider the impact of genes, not in isolation, but as part of a multifactorial miasma including both other genetic factors and the environment"[9]. Because most studies are based on clinical samples or samples that are not nationally representative and each sample is likely differentially exposed to particular environmental conditions, certain genes may only have effects when they are paired with certain environments[38]. Another possible reason for the lack of reproducibility in this arena is population stratification. Indeed, the allelic frequencies of some genes vary significantly across different racial/ethnic categories[39], and genetic effects may vary across racial/ethnic groups because of (a) differences in allelic distributions and (b) differences in exposure to environmental risk factors[38].

Thus, there is an urgent need to examine the associations between certain genes and aggressive behaviour in samples of various racial/ethnic groups. In this study, we examined the single-gene effects of the *AR* gene and three dopaminergic genes (*DAT1*, *DRD2*, and *COMT*) as well as the two-gene associations of components of the dopaminergic system with aggression in a sample of Datoga, who are traditional East African pastoralists.

Table 1 | Descriptive statistics of aggression (AQ) subscales

	Basic Statistics		
	PhA	Ang	Hostil
N	138	135	138
Mean	28.710	22.859	29.138
Std. Dev.	4.890	4.302	5.109
Minimum	16	11	13
Maximum	42	34	40

PhA – physical aggression, Ang – anger, Hostil – hostility.

The following hypotheses were tested:

1. Men showing lower *AR* CAG repeat numbers exhibit higher aggression scores, particularly for physical aggression and negative emotions.
2. Male carriers of the +10 type of *DAT1* (homozygous or heterozygous) present higher aggression scores.
3. Men heterozygous for *DRD2* display higher aggression scores compared to both types of homozygotes.
4. Men homozygous for the high-activity Val/Val genotype of *COMT* show higher aggression scores.
5. Two-gene interaction effects between the three genes in the dopaminergic system (*DAT1*, *DRD2*, and *COMT*) provide important information on the associations of these genes with various types of aggression.

Results

The means and standard deviations for Buss and Perry's Aggression Questionnaire subscales were computed as well as minimum, and maximum scores for Datoga males (Table 1).

We performed a simple regression analysis to test the single-gene effect of the *AR* gene on aggression and detected significant effects of the *AR* CAG polymorphism on physical aggression and hostility, with carriers of smaller numbers of CAG repeats showing a higher rating (Tables 2, and Figs. 1a,b,c). The results for *AR* and the self-rated scores for anger exhibited a trend in the same direction (Table 2). Therefore, our data show that men with fewer CAG repeats behave more aggressively than their fellow males with a greater number of *AR* CAG repeats.

We split our sample according to *DAT1* genotype, as suggested previously by a number of authors[26,30,32]. The first group included men with the 9/9 genotype of the *DAT1* (−10) type, and the second group included individuals with the 9/10 or 10/10 genotype of the *DAT1* (+10) type. One-way ANOVA with *DAT1* type as the independent variable and aggression traits as the dependent variables revealed no significant association between the +10 type and all AQ scales (Table 3).

Table 2 | Simple regression analyses. Main effects of *AR* gene polymorphism on physical aggression, anger and hostility in Datoga males

AQ Scales	N	R^2	β	t	P
PhA	87	0.105	−0.3234	−3.0189	0.0042**
Ang	85	0.080	−0.2827	−2.6025	0.0197^{+}
Hostil	86	0.069	−0.2531	−2.3104	0.0142*

PhA – physical aggression, Ang – anger, Hostil – hostility, N – the sample size, R^2 – proportion of the total variability explained by the factor effect, β – coefficient of regression, t – *t*-statistic;
*$P \leq 0.05$;
**$P \leq 0.01$;
$^{+}P \leq 0.1$ (adjusted for multiple correlated outcomes).

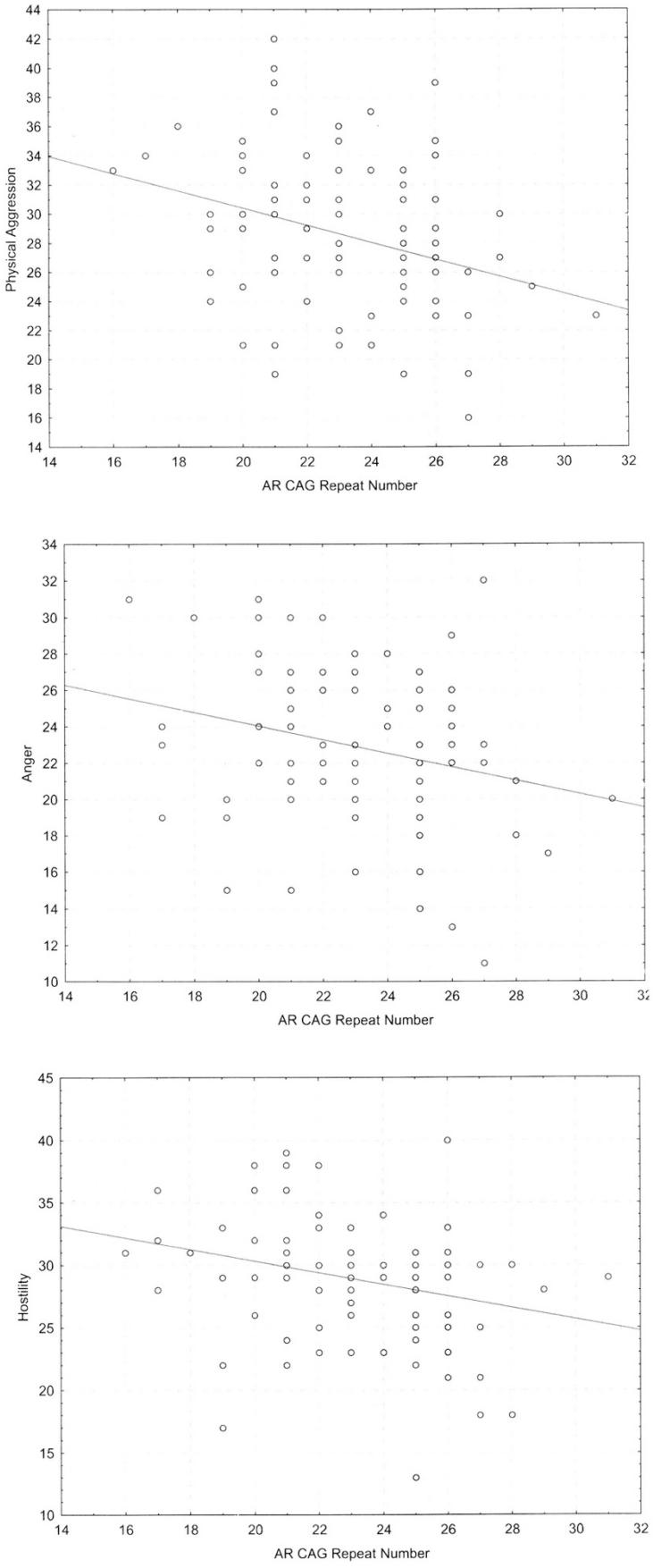

Figure 1 | The distributions of the physical aggression (A), anger (B), and hostility (C) subscales as correlated with the number of CAG repeats in the *AR* gene. The ordinate numbers designate the self-rated scores according to the subscales of Buss and Perry's Aggression Questionnaire.

Table 3 | One-way ANOVA. Main effects of *DRD2*, *DAT1*, and *COMT* genes on physical aggression, anger and hostility in Datoga males

AQ Scale	Source of variability	DF	SS	MS	F	P
PhA	*DAT1*	1	92.43	92.43	3.383	0.0688
	Error	101	2759.96	27.33		
	DRD2	1	14.10	14.10	0.502	0.4804
	Error	101	2838.29	28.10		
	COMT	2	0.93	0.46	0.016	0.9837
	Error	101	2862.06	28.34		
Ang	*DAT1*	1	96.04	96.04	5.099	0.0262
	Error	98	1845.80	18.83		
	DRD2	1	249.64	249.64	14.457	**0.0002**
	Error	98	1692.20	17.27		
	COMT	2	28.48	14.24	0.726	0.4865
	Error	98	1922.51	19.62		
Hostil	*DAT1*	1	89.37	89.37	3.242	0.0748
	Error	100	2756.47	27.56		
	DRD2	1	146.83	146.83	5.440	0.0217
	Error	100	2699.01	26.99		
	COMT	2	12.53	6.26	0.218	0.8042
	Error	100	2868.50	28.69		

PhA – physical aggression, Ang – anger, Hostil – hostility, DF – degrees of freedom, SS – sum of squares, MS – mean square, F – F-test, P – probability.

Our sample was also split into three groups based on *DRD2* polymorphisms, with group 1 including men with the A1/A1 genotype, group 2 consisting of men with the A1/A2 genotype, and group 3 comprising individuals with the A2/A2 genotype, following the divisions originally employed in a previous study by Guo and colleagues[26]. Significant single-gene effects of *DRD2* in the Datoga men were found only with regard to anger (Fig. 2). Lastly, we divided our sample into three groups according to *COMT* polymorphisms: group 1 (V/V), group 2 (V/M), and group 3 (M/M); however, no single-gene effect of *COMT* was identified in this study (Table 3).

We subsequently performed a factorial two-way ANOVA to test both the main and interaction effects of the three genes (*DAT1*, *DRD2*, and *COMT*) on three aggression subscales (Tables 4). The two-gene model revealed two significant main effects of the *DRD2* gene on anger and hostility, showing a tendency for a main effect of *DAT1* on hostility. Tendencies of *DAT1* × *COMT* interaction effects on anger and hostility, but not on physical aggression, were also observed (Fig. 3a,b).

Discussion

Overall, we found that the polymorphisms in all four genes tested in this study affected the self-rated aggression scores of the Datoga men, with either single-gene effects or interaction effects between these genes being detected. Our data also confirmed our initial hypothesis regarding the single-gene effect of *AR* on the self-rated scores for the three AQ scales. Our results regarding the association between aggression and the number of CAG repeats in *AR* were in the predicted direction: the levels of physical aggression, anger, and hostility among the Datoga men were higher in individuals with fewer CAG repeats. Interestingly, we recently demonstrated that individuals with a small number of CAG repeats are overrepresented among world-class judo sportsmen compared to the general Russian population[40]. At the same time, these data contradict those obtained by Hurd, Vaillancourt, and Dinsdale[41] in Canadian students, where men with more sensitive androgen receptors tended to score lower for physical aggression and anger. The results also contradict our early findings in Hadza men, for whom no correlations were found between the AQ subscales and the number of *AR* CAG repeats[42]. A reasonable explanation for these differences may be the existence of gene–environment effects and may be particularly attributable to differences in cultural attitudes toward aggression among the Hadza and Datoga populations.

The significant single-gene effects of *DAT1* polymorphisms on anger and hostility were in the direction predicted in the second hypothesis. Therefore, not only is +10 DAT1 overrepresented in samples of antisocial adolescents in the USA[31], but it is possible that these genotypes are positively selected in pastoralist cultures because such personalities are better suited to both cattle raiding and cattle protection. Although significant single-gene effects of the *DRD2* polymorphism on anger and hostility were also demonstrated, contrary to our expectations, the carriers of the heterozygous type displayed the same high levels of these characteristics as the homozygous A2/A2 men. We did not find any single-gene effect of *COMT* in this study; therefore, our fourth hypothesis should be rejected. Lastly, we demonstrated tendencies toward interaction effects between *DAT1* and *COMT* with regard to anger and hostility in Datoga males. According to our findings, these interaction effects are only essential for carriers of the different COMT genotypes and the –10 DAT1 type.

Guo and colleagues[26] demonstrated that an interaction between *DAT1* and *DRD2* produces essentially the same effect on aggression

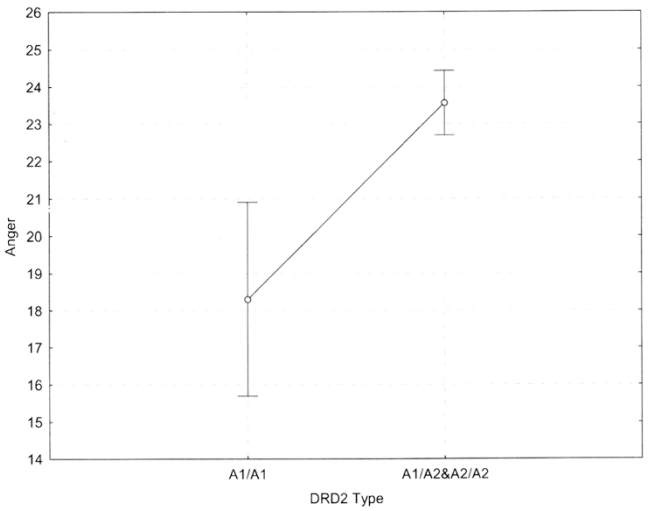

Figure 2 | The distribution of the anger subscale as correlated with the *DRD2* type (1 – A1/A1 and 2 – A1/A2 and A2/A2). The designations of the numbers are described in Figure 1.

Table 4 | Two-way ANOVA. Main effects of *DRD2*, *DAT1*, and *COMT* genes and effects of interactions between them on physical aggression, anger and hostility in Datoga males

AQ Scale	Source	DF	SS	MS	F	P
PhA	*DAT1*	1	1.589	1.589	0.058	0.8101
	DRD2	1	7.252	7.252	0.265	0.6079
	DAT1 × *DRD2*	1	36.422	36.422	1.330	0.2515
	Error	99	2710.475	27.379		
	DAT1	1	64.30	64.30	2.444	0.1215
	COMT	1	0.55	0.55	0.021	0.8853
	DAT1 × COMT	1	1.40	1.40	0.053	0.8182
	Error	90	2368.26	26.31		
	DRD2	1	18.25	18.25	0.678	0.4125
	COMT	1	3.70	3.70	0.137	0.7117
	DRD2 × COMT	1	3.10	3.10	0.115	0.7352
	Error	90	2423.40	26.93		
Ang	*DAT1*	1	63.645	63.645	3.8444	0.0528
	DRD2	1	134.045	134.045	8.0969	**0.0054**
	DAT1 × *DRD2*	1	6.876	6.876	0.4153	0.5208
	Error	96	1589.284	16.555		
	DAT1	1	41.85	41.85	2.312	0.1320
	COMT	1	42.10	42.10	2.326	0.1309
	DAT1 × COMT	1	138.50	138.50	7.651	*0.0069*
	Error	87	1574.94	18.10		
	DRD2	1	179.80	179.80	10.195	**0.0020**
	COMT	1	10.85	10.85	0.615	0.4349
	DRD2 × COMT	1	4.38	4.38	0.248	0.6197
	Error	87	1534.35	17.64		
Hostil	*DAT1*	1	176.260	176.260	6.856	*0.0102*
	DRD2	1	221.310	221.310	8.609	**0.0042**
	DAT1 × *DRD2*	1	90.753	90.753	3.530	0.0632
	Error	98	2519.379	25.708		
	DAT1	1	3.50	3.50	0.136	0.7129
	COMT	1	117.70	117.70	4.584	0.0350
	DAT1 × COMT	1	157.73	157.73	6.143	*0.0151*
	Error	89	2285.36	25.68		
	DRD2	1	158.38	158.38	6.100	0.0154
	COMT	1	12.05	12.05	0.464	0.4975
	DRD2 × COMT	1	4.21	4.21	0.162	0.6881
	Error	89	2310.91	25.97		

PhA – physical aggression, Ang – anger, Hostil – hostility, DF – degrees of freedom, SS – sum of squares, MS – mean square, F – F-test, P – probability.

observed in single-gene models, which was confirmed in our analysis of anger and hostility.

However, we did not test putative associations of *AR* gene polymorphisms with the three genes involved in the dopaminergic system, as there is still no evidence of their functional relationship.

Because these data were collected in a single population and due to the highly similar cultural and social environments of the respondents, we believe that we were able to minimise the inter-individual variation in the environmental influence. Moreover, only males were included in our analysis, as there is convincing evidence to support significant sex-based differences in gene–environment interactions[43]. The Datoga are polygynous pastoralists, and the trajectories for achieving higher reproductive success are radically different in men and women. In contrast to the egalitarian and relatively peaceful Hadza hunter–gatherers, the men of the Datoga, another indigenous culture in the same region[44,45], have been selected for their aggression, particularly their physical aggression, as have other African pastoralists[46,47]. Indeed, men with fierce characters and warrior skills may be more successful in their mating and parental efforts because they can acquire and protect cattle, consequently showing higher reproductive success (more wives and children)[48]. Furthermore, our data suggest a possible link between physical aggression and direct fitness caused by strong sexual selection in Datoga men, while such a relationship most likely no longer exists in Westernised societies.

Of course, the present work has a number of limitations. First, the sample size was not large. However, because all our respondents were from the same tribal community, practiced traditional rites, participated in the traditional economy, and lived in the same area, the inter-individual variability in this respect was somewhat limited. As mentioned above, previous findings regarding the association between genes and particular behavioural traits are highly contradictory[36,37]. One possible reason for these disparities may be rooted in environmental factors[38], and the present data together with our data on the Hadza[42] illustrate a situation favouring this suggestion. Additionally, particular polymorphic loci may account for a relatively small proportion of phenotypic variance, and these effects may not be significant in relatively small samples of individuals with different socialisation experiences. If more than two genes/polymorphisms and many behavioural traits are selected for analysis, the necessity of making corrections for multiple comparisons is problematic. One possible solution for this situation is to obtain a very large sample; another possibility is the generation of biologically founded multilocus genetic profiles reflecting the cumulative effects of multiple polymorphic loci of known functionality on a specific behaviour[49]. In accord with this approach, Beaver and Chavian created the Genetic Risk Index for criminal involvement in Hispanics based on three dopaminergic gene polymorphisms and demonstrated its effectiveness[38]. Other authors proposed the construction of a multilocus genetic profile score based on five functional polymorphic loci for dopamine signalling and reported that it accounted for 10.9% of the inter-individual variability in reward-related VS reactivity[50]. The dopamine (DA) index was found to be associated

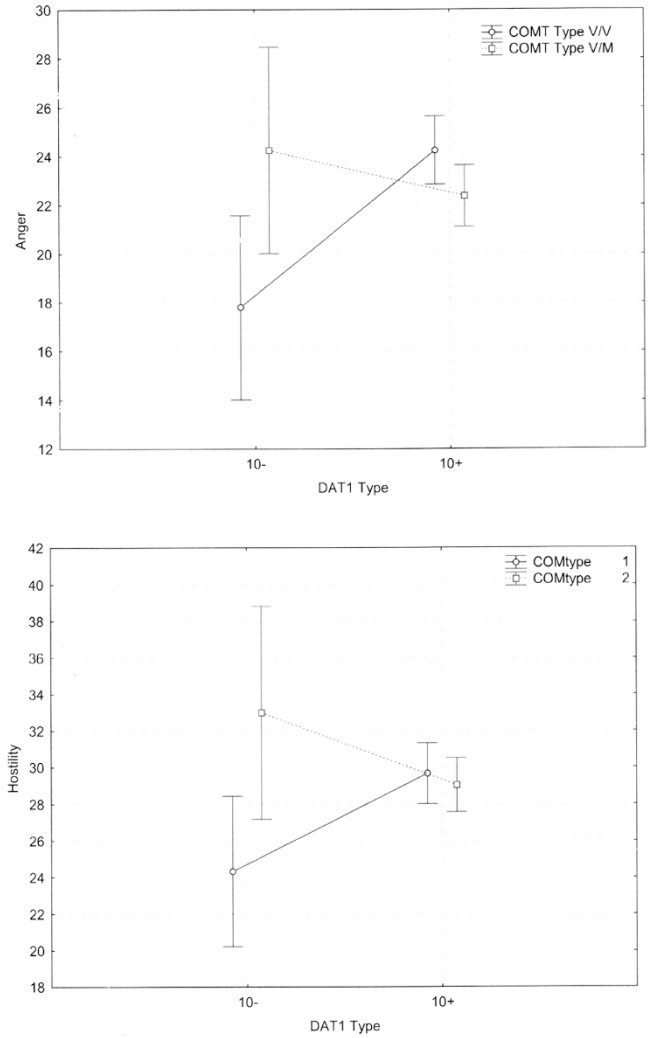

Figure 3 | The distribution of the anger (A) and hostility (B) as correlated with the *DAT1* × *COMT* effect. The designations of the ordinate numbers are described in Figure 1.

with ventral striatum activity in an experimental study and was recently applied in a psychiatric study. However, we decided not to use this index in our work because we do not know how it may be associated with the activity of other brain structures that are activated during aggression and their control in humans, particularly the prefrontal zone[51]. We refrained from applying such an approach in this study because (i) the initial assumption that particular polymorphisms act additively and not interactively to influence dopamine signalling should be tested in more detail, and (ii) more evidence is needed that all of the loci used to generate the multilocus genetic profile score are truly invested equally in behaviour.

Methods

Ethics statement. Institutional approvals, including those from the University (Moscow State University Ethics Committee) and local governmental agencies (including the Tanzanian Commission for Science and Technology), were obtained prior to conducting this study. All subjects gave their informed, verbal consent prior to participation. Verbal consent was deemed appropriate given the low literacy rates among traditional Datoga and was specifically approved by the University EC and Tanzanian agency.

Study sample and data collection. The data were collected during field studies from 2006 to 2012 in the United Republic of Tanzania. The sample analysed here includes 138 adult Datoga men, with a mean age of 34.09 ± 12.12 years (median of 33.5 years; range, 18–70 years).

The participants were personally interviewed to determine their age, sex, ethnicity, and personal history, as described previously[42]. All participants gave their consent prior to participating, and the average pay per subject was equal to US$2. All interviews were conducted in a one-to-one dialogue with the respondents. All questions were read aloud and explained if necessary. Many of the Datoga men commented in these interviews that aggression is an appropriate way of 'saving face', for both themselves and their relatives, and of protecting their property. When asked about family violence, all the men mentioned that they were physically punished during childhood by their parents (father, mother, or both). Self-rated aggression scores were obtained using Buss and Perry's Aggression Questionnaire (AQ)[52], which consists of 29 statements, grouped into four subscales: physical aggression (nine items), verbal aggression (five items), anger (seven items), and hostility (eight items). The first two subscales, physical and verbal aggression, represent direct aggression, and the other two subscales pertain to the individual's emotional basis for aggressive behaviour. AQ uses a Likert scale, ranging from 1 (extremely uncharacteristic) to 5 (extremely characteristic). This tool was employed because the original English version of the AQ has shown moderate construct validity and high test–retest reliability[53] and has been widely applied in aggression studies. In the present work, we used the ki-Swahili version of the AQ, which was initially administered by us and was previously used in our research on the Hadza[42]. For the details of the data collection procedure, see Butovskaya et al[42]. Here, we present data for three subscales of the AQ: physical aggression, anger, and hostility. Cronbach's Alpha values for these behavioural measures were as follows: physical aggression, 0.64; anger, 0.63; and hostility, 0.66.

Although the main conclusions drawn in this work regarding the subjects' aggression profiles were based on self-reported scores, rather than on direct observations, we do not think that this method of data collection significantly distorted the results. A meta-analysis performed earlier by Book and colleagues[54] demonstrated that "the measure of aggression (behavioural or self-report) did not have any effect on the relationship between testosterone and aggression," and there is no reason to assume that this situation differs with regard to genetic data.

Genotyping. Buccal epithelium samples were collected for DNA analysis during the field surveys, and DNA was later isolated using the DIAtom™ DNA Prep 200 extraction kit (IsoGene Lab, Moscow, Russia), which is intended for the isolation of DNA from various biological materials, according to the manufacturer's protocol.

AR CAG repeats, *DRD2* Taq1A, the *DAT1* VNTR 3′-noncoding region and *COMT* Val-158-Met loci polymorphisms were typed via polymerase chain reaction (PCR), which was performed with the GenePak® PCR MasterMix Core (IsoGene Lab, Moscow, Russia) reagent kit, according to the recommendations of the manufacturer[55].

Amplification of the target loci was performed using an MJ Research PTC-100 thermocycler with the following program: 1 cycle of 1 min at 94°C for denaturation, 30 sec at X°C for annealing, and 30 sec at 72°C for synthesis. This was followed by 30 cycles of 30 sec at 94°C, 30 sec at X°C, and 15 sec at 72°C, with a final elongation at 72°C for 3 min. X denotes the annealing temperatures of individual primer pairs (Table 5).

The reaction products for the *AR* locus were analysed using an ABI PRISM 3100-Avant automated DNA sequencer (Applied Biosystems, Foster City, USA).

To identify the TaqI polymorphism (T/C), the *DRD2* amplification product was subdivided into 10 μl aliquots, one of which was treated with the TaqI restriction endonuclease overnight at 65°C. For the *COMT* locus, the PCR products were also

Table 5 | Summary of primers for the PCR amplification of the four loci studied

Locus	Primer Sequences	Annealing Temperatures
AR(CAG)$_n$	F*: 5′-(FAM)-TCCAGAGCGTGCGCGAAGTGAT-3′ R: 5′-(FAM)-CGACTGCGGCTGTGAAGGTTG-3′	56°C
DRD2-Taq1 A	F: 5′-CCGTCGACGGCTGGCCAAGTTGTCTA-3′ R: 5′-CCGTCGACCCTTCCTGAGTGTCATCA-3′	68°C
DAT1 VNTR	F: 5′-TGCGGTGTAGGGAACGGCCTGAG-3′ R: 5′-CTTCCTGGAGGTCACGGCTCAAGG-3′	68°C
COMT M^{158}V	F: 5′-TACTGTGGCTACTCAGCTGTGC-3′ R: 5′-GTGAACTGTGTGTGAACACC-3′	55°C

*F – forward primer, R – reverse primer.

subdivided into 10 µl aliquots, and one of these aliquots was treated with NlaIII overnight at 37°C.

The amplification and restriction products were separated via electrophoresis in a 2% (*DRD2* and *DAT1*) or 3% (*COMT*) agarose gel and stained with ethidium bromide. The results were photographed and analysed with a BioDocAnalyze device (Biometra, Goettingen, Germany).

Statistics. Statistical treatment of the data was performed with SPSS 13.0 for Windows (IBM, Armonk, NY, USA). The means and standard deviations were computed for the studied traits (Table 1). The Kolmogorov–Smirnov test was employed to test whether our behavioural data were normally distributed. As all of the obtained probability values were greater than 0.1, parametric methods of statistical analysis were suitable for evaluating the dependence of the aggression subscales on the genotypes. The scores at all four aggression subscales were positively correlated with one another (with r values ranging from 0.386 to 0.497); thus, we used the correction for the critical p value for multiple correlated outcomes (http://gump.qimr.edu.au/general/daleN/matSpD/) previously applied by Hurd, Vaillancourt, and Dinsdale[41]. The adjusted critical p value for all of the aggression subscales was 0.01695. Because we tested the association of the *AR* gene and a set of three dopaminergic genes on aggression scales separately, the following corrections of the critical p-values were suggested. The adjusted critical value for *AR* was set to $p \leq 0.017/1(\text{gene}) = 0.017$ (two-tailed), whereas $p \leq 0.034$ was regarded as a trend. The significance levels for the three dopaminergic genes were calculated as $p \leq 0.017/3(\text{genes}) = 0.0057$ (two-tailed); a level of probability ranging from 0.0057 to 0.0113 was considered a tendency.

A linear regression was applied to analyse the associations of the three aggression subscales with the number of CAG repeats in the *AR* gene. One-way and two-way ANOVAs were conducted to determine the effects of the three candidate dopamine genes on the variations in the examined behavioural traits. We did not test the interaction effects of *AR* and the three dopamine genes in association with aggression because they represent different functional systems.

A genetic population analysis revealed agreement with Hardy-Weinberg equilibrium for the genotypes of the *DAT1* and *COMT* genes (Hobs = 0.505/Hexp = 0.505, p = 0.835, and Hobs = 0.491/Hexp = 0.443, p = 0.298, respectively), but not for the *DRD2* gene (Hobs = 0.692/Hexp = 0.495, $p \ll 0.001$, with a corrected level of significance of $\alpha/3 - 0.05/3 = 0.017$).

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Author contributions

Conceived and designed the experiments: M.L.B., A.P.R., A.M.K., V.A.V. Performed the experiments: M.L.B., V.A.V., E.M.S., D.V.S. Data collection: M.L.B., D.V.K., V.N.B. Supporting data collection: A.M. Analyzed the data: M.L.B., A.M.K., O.E.L. Wrote the paper: M.L.B.

Additional information

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