Linked *OXTR* Variants Are Associated With Social Behavior Differences in Bonobos (*Pan paniscus*)

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Revision Summary:

The original manuscript version underwent peer-review at Frontiers in Behavioral Neuroscience. As a part of this process, a reviewer requested that the sequence files be included in the manuscript. While preparing these files, Dr. Hudson identified several errors in his work regarding the individual bonobo genotypes reported in the original version of the manuscript. Upon discovery of the errors, Dr. Skiba and Dr. Taglialatela conducted a new genetic analysis using the raw files and subsequently corrected the misreported information - updating the editorial team and including the raw sequences with the revised manuscript. In addition to requested edits for improvement and clarity by the peer-reviewers, this revised manuscript corrects several errors in the human and bonobo reference genomes, the SNP locations, and the individual genotypes reported in the original manuscript. Information in the figures, main text, and supplementary materials has been corrected in this revised version. Dr. Hudson and his lab member, Mr. Hansen, were unable to identify how the errors in data reporting occurred, and neither party contributed to the revised version of this manuscript. The co-author list has been adjusted to reflect contributions to the corrected analyses and revised manuscript. We are deeply grateful to the editorial team and reviewers at Frontiers in Behavioral Neuroscience for their thorough peer-review of this work - improving the overall quality of the manuscript and leading to the discovery and correction of substantial errors in the original version.

Abstract

Single-nucleotide polymorphisms (SNPs) in forkhead box protein P2 (*FOXP2*), oxytocin receptor (*OXTR*), and arginine vasopressin receptor gene 1A (*AVPR1A*) have been associated with linguistic and social development in humans, as well as symptom severity in autism. Studying biobehavioral mechanisms in the species most closely related to humans can provide insights into the origins of human communication, and the impact of genetic variation on complex behavioral phenotypes. Here, we tested the hypothesis that similar genetic factors underlie social communication differences in both bonobos (*Pan paniscus*) and humans. We analyzed Sanger sequencing results to determine if bonobos exhibit individual variation at 10 loci across *FOXP2*, *OXTR*, and *AVPR1A* that have been implicated in human social development and behavior. We identified a novel variant in bonobo *FOXP2*, as well as three novel variants in bonobo *OXTR* that were 19-184 base pairs away from the target human SNPs. We also found a linked SNP combination (TGA) across the 3 novel bonobo *OXTR* sites at high frequency (65%) in the study population, including 6 homozygous bonobos. When comparing the combined *OXTR* genotypes, we found significant group differences in social behavior; bonobos with two copies of the TGA combination were more social than bonobos with one copy and bonobos with zero copies of the TGA combination. Taken together, our findings suggest that these *OXTR* variants may influence individual-level social behavior in bonobos and support the notion that linked genetic variants are promising biomarkers for differences in human social communication.

1 Introduction

Autism spectrum disorder (ASD) is characterized by social communication deficits and restricted, repetitive behaviors (RRBs) that impact daily functioning and can persist into adulthood. Determining the genetic factors that impact individual-level social communication is critical to our understanding of ASD and other neurodevelopmental disorders and may aid in identifying children at risk of developing social and linguistic impairments. The Simons Foundation created a database of genes associated with aspects of the ASD behavioral phenotype – SFARI Gene [1] – providing a systematic assessment of evidence for individual ASD-related genes [2]. This growing database highlights the complexity of human neurodevelopmental disorders, like autism, and how challenging it can be to identify biomarkers in humans. To help address the potential for false positives in large genetic datasets, the SFARI gene scoring system ranks genes as 1, 2, or 3 (high confidence, strong candidate, or suggestive evidence) based on the strength of the evidence supporting the gene's link to autism risk, as well as the frequency of replicated results [1,2].

A potential biological factor underlying individual differences in social communication is the forkhead box protein P2 (*FOXP2*; SFARI Gene Score 1). *FOXP2* is one of the first genes to be associated with human language disorders and fine orofacial motor control [3-5]. Most notably, researchers have determined that *FOXP2* is critical to the developmental processes underlying speech and language [3,4]. In addition, Haghighatfard and colleagues [6] found that lower *FOXP2* expression was associated with executive dysfunction in children diagnosed with ASD. Thus, it is possible that polymorphisms in *FOXP2* impact gene regulation and protein expression, and therefore may underlie individual-level differences in social communication [7].

Much like in the case of *FOXP2*, several studies have demonstrated the important role that the oxytocin receptor gene (*OXTR*; SFARI Gene Score 2) plays in social bond formation and social motivation [8-11]. In particular, researchers have documented relations between OXTR variation and social affiliation [12], vocal symptoms [13], as well as social communication impairments associated with ASD [14]. There is also considerable evidence that *OXTR* SNPs are related to empathy, prosocial temperament, social sensitivity, and stress reactivity in individuals diagnosed with ASD [15]. Variations in the arginine vasopressin receptor gene 1A (*AVPR1A*; SFARI Gene Score 2) have also been linked to differences in social communication in both humans and nonhuman mammals [16-22]. Over the course of hominid evolution, there is evidence indicating a tandem duplication of a non-coding, 5' flanking region of *AVPR1A* (DupB) [17] in the five great apes (orangutans, gorillas, bonobos, and humans). The DupB region (~350BP) contains a complex (CT)4-(TT)- (CT)8-(GT)24 polymorphic variable motif, known as the RS3 microsatellite [17]. In their 2008 study [17], Donaldson and colleagues found that among hominids (orangutans, gorillas, chimpanzees, bonobos, and humans), chimpanzees are unique in that individuals either have two copies, one copy, or zero copies of the DupB microsatellite-containing element in the 5' flanking region of *AVPR1A*. Hopkins and colleagues found evidence that chimpanzees lacking the DupB region perform significantly poorer on social cognition tasks [19] and responded less to joint attention cues [23] than chimpanzees with at least one copy of the DupB region.

In contrast to the three DupB genotypes observed in chimpanzees, bonobos and humans are homozygous for the microsatellite (DupB+). While all bonobos and humans exhibit both copies of the DupB region, individuals vary in the length of the RS3 microsatellite [17]. In humans, allelic variations in *AVPR1A* RS3 microsatellite length have been associated with social behavior and pair-bonding, as well as individual differences in aspects of social cognition

[14,22,24,25]. Specifically, individuals with a shorter RS3 microsatellite were less likely to act altruistically and reported higher levels of social conflict with their siblings, than did individuals with a longer RS3 microsatellite length [16,26]. All told, converging data across multiple disciplines have demonstrated that *AVPR1A* variations are associated with individual variability in social behavior, communication, and social cognition in humans and nonhuman great apes [16-17,21,27].

Collectively, these studies suggest that variation in *FOXP2*, *OXTR*, and *AVPR1A* influence social communication development and social functioning in humans. To better understand the impact of genetic variants on complex behavioral phenotypes, burgeoning evidence supports the study of these biobehavioral mechanisms in nonhuman animals [28-30]. Indeed, variation in *FOXP2*, *OXTR*, and *AVPR1A* have been associated with differences in social behavior and/or social communication in rodents [31,32], zebrafish [33], great apes [17,20,21,34,35], and zebra finches [36]. Seminal work in rodents, including knock-out experiments, account for much of what we know about ASD-associated variants and other social communication related genes [37-41]. However, recent evidence suggests that common animal models of neurodevelopmental disorders (e.g., rodents and invertebrates) are limited in their comparability to human social communication [42,43] and may be too phylogenetically distant from humans to advance early identification and intervention techniques [44].

Bonobos, along with chimpanzees, are the closest living relatives to humans [45] and are regarded as having one of the most complex social communication systems in the animal kingdom [46,47]. Although bonobos cannot be diagnosed with human neurodevelopmental disorders, they do show significant individual-level variability in social engagement [48-50], communicative production [51,52], and repetitive behaviors [50,53]. Several studies have identified links between ASD-associated genes and social communication in bonobos [9,54,55], as well as in chimpanzees [56-58]. There is also evidence that oxytocin is related to socio-sexual behavior in female bonobos [59]. Thus, bonobos are a promising model species for investigating the impact of genetic variants on human neurodevelopment and social communication.

Identifying biological factors that underlie individual-level social communication is critical to our understanding of autism and other neurodevelopmental disorders. To this end, we aimed to determine if bonobos - our closest living relatives - exhibit single nucleotide variation in *FOXP2, OXTR*, and *AVPR1A*, which are implicated in autism or differences in social communication. Given that bonobos live in large, dynamic social groups and that they produce complex communicative signals of various types (vocalizations, facial expressions, manual gestures, and multi-source signals), we hypothesized that bonobos may exhibit allelic variation in *FOXP2*, *OXTR*, and *AVPR1A*.

2 Materials and methods

2.1 Genetic analyses

Biological samples were collected from 29 bonobos (7 whole blood samples and 22 buccal samples) living at the Ape Cognition and Conservation Initiative (n=7; IACUC protocol #210305-01), the Columbus Zoo and Aquarium (n=6), and the Milwaukee County Zoo (n=16). Whole blood samples were collected under anesthesia during the bonobo's routine physical exam (n=6), except for one blood sample that was collected from an awake bonobo via voluntary presentation (n=1). Buccal samples were collected by swabbing the inner cheek or lower lip for 10-15 seconds with a QIAGEN OmniSwab (n=22). All buccal samples were taken from awake bonobos that presented voluntarily for sample collection. Of the 22 buccal samples, 16 yielded

DNA of a quality that was too low for Polymeric Chain Reaction (PCR) amplification. This was likely due to the level of bacteria in the buccal cavity of the bonobos, as well as the collection method; samples from Ape Initiative were repeated following the first attempt at DNA extraction and were collected from the bonobos' lower lips after rinsing their mouths with water (as opposed to collecting the samples from the bonobos' inner cheeks without rinsing with water beforehand). These repeated samples were sufficient for DNA extraction, resulting in a total of 13 biological samples for the genetic analyses (whole blood n=7, buccal swab n=6).

Autism-associated genes included in this study were *FOXP2*, *OXTR*, and *AVPR1A*. Primer pairs were designed using NCBI Primer-BLAST [60] and ApE [61] to flank each SNP by ~250 base pairs (giving approximately 500bp amplicons) and ordered from Thermo Fisher Scientific. Primer pairs that were included in the study met the following criteria: 1) the PCR product was at least 250 base pairs long, 2) the melting temperature (Tm) of the primer was 60°C and the GC% was approximately 50%, and 3) the primer did not have hair-pin formations or self-annealing sites and was not 3' prime complimentary. This resulted in the inclusion of 10 human loci: *FOXP2* rs6980093, *OXTR* rs2270463, rs237877, rs237878, rs35062132, rs2254295, rs237894, rs237895, and rs237900, and *AVPR1A* rs3803107 (Table 1). See Supplementary Material 1 for a list of human SNPs that did not meet the criteria.

DNA was extracted from bonobo whole blood and buccal samples, amplified by PCR, resolved by gel electrophoresis, gel purified (Zymo Gel DNA Recovery Kit) and sent to Genewiz for Sanger sequencing (see Supplementary Material 2 for details). Sequences for the bonobo reference genome (May 2020 (Muhdiblu_PPA_v0/panPan3)) and the human reference genome (Dec. 2013 (GRCh38/hg38)) were obtained from the UCSC Genome Browser [62]. To determine SNP presence, individual bonobo Sanger sequences were aligned to the other bonobos from our sample, the bonobo reference genome, and the human reference genome, using Clustal Omega [63]. See Supplementary Material 3 for the novel bonobo SNP visualizations in Clustal Omega, and Supplementary Material 4 for the raw bonobo sequences and reference sequences used in the alignments. Heterozygotes were identified by visual inspection of the Sanger chromatograms in 4Peaks [64] and confirmed via peak height ratios >33% to minimize the likelihood of false positives. Peak height ratios were calculated by dividing the height of the smaller peak by the height of the larger peak. Individual genotypes were cross-checked based on known pedigrees. See Supplementary Material 5 for the relatedness between each subject.

2.2 Behavioral data

To determine if any observed genetic variation was linked to social behavior, we utilized previously collected observational data that were available for 12 of the 13 subjects [50,65]. Focal observations were not available for subject M15 because the individual was under the age threshold of 2 years at the time of data collection. Bonobos included in the behavioral analyses ranged in age from 6 to 40 years old, with a mean age of 20.88 years (female n=6, female mean age=21.58 years; male n=6, male mean age=20.17 years). The bonobos were housed in their indoor and outdoor enclosures, which featured environmental enrichment (e.g., artificial vines and sleeping platforms) that promoted species-typical behaviors (e.g., climbing and nest building). Facility care staff were responsible for monitoring the health and well-being of the bonobos, and any behavioral concerns or signs of distress noted by the researchers were reported immediately to the care staff. No modifications were made to the animals' housing or social groupings for this study, and data collection did not interfere in any way with the bonobo/care staff's daily schedule.

For this study, we utilized eight 10-minute focal observations (i.e., observing the behavior of a single individual) from each subject to assess group differences in communicative production and in social proximity – an established method for measuring social relationships in nonhuman primates that encapsulates both social interest and engagement [66-68]. Social proximity was recorded instantaneously at 1-minute increments starting at time zero, which resulted in 11 proximity data points per observation. Communicative production (i.e., the total number of gestures, facial expressions, and multi-source signals, including vocalizations + gestures, gestures + facial expressions, and facial expressions + vocalizations, produced by the focal individual) was recorded on an all-occurrence basis. See Table 2 for social proximity measures.

2.3 Statistical analyses

For each observation, the total number of communicative signals produced was summed, and a social proximity score (ranging from 0-3), was calculated using the following formula, where 11 is the total number of proximity data points per focal follow:

$$\frac{(3*N\ touching\ data\ points)+(2*N\ socially\ close\ data\ points)+(1*N\ socially\ peripheral\ data\ points)+(0*N\ isolated\ data\ points)}{(11-N\ cannot\ be\ determine\ data\ points)}$$

Kruskal-Wallis H tests and Wilcoxon rank tests were utilized to assess group differences in social behavior and communicative production. To rule out rearing influences on any observed differences in bonobo social behavior, we grouped bonobos based on whether they were raised primarily by their mother (typical rearing, n=7), or were raised primarily by humans and/or captured from the wild (atypical rearing, n=5). To rule out age influences on any observed differences in bonobo social behavior, we grouped bonobos based on whether they were a subadult (<14 years old, n=4), adult (14-34 years old, n=6), or elder (>34 years old, n=2), at the time the behavioral observations were collected.

3 Results

3.1 Genetic variation

Analyses revealed a novel SNP in bonobo *FOXP2*, 78bp downstream of *FOXP2* rs6980093 (human chr7:114522685; A/G). Five bonobos were heterozygous at this location (G/A), one bonobo was homozygous (G/G), and the rest were homozygous (A/A; bonobo 5' flanking sequence CACTCGTATCACATTATAAT A/G; Figure 1). Analyses did not reveal individual variation in *AVPR1A*; all of the bonobos were homozygous for the same allele at this position (G/G).

Our analyses revealed genetic variation in bonobo *OXTR* at three novel positions (Figure 2A). Specifically, we identified a novel SNP 73bp upstream of rs237877 (chr3:8741201; C/T) and 184bp upstream of rs237878 (chr3:8741312; T/A/C); 11 of the 13 bonobos were homozygous (T/T) and 2 bonobos were heterozygous (T/G; 5' flanking sequence TTGCAGCTATCACCTCATTT T/G; Novel *OXTR* SNP #1). We also identified a novel SNP 19bp downstream of human SNP rs35062132 (chr3:8753201; G/A/C). In our sample, 11 bonobos were homozygous (G/G), and 2 bonobos were heterozygous (G/A; 5' flanking sequence CGATGGCTCAGGACAAAGGA G/A; Novel *OXTR* SNP #2). In addition, a novel SNP was observed 19bp upstream of rs237900 (chr3:8767010; G/A). Analyses revealed 3 genotypes at this locus; 8 bonobos were homozygous (A/A), 3 bonobos were heterozygous (A/C), and 2 bonobos were homozygous (C/C; 5' flanking sequence GCCCAAGGACTGTGCTAAGG A/C; Novel *OXTR* SNP #3). Collectively, we observed a linked SNP combination (TGA) across the 3 novel bonobo *OXTR* sites at high frequency (65%) in the study population, including 6 homozygous bonobos (Figure 3). Observed genotypes across the novel *OXTR* sites included

TGA (65%), TGC (27%), and GAA (8%). See Table 3 for a complete list of genotypes and Table 4 for the novel bonobo SNP frequencies.

3.2 Behavioral differences

The behavioral analyses excluded one participant for whom behavioral data were not available – subject M15 (two copies of *OXTR* TGA and heterozygous at the novel *FOXP2* SNP). For *OXTR*, individual bonobos were grouped based on the number of TGA copies for the novel *OXTR* SNPs (Figure 3) – zero (n=2), one (n=5), or two (n=5; Table 5). Kruskal-Wallis test results indicated a significant *OXTR* group difference in social proximity score (H (2) = 15.461, $p < 0.001$; Figure 4; Table 6). Bonobos with two copies of TGA were more social (*Mdn* = 2.36) than individuals with one copy (*Mdn* = 1.77) and individuals with zero copies of TGA (*Mdn* = 1.55). A post-hoc Wilcoxon rank test with Benjamini-Hochberg adjustment revealed a significant difference in social proximity scores between bonobos with zero TGA copies and bonobos with two TGA copies ($p < 0.001$), and between bonobos with two copies and one copy ($p = 0.007$), but not between one copy and zero copies ($p = 0.156$). There was not a significant *OXTR* group difference in communicative production (H (2) = 2.888, $p = 0.236$; Figure 5, Table 7). For *FOXP2*, individuals were grouped based on whether they were homozygous (A/A, n=7), heterozygous (G/A, n=4), or homozygous (G/G, n=1) at the *FOXP2* SNP locus. Kruskal-Wallis test results indicated that there was not a significant *FOXP2* group difference in social proximity score (H (2) = 3.896, $p = 0.143$, Figure 6A), nor in communicative production (H (2) = 0.942, $p = 0.625$, Figure 6B). Post-hoc analyses indicated that rearing (atypical or typical) and age (subadult, adult, or elder) did not influence the observed differences in bonobo social behavior; there were no significant differences in social proximity score based on rearing ($W = 1071$, $p = 0.717$; Figure 7) or based on age (H (2) = 1.345, $p = 0.510$; Figure 8).

4 Discussion

Determining the biological factors underlying individual-level social communication is key to understanding autism and other neurodevelopmental disorders and may aid in identifying children at risk of developing social communication impairments. Studying biobehavioral mechanisms in captive, nonhuman animals, permits researchers to elucidate the genetic contributions to complex behavioral phenotypes, while minimizing confounding rearing and environmental factors. Thus, we aimed to determine if one of the species most closely related to humans, the bonobo, exhibits SNPs in *FOXP2, OXTR*, and *AVPR1A* at known human loci.

Despite whole genomic investigations in all four nonhuman species of great ape, no SNPs have been identified in bonobo *FOXP2* to date. We are the first to report a SNP in bonobo *FOXP2* (near rs6980093) and we identified two novel genotypes in our sample. Although we did not find behavioral differences based on the bonobos' *FOXP2* genotypes, we are encouraged by the identification of variation in bonobo *FOXP2* (75bp from the ASD-linked human SNP) and believe that further investigation into the bonobo *FOXP2* gene and measures of bonobo vocal communication might yield promising results.

The human *FOXP2* SNP rs6980093 is an intronic polymorphism, suggesting it may be involved in regulating *FOXP2* gene expression [7,69]. Several studies have identified relations between *FOXP2* rs6980093 variation and speech production [68], cortical activation in language-related regions [71], as well as speech and language learning abilities [7,72]. The identification of variation in bonobo *FOXP2* is a foundational step in understanding the impact of *FOXP2* on social communication in humans and our closest living relatives. Bonobos have the largest vocal repertoire size of the nonhuman great apes [73] and modify their communicative signals

depending on social context [74-76]. In addition, multiple reports in a single bonobo support the notion that bonobos are able to understand spoken English words and sentences [77-79], as well as degraded and computer-generated speech [80]. Thus, it is possible that *FOXP2* alleles modulate *FOXP2* expression in bonobos and that differential *FOXP2* regulation impacts individual variability in bonobo vocal communication.

Specific *OXTR* variants have been linked to social functioning [81] and symptom severity [14,15] in autism. In our sample of bonobos, we identified a novel SNP between human *OXTR* rs237877 and rs237878. Variants at these positions have been linked to human levels of extraversion [8] and reward responsiveness [82] in typically developed adults, as well as neurological responses to oxytocin in autistic adults [83]. In addition, we identified a novel SNP in bonobo *OXTR* close to the human SNP rs35062132. Eleven of the 13 bonobos in our sample exhibited the G/G genotype. In humans, the C/G genotype of *OXTR* rs35062132 was associated with an increased risk of ASD and proposed to be a biological basis for individual differences in social behavior [84]. Although they identified three genotypes in their sample, Egawa and colleagues did not find a relation between these genetic variants and ASD [85]. Hence, further investigations are needed to understand the role rs35062132 variation plays in social communication development and to determine if the rs35062132 G allele is a risk factor for autism [84,85].

We also found a novel SNP in bonobo adjacent to rs237900. In humans, rs237900 variants were associated with reaction times for nonverbal-reaction-based-judgements, but not with nonverbal communication scores [83]. When controlling for sex, researchers found a significant rs237900 variant difference in harm avoidance. Female participants with the alternate (T) allele scored significantly higher on harm avoidance than all other participants [86]. Interestingly, rs237900 variants have also been linked to differences in DNA methylation of the *OXTR* gene [87]. Findings linking social functioning to another SNP in *OXTR,* rs2254298, are also well-established [88-91]. Yang and colleagues found differences in serum oxytocin levels based on the rs2254298 genotype [11]. However, the role that rs2254298 variants play in autism remains unclear. Specifically, results differ among human populations; the “A” allele was associated with autism in Japanese [92] and Chinese [93] populations, whereas the “G” allele was considered a risk factor in a population of autistic children and adolescents of European ancestry [94]. Parker and colleagues also identified links between rs2254298 variants and social impairments in children with and without ASD [95]. In both groups, individuals with the “A” allele exhibited greater social impairments than individuals without the “A” allele. Collectively, these findings suggest that specific *OXTR* variants might be promising biomarkers for social communication dysfunction in humans and highlight the need for alternative approaches to assessing the impact *OXTR* variants have on complex behavioral phenotypes, like those observed in ASD [95-97].

In addition to finding 3 novel *OXTR* sites, we found a linked SNP combination (TGA) across the *OXTR* sites at high frequency (65%) in the study population, including 6 homozygous bonobos. Linked *OXTR* variants have also been found in children, adolescents, and young adults diagnosed with ASD [12]. In addition, Wu and colleagues identified linkage among two *OXTR* variants in autistic people from the Chinese Han population [93]. Thus, we encourage researchers interested in the biomarkers of human social communication disorders to consider the relative influence of individual SNPs and their collective contribution to complex behavioral phenotypes.

If the observed TGA combination positively impacts bonobo sociality, we would expect to see behavioral differences based on these genotypes. In our sample, bonobos with two copies of the TGA combination had higher social proximity scores (i.e., they spent more time in close proximity to conspecific social partners) than bonobos with zero copies and bonobos with one copy of the TGA combination. This result supports previous conclusions that *OXTR* plays a pivotal role in bonobo social behavior [9,35,59] and is consistent with findings in humans that indicate that linked *OXTR* variants are associated with differences on the social responsiveness and repetitive behavior scales in autistic children [98]. In chimpanzees, researchers did not identify a link between *OXTR* variation and social behavior [99].

Our study complements several studies with rhesus macaques, including an investigation where an *OXTR* haplotype was associated with cognitive and behavioral differences [100]. Our results are also similar to data collected from children, adolescents, and young adults diagnosed with ASD; a haplotype comprised of four *OXTR* loci was associated with greater impairments in social interaction and communication in autistic individuals [12]. Thus, our findings suggest that the *OXTR* TGA combination may have been selected for in bonobos. It is also possible that the high frequency of the TGA combination is the product of a founder effect. Our collective results highlight the importance of considering multiple genetic variants in a given study and the benefits of multi-loci investigations in nonhuman great apes.

Given the documented relations between *OXTR* and social functioning, researchers have proposed that oxytocin can help facilitate social information processing in individuals with ASD. Indeed, evidence exists that oxytocin treatment can improve social abilities in children diagnosed with ASD [101] and that oxytocin infusions can reduce repetitive behaviors in autistic adults [83,102]. Researchers have also determined that oxytocin treatment efficacy differs between people, with the greatest improvements to social behavior occurring in individuals with the lowest pretreatment oxytocin blood concentration levels [101]. Along with previous evidence of a low social, high repetitive behavior phenotype in bonobos [50], our findings suggest that bonobos are an exemplary species for evaluating the efficacy of oxytocin interventions in the treatment of social communication dysfunction. Notwithstanding, further studies are needed to determine the specific characteristics that impact oxytocin efficacy and to identify biomarkers that predict which individuals will benefit the most from oxytocin treatments [82,101].

Unlike our findings in *FOXP2* and *OXTR*, we did not identify single-nucleotide variability in bonobo *AVPR1A*. With that said, we only targeted one human *AVPR1A* SNP (rs3803107) - sequencing ~250 bp surrounding this locus. Other primer sets designed to amplify additional ASD-related SNPs in *AVPR1A* failed to work. Associations between *AVPR1A* and social behavior are well-documented in chimpanzees [57,99,103], and to a lesser extent bonobos [20]. However, the nonhuman great ape literature has centered around the RS3 microsatellite, given that chimpanzees are polymorphic for a secondary deletion of the DupB microsatellite-containing element in the 5' flanking region of *AVPR1A* [17,20,103]. Many of these studies in chimpanzees and bonobos utilized personality questionnaire data, where human caregivers made a subjective estimate of each ape's social behavior [20,56,99,103]. Additional objective assessments of individual-level social communication in nonhuman great apes are needed.

Seminal work in rodents accounts for much of what we know about *OXTR* and other ASD-related genes. However, many of these studies require substantial modification of the gene and/or the receptors or are limited in their translatability to human development and the complex phenotype of ASD [38,40,42]. In this study, we identified naturally occurring linkage among three novel *OXTR* variants and documented differences in bonobo social behavior based on this

368 combined *OXTR* genotype. We also demonstrated that it is possible to detect genetic variability,
369 variant linkage, and behavioral differences in even small samples of nonhuman great apes. If
370 researchers incorporate our modified technique of rinsing the animal's buccal cavity beforehand
371 and swabbing the individual's lower lip, they should be able to obtain voluntary biological
372 samples that allow for sufficient DNA extraction from any nonhuman great ape living in human
373 care. We encourage the inclusion of bonobos in future studies and suggest incorporating
374 additional objective assessments of individual-level social communication (e.g., social attention,
375 joint engagement, communicative proficiency). Singular investigations into Sanger sequencing
376 data may underestimate individual variability across the target genes. Thus, we emphasize the
377 need for a publicly accessible database to report SNPs identified in nonhuman primates and to
378 contribute to larger scale genomic studies in nonhuman great apes with future research.

379 **4.1 Conclusions**

380 Here, we are the first to report a SNP in bonobo *FOXP2* – a gene necessary for typical
381 linguistic development in humans. We also identified three novel SNPs in bonobo *OXTR* and
382 demonstrated linkage among these *OXTR* variants. Our results indicate that individuals with two
383 copies of the *OXTR* TGA combination are more social than individuals with one copy or zero
384 copies of the TGA combination. Our collective findings suggest that these *OXTR* variants
385 influence individual-level social communication in bonobos and support the notion that linked
386 *OXTR* variants could be promising biological factors for identifying humans at risk of
387 developing social communication deficits. This study also highlights the advantages of studying
388 biobehavioral mechanisms in the species most closely related to humans and indicates that
389 bonobos are a suitable model for testing hypotheses about the etiology of ASD and other human
390 neurodevelopmental disorders.
391

392 **5 Figure Legends**

393 **5.1 Figure 1. SNP in Bonobo *FOXP2*.** Identification of a single-nucleotide polymorphism in
394 bonobo *FOXP2* (A) Diagram of the human *FOXP2* gene showing relative location of the SNP
395 rs6980093. Arrow shows direction of gene transcription. Short vertical bars (grey) show
396 transcriptional start sites. Black vertical bars show exons. (B) Alignment of the human and
397 bonobo references genomes, along with individual sequencing data from subjects in this study.
398 Numbers refer to the human reference genome location on chromosome seven. (C, D)
399 Representative Sanger sequencing chromatograms across the SNP, showing (C) a homozygous
400 sample (F18), and (D) a heterozygous sample (F05).

401 **5.2 Figure 2. SNPs in Bonobo *OXTR***: Identification of single-nucleotide polymorphisms in
402 bonobo *OXTR* (A) Diagram of the human *OXTR* gene showing relative locations of SNPs
403 rs237877, rs237878, rs35062132, and rs237900, as well as the novel bonobo SNPs. Arrow shows
404 direction of gene transcription. Untranslated regions are shown in grey. Black oblongs show
405 exons. (B) Alignment of the human and bonobo references genomes, along with individual
406 sequencing data from subjects in this study. Numbers refer to the human reference genome
407 location on chromosome three. (C, D) Representative Sanger sequencing chromatograms across
408 the novel SNP, showing (C) a homozygous sample (F13), and (D) a heterozygous sample (M13).

409 **5.3 Figure 3. Bonobo Pedigree**: Pedigree of the 13 subjects, with corresponding *OXTR*
410 genotypes at the three novel SNPs in bonobo *OXTR*. Bonobos with zero copies are in blue,
411 bonobos with one copy are in yellow, and bonobos with two copies of the TGA combination are
412 in red.

5.4 Figure 4. *OXTR* Group Differences in Bonobo Social Behavior: Observed group differences in social behavior based on the number of *OXTR* TGA copies. Average social proximity scores with standard error bars (A) and raw social proximity scores (B) for bonobos with zero, one, and two copies of the TGA combination.

5.5 Figure 5. *OXTR* Groups and Bonobo Communicative Production: Communicative production based on the number of *OXTR* TGA copies. Average communicative production with standard error bars (A) and raw communicative production totals (B) for bonobos with zero, one, and two copies of the TGA combination.

5.6 Figure 6. *FOXP2* Groups and Bonobo Social Communication: Social proximity and communicative production based on *FOXP2* genotype. Raw social proximity scores (A) and raw communicative production totals (B) for bonobos that were homozygous (G/G), heterozygous (A/G), and homozygous (A/A) at this position.

5.7 Figure 7. Rearing and Bonobo Social Behavior: Social proximity scores based on rearing. Average social proximity scores with standard error bars (A) and raw social proximity scores (B) for bonobos that were raised primarily by their mothers (typical) and bonobos that were raised primarily by humans or taken from the wild (atypical).

5.8 Figure 8. Age and Bonobo Social Behavior: Social proximity scores based on age. Average social proximity scores with standard error bars (A) and raw social proximity scores (B) for subadult (<14 years old), adult (14-34 years old), and elder (>34 years old) bonobos.

6 Tables

6.1 Table 1: Selected Human SNPs: Name, position, relative allele frequency, and variant type for the selected human single nucleotide polymorphisms. Human alleles that were only found in a small subset of studies have rounded frequences of zero (.00).

Single Nucleotide Polymorphism	Position in Human Reference Genome	Allele Frequency in Humans	Variant Type	Bonobo Nucleotide
FOXP2 rs6980093	chr7:114522685	G (.41) / A (.59)	Intron	A (1.00)
OXTR rs2270463	chr3:8733391	G (.77) / T (.23)	Intron	G (1.00)
OXTR rs237877	chr3:8741201	C (.69) / T (.31)	Intron	T (1.00)
OXTR rs237878	chr3:8741312	T (.78) / C (.22) / A (.00)	Intron	C (1.00)
OXTR rs35062132	chr3:8753201	G (1.00) / A (.00) / C (.00)	Missense	G (1.00)
OXTR rs2254295	chr3:8760606	T (.87) / C (.13)	Intron	T (1.00)
OXTR rs237894	chr3:8764845	G (.76) / C (.24)	Intron	G (1.00)
OXTR rs237895	chr3:8765737	T (.37) / C (.63)	Intron	C (1.00)
OXTR rs237900	chr3:8767010	G (.64) / A (.36)	Intron	G (1.00)
AVPR1A rs3803107	chr12:63147054	G (.83) / A (.17)	3 Prime UTR	G (1.00)

6.2 Table 2: Social Proximity Measures

Proximity Measure	Description
Close-Touching	The focal individual is <1.5 meters from the nearest conspecific.
Socially Close	The focal individual is 1.5-3 meters from the nearest conspecific.
Socially Peripheral	The focal individual is 3-5 meters from the nearest conspecific.
Isolated	The focal individual is >5 meters from the nearest conspecific.

441
442 **6.3 Table 3: Individual Sequencing Data:** Individual sequencing data for the novel SNPs
443 identified in bonobo *FOXP2* and *OXTR* and for the selected human loci.
444

Bonobo ID	**F14**	**F16**	**M14**	**F13**	**M10**	**F05**	**M13**	**F18**	**M15**	**M12**	**M18**	**F10**	**M19**
Novel SNP *FOXP2*	A/A	A/A	A/A	A/A	A/A	A/G	G/G	A/A	A/G	A/G	A/G	A/G	A/A
Novel SNP *OXTR* #1	T/T	T/G	T/T	T/T	T/T	T/T	T/G	T/T	T/T	T/T	T/T	T/T	T/T
Novel SNP *OXTR* #2	G/G	G/A	G/G	G/G	G/G	G/G	G/A	G/G	G/G	G/G	G/G	G/G	G/G
Novel SNP *OXTR* #3	A/A	A/A	A/A	A/C	C/C	A/A	A/A	A/C	A/A	A/C	C/C	A/A	A/A
***FOXP2* rs6980093**	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A
***OXTR* rs2270463**	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G
***OXTR* rs237877**	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T
***OXTR* rs237878**	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C
***OXTR* rs35062132**	GG	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G
***OXTR* rs2254295**	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T
***OXTR* rs237894**	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G
***OXTR* rs237895**	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C
***OXTR* rs237900**	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G
***AVPR1A* rs3803107**	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G

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446 **6.4 Table 4: Allele Frequencies in Bonobos:**
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Novel Bonobo SNP	Nearest Human SNP(s)	Allele Frequency in Bonobos
Novel SNP *FOXP2*	rs6980093	A (.73) / G (.27)
Novel SNP *OXTR* #1	rs237877, rs237878	T (.92) / G (.08)
Novel SNP *OXTR* #2	rs35062132	G (.92) / A (.08)
Novel SNP *OXTR* #3	rs237900	A (.73) / C (.27)

6.5 Table 5: *OXTR* Combined Genotypes: Individual identifications and corresponding ages for bonobos with zero, one, and two copies of the *OXTR* TGA combination.

TGA Copies	Zero	One	Two
Bonobo IDs	M10, M18	F13, F16, F18, M12, M13	F05, F10, F14, M14, M19
Ages in Years	40, 10	23, 10, 13, 22, 20	35, 24, 24, 23, 6

6.6 Table 6: Social Proximity Scores: Social proximity scores for each of the bonobo's focal observations.

Bonobo ID	F14	F16	M14	F13	M10	F05	M13	F18	M12	M18	F10	M19
Proximity Score 1	2.73	1.70	1.18	0.64	1.82	3.00	3.00	1.64	0.00	0.55	3.00	2.36
Proximity Score 2	2.27	0.82	1.00	0.55	2.18	3.00	1.36	3.00	1.27	1.00	3.00	2.09
Proximity Score 3	3.00	1.45	1.09	0.82	0.64	2.73	2.09	3.00	0.00	0.00	3.00	2.00
Proximity Score 4	2.36	3.00	1.09	3.00	2.73	3.00	1.45	2.27	3.00	0.00	3.00	2.46
Proximity Score 5	1.45	2.18	1.91	2.82	1.64	3.00	0.27	3.00	0.45	0.45	3.00	2.18
Proximity Score 6	1.50	2.82	2.36	2.64	2.00	2.45	1.27	1.82	1.73	1.55	3.00	1.55
Proximity Score 7	2.90	1.00	2.00	0.82	0.00	3.00	0.36	2.91	2.18	2.18	2.45	2.55
Proximity Score 8	2.82	1.73	1.45	1.73	1.55	1.18	1.00	2.55	1.73	2.73	2.27	2.82

6.7 Table 7: Communicative Production: Number of communicative signals produced during each of the bonobo's focal observations.

Bonobo ID	F14	F16	M14	F13	M10	F05	M13	F18	M12	M18	F10	M19
Communicative Production 1	1	2	0	0	1	3	0	6	0	4	0	8
Communicative Production 2	0	1	0	1	4	6	4	1	9	4	0	16
Communicative Production 3	1	0	0	0	15	4	4	1	0	1	4	0
Communicative Production 4	1	2	20	1	2	6	1	5	1	0	0	4
Communicative Production 5	0	2	1	0	2	15	4	2	1	3	11	4
Communicative Production 6	1	0	0	0	6	2	9	7	0	8	0	0
Communicative Production 7	6	4	0	1	2	3	2	0	20	2	2	0
Communicative Production 8	6	4	0	2	0	1	0	9	0	9	0	13

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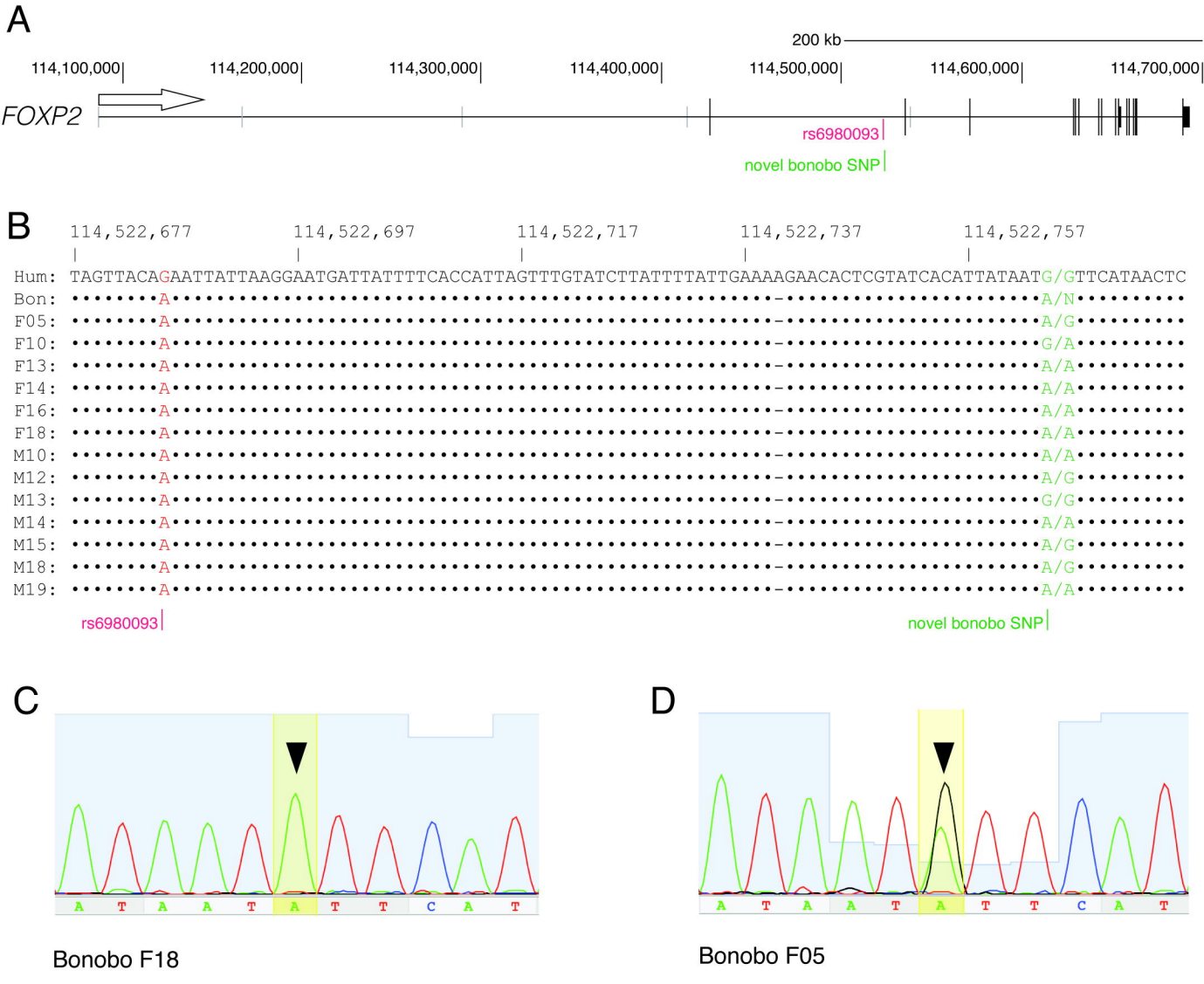
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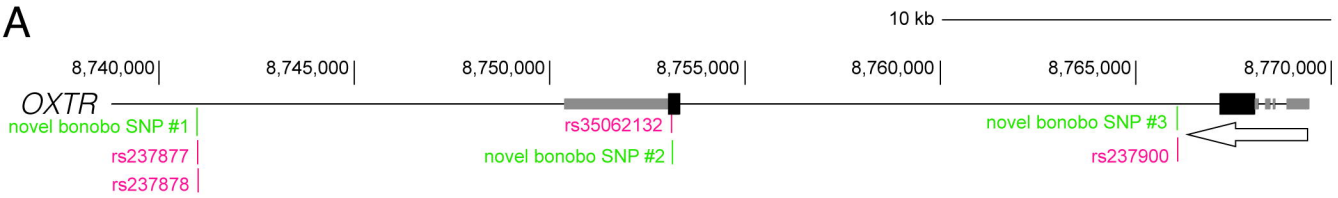

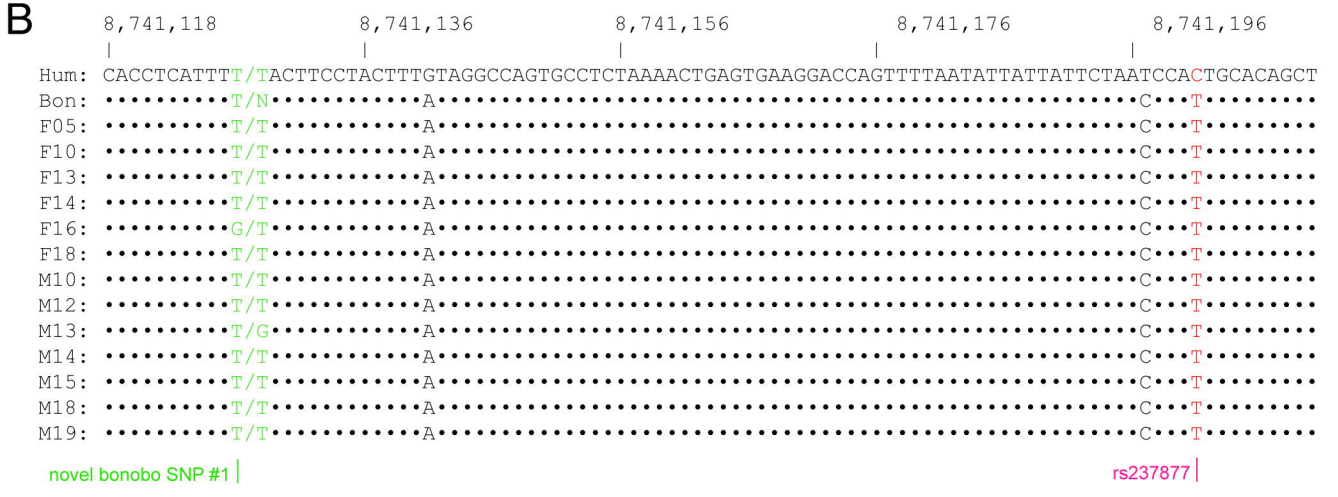

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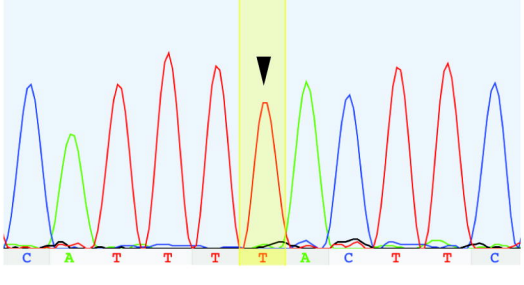

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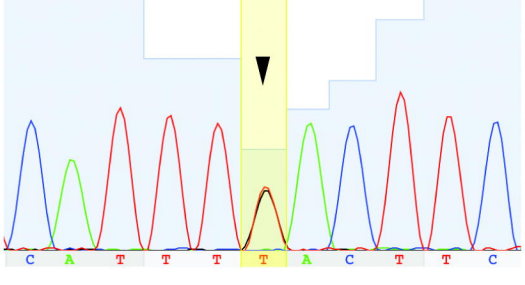

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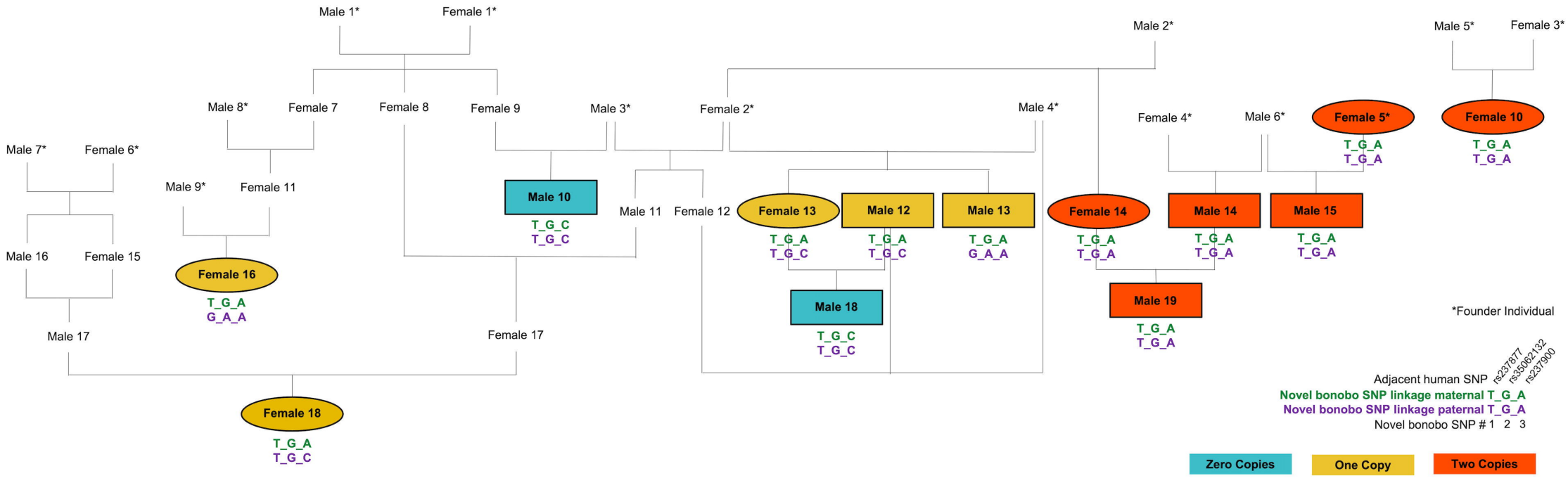
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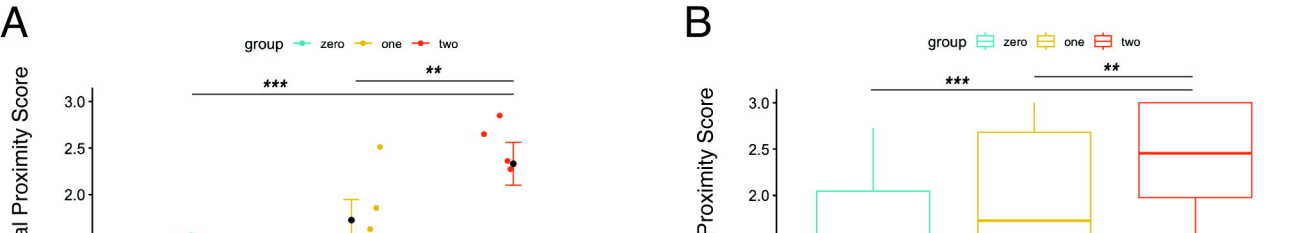
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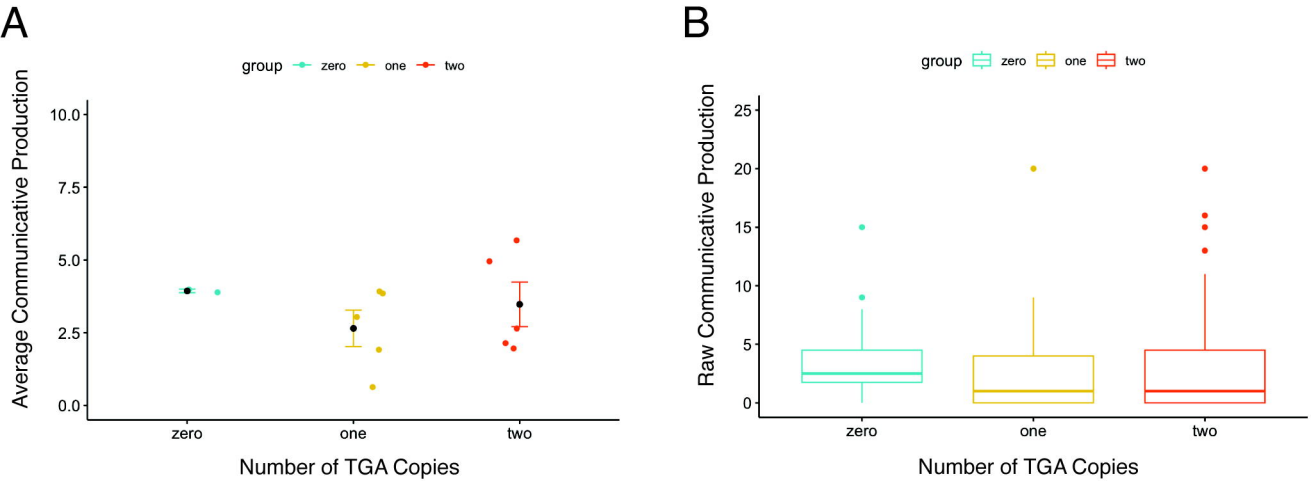
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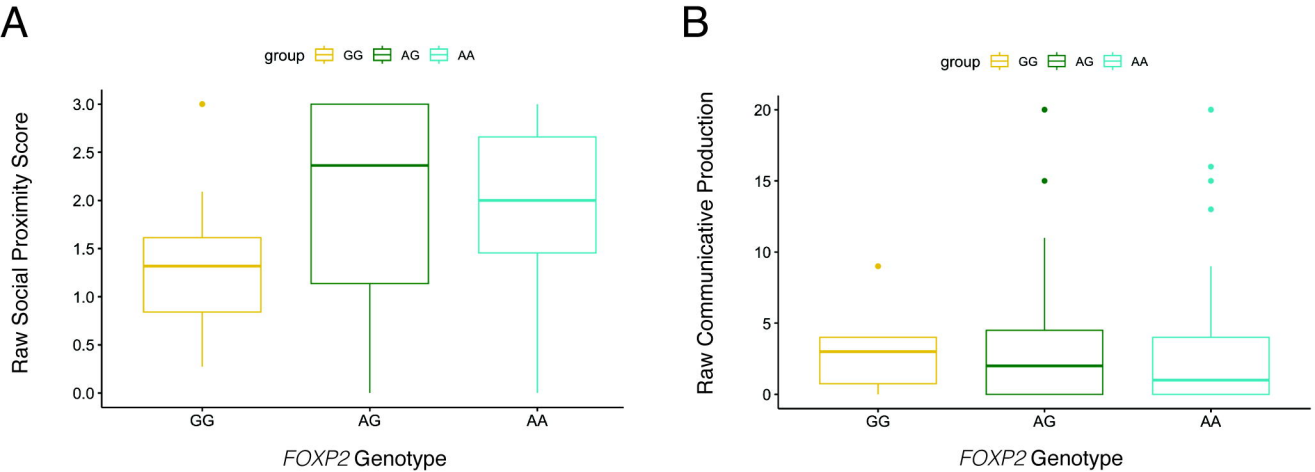
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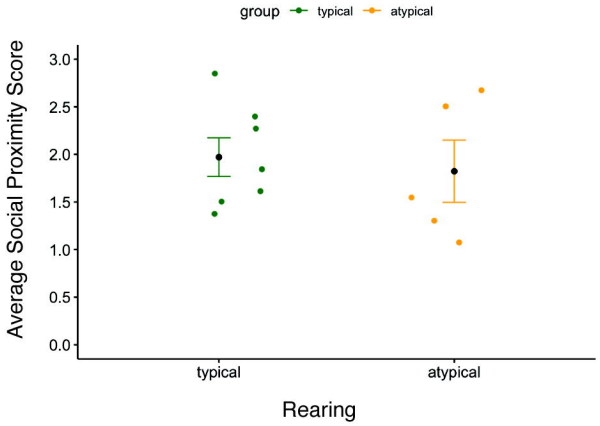
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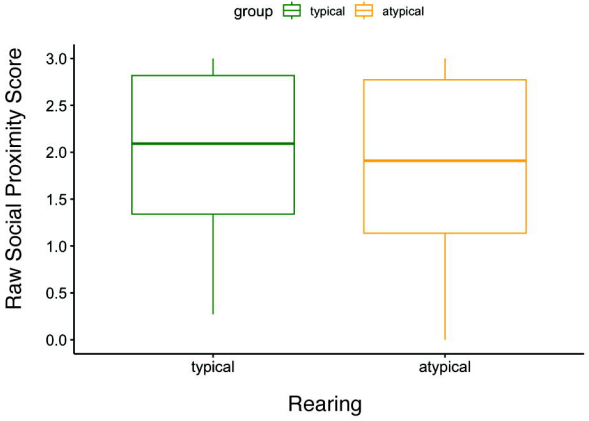
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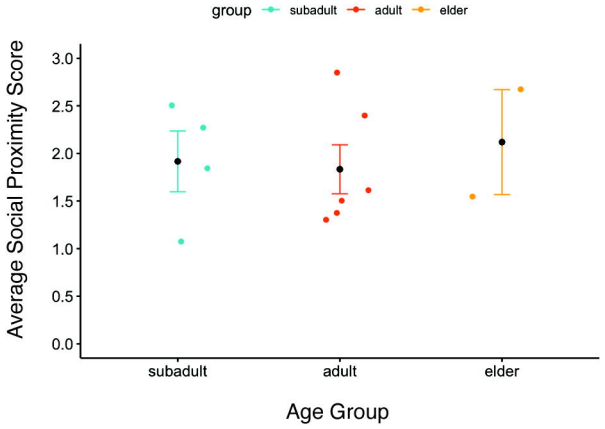
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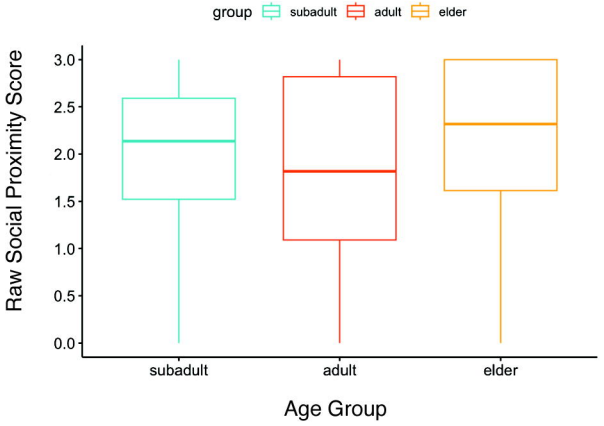
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