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 cultures1–4, which is most likely attributable to the greater impulsiveness of men and a stronger fear of physical danger in women5,6. A meta-analysis of 24 genetic studies on aggression demonstrated that heritability accounts for approximately 50% of the variance in aggression7,8, with the heritability of aggressive behaviour being higher in men than in women9. 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 others10–12.

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 brain13,14. 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 subjects16. Indeed, an increased number of these repeats in AR results in receptors with lower androgen sensitivity17,18. 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 domain19. 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 men20. 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. 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.

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.

DAT1 and DRD2 polymorphisms were examined in adolescents with pathological aggression, and it was found that carriers of the DAT1 10/10 genotype were underrepresented in a sample of aggressors compared to the control group and that DRD2 A1/A1 and A1/A2 were also underrepresented in aggressors. Vaughn and colleagues 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. Guo and co-authors 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 traditional East African pastoralists.

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. 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 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.

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

| AQ Scales | N  | R²  | 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, R² – proportion of the total variability explained by the factor effect, β – coefficient of regression, t – t-statistic, P – probability value.

**P < 0.01; **P < 0.001; *P < 0.05; P = 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| 14.57| 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. 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. At the same time, these data contradict those obtained by Hurd, Vaillancourt, and Dinsdale 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. 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, 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 showed that an interaction between DAT1 and DRD2 produces essentially the same effect on aggression...
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. 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 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, have been selected for their aggression, particularly their physical aggression, as have other African pastoralists. 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). 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. One possible reason for these disparities may be rooted in environmental factors, and the present data together with our data on the Hadza 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. 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. 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. The dopamine (DA) index was found to be associated

| 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 | 4.244 | 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 |       |     |
|          | 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 |       |     |
|          | 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.
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. All participants gave their consent prior to participation, 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), 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 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. For the details of the data collection procedure, see Butovskaya et al. 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, 0.65; 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 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 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.

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 Taq1 polymorphism (T/C), the DRD2 amplification product was subdivided into 10 µl aliquots, one of which was treated with the Taq1 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) | F: 5′-(FAM)-TCCAGAGCGGCTGCGGAAGTGA-3′<br>R: 5′-CGACTGCGCTGGAAGGTTG-3′ | 56°C                   |
|         | F: 5′-CGCAGGCCCCTTCTGAGTGAATGCA-3′<br>R: 5′-CTCCGTGGGTCGACGGC-3′ | 68°C                   |
| DAT1 VNTR | F: 5′-TGGCGGTCTAGGGAACGGGCTG-3′<br>R: 5′-TTGAACTGTGTGTAACACC-3′ | 55°C                   |
| COMT M158V | F: 5′-TACTGTGCTACTCGAGCTGTG-3′<br>R: 5′-TCCAGAGCGGCTGCAAGGTCG-3′ | 68°C                   |

*F – forward primer, R – reverse primer.
subdivided into 10 μl aliquots, and one of these aliquots was treated with NalII 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 independence 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/daneN/matSpdf/). We previously applied for Hurd, Vaillancourt, and Dinndal55. 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 ≤ 0.0171/(gene) = 0.017 (two-tailed), whereas p ≤ 0.034 was regarded as a trend. The significance levels for the three dopaminergic genes were calculated as p ≤ 0.017/3 = 0.0057 (two-tailed); a level of probability ranging from 0.0057 to 0.0131 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 ANOVA were conducted to determine the effects of the three candidate dopamine genes and the interaction of them on 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 = 0.85, and Hobs/Hexp = 0.443, p = 0.298, respectively), but not for the DRD2 gene (Hobs = 0.692/Hexp = 0.495, p ≤ 0.001, with a corrected level of significance of a/3 = 0.05/3 = 0.017).

1. Archer, J. Sex differences in aggression in real-world settings: a meta-analysis review. Rev Gen Psychol 8, 291–322 (2004).
2. Bjorkqvist, K. Sex differences in physical, verbal and indirect aggression: A review of recent research. Sex Roles 30, 177–188 (1994).
3. Butovskaya, M. L., Timentschik, V. & Burkev, V. Aggression, conflict resolution, popularity, and attitude to school in Russian adolescents. Aggressive Behav 33, 170–183 (2007).
4. Butovskaya, M. L., Burkev, V. N. & Mabulla, A. Sex differences in 2D: 4D ratio, aggression and conflict resolution in African children and adolescents: a cross-cultural study. J Aggress Conflict Peace Res 2, 17–31 (2010).
5. Daly, M. & Wilson, M. Risk-Taking, Intrasexual Competition, and Homicide Freidman, J. A., Kamboh, M. I., Waziri, C. & Leger, D. W. (eds.) Volume 47 of the Nebraska Symposium on Motivation: Evolutionary Psychology and Motivation, 1–36 (Lincoln, NE: University of Nebraska Press, 2001).
6. Knight, G. P., Guthrie, I. K., Page, M. C. & Fabes, R. A. Emotional arousal and gender differences in aggression: A meta-analysis. Aggressive Behav 28, 366–393 (2002).
7. Miles, D. R. & Carey, G. Genetic and environmental architecture of human aggression. J Pers Soc Psychol 72, 207–217 (1997).
8. Rhee, S. H. & Waldman, I. D. Genetic and environmental influences on antisocial behavior: a meta-analysis of twin and adoption studies. Psychol Bull 128, 490–529 (2002).
9. Craig, I. W. & Halton, K. E. Genetics of human aggressive behaviour. Hum Genet 126, 101–113 (2009).
10. van Gestel, S. et al. Epistatic effect of genes from the dopamine and serotonin systems on the temperament traits of Novelty Seeking and Harm Avoidance. Mol Psychiatri 7, 448–450 (2002).
11. Trainor, B. C., Sisk, C. L., Nelson, R. J. Hormones and the Development and Expression of Aggressive Behavior Pfaff, D. W., Arnold, A. P., Egen, A. M., Fahrbach, S. E. & Rubin, R. T. (eds.) Volume 1 of the Hormones, Brain and Behavior (2nd ed.), 167–203 (San Diego, USA: Academic Press, 2009).
12. Vasiliev, V. A. Molecular psychogenetics of deviant aggressive behavior in humans. Russ J Genet 47, 1023–1032 (2011).
13. Cohen Bendahan, C., Van de Beek, C. C. & Bergenbaum, S. Prenatal sex hormones effects on child and adult sex-typed behavior: methods and findings. Neurosci Biobehav R 29, 353–384 (2005).
14. Ryan, B. C. & Vandenberg, J. G. Intrauterine position effects. Neurosci Biobehav R 26, 665–678 (2002).
15. Mønning, I. T., Henn, P., Venkatramana, P., Martin, S. & Singh, D. Second to fourth digit ratio: ethnic differences and family size in English, Indian and South African populations. Ann Hum Biol 30, 579–588 (2003).
16. Rajender, S. et al. Reduced CAG repeats length in androgen receptor gene is associated with violent criminal behavior. Int J Legal Med 122, 367–373 (2008).
Acknowledgments
This study was partly supported by RFHR (project no. 12-01-00032), the Federal Innovation Program (no. 16.740.11.0172), the Ministry of Education and Science of the Russian Federation (project no. 8776), the RAS Program “Molecular and Cell Biology”, RFBR (projects no. 12-04-31869 and 13-04-00858), and the RF President’s Program (no. 5233.1201.4). This study would have been utterly impossible without the tolerance and constant help of our Datoga friends.

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
Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Butovskaya, M.L. et al. Aggression and polymorphisms in AR, DAT1, DRD2, and COMT genes in Datoga pastoralists of Tanzania. Sci. Rep. 3, 3148; DOI:10.1038/srep03148 (2013).

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