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

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Abstract

Single-nucleotide polymorphisms (SNPs) in forkhead box protein P2 (*FOXP2*) and oxytocin receptor (*OXTR*) genes have been associated with linguistic and social development in humans, as well as to symptom severity in autism spectrum disorder (ASD). 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 aimed to determine if bonobos (*Pan paniscus*) exhibit individual variation in *FOXP2* and *OXTR* loci that have been associated with human social development and behavior. Although the ASD-related variants were reported in 13-41% of the human population, we did not find variation at these loci in our sample of 13 bonobos. However, we did identify a novel variant in bonobo *FOXP2*, as well as four novel variants in bonobo *OXTR* that were 17-184 base pairs from the human ASD variants. We also found the same linked, homozygous allelic combination across the 4 novel *OXTR* SNPs (homozygous TGTC) in 6 of the 13 bonobos, indicating that this combination may be under positive selection. When comparing the combined *OXTR* genotypes, we found significant group differences in social behavior; bonobos with zero copies of the TGTC combination were less social than bonobos with one copy of the TGTC 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 risk factors for social communication deficits in humans.

Keywords: *Autism, Bonobo, Genetics, Great Ape, Oxytocin, Social Behavior, Social Communication*

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.

A potential biological factor underlying individual differences in social communication is the forkhead box protein P2 (*FOXP2*). *FOXP2* is one of the first genes to be associated with human language disorders and fine orofacial motor control[3,4,5]. Most notably, researchers have determined that *FOXP2* is critical to the developmental processes underlying speech and language[3,4]. In addition, Haghighatfard and colleagues (2022) found that lower *FOXP2* expression was associated with executive dysfunction in children diagnosed with ASD[6]. Thus, it is possible that polymorphisms in *FOXP2* that affect gene regulation and protein expression 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*) plays in social bond formation and social motivation[8,9,10,11]. 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]. Collectively, these studies suggest that variation in *FOXP2* and *OXTR* 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[16,17,18]. Indeed, variation in *FOXP2* and *OXTR* have been associated with differences in social behavior and/or social communication in rodents[19,20], zebrafish[21], great apes[22,23], and zebra finches[24]. 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[25,26,27,28,29]. 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[30,31] and may be too phylogenetically distant from humans to advance early identification and intervention techniques[32].

Bonobos, along with chimpanzees, are the closest living relatives to humans and are regarded as having one of the most complex social communication systems in the animal kingdom. While bonobos cannot be diagnosed with human neurodevelopmental disorders, they do show significant individual-level variability in social engagement[33,34], communicative production[35,36], and repetitive behaviors[34,37]. Several studies have identified links between ASD-associated genes and social communication in

70 bonobos[9,38,39]. There is also evidence that oxytocin is related to socio-sexual behavior in
71 female bonobos[40]. Thus, bonobos are a promising candidate for investigating the impact
72 of genetic variants on human neurodevelopment and social communication.
73 Identifying biological factors that underlie individual-level social communication is
74 critical to our understanding of autism and other neurodevelopmental disorders. To this
75 end, we aimed to determine if bonobos - our closest living relatives - exhibit single
76 nucleotide variation in *FOXP2* and *OXTR* at known human loci that have been
77 implicated in autism or differences in social communication. Given that bonobos live in
78 large, dynamic social groups and that they produce complex communicative signals of
79 various types (vocalizations, facial expressions, manual gestures, and multi-source
80 signals), we hypothesized that bonobos would exhibit allelic variation in *FOXP2* and
81 *OXTR*.
82
83 **Materials and Methods**
84 Genetic Analyses
85 Biological samples were collected from 29 bonobos (7 whole blood samples and
86 22 buccal samples) living at the Ape Cognition and Conservation Initiative (n=7; IACUC
87 protocol #210305-01), the Columbus Zoo and Aquarium (n=6), and the Milwaukee
88 County Zoo (n=16). Whole blood samples were collected under anesthesia during the
89 bonobo's routine physical exam (n=6), except for one blood sample that was collected
90 from an awake bonobo via voluntary presentation (n=1). Buccal samples were collected
91 by swabbing the inner cheek or lower lip for 10-15 seconds with a QIAGEN OmniSwab

92 (n=22). All buccal samples were taken from awake bonobos that presented voluntarily
93 for sample collection.

94 Autism-associated genes included in this study were *FOXP2* and *OXTR*. Primer
95 pairs were designed using NCBI Primer-BLAST[41] and ApE[42] to flank each SNP by ~250
96 base pairs (giving approximately 500bp amplicons) and ordered from Thermo Fisher
97 Scientific. A total of 9 human SNP loci were included: *FOXP2* rs6980093, and *OXTR*
98 rs2270463, rs237877, rs237878, rs35062132, rs2254295, rs237894, rs237895, and
99 rs237900 (Table 1).

100 Table 1: Selected Human SNPs

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

101

102 Table 1: The name, position, relative allele frequency, and variant type for the selected
103 human single nucleotide polymorphisms.

104 DNA was extracted from bonobo whole blood and buccal samples, amplified by
105 PCR, resolved by gel electrophoresis, gel purified (Zymo Gel DNA Recovery Kit) and
106 sent to Genewiz for Sanger sequencing (see Supplementary Material 1 for details). To

determine SNP presence, individual Sanger sequences were aligned to the bonobo reference genome using ApE and the human reference genome [Dec. 2013 (GRCh38/hg38)] using the BLAT tool[43]. Heterozygotes were identified by visual inspection of the Sanger chromatogram and confirmed by sequencing the reverse strand.

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[34,44]. In short, eight 10-minute focal observations (i.e., observing the behavior of a single individual) from each subject were used to assess group differences in social proximity – an established method for measuring social relationships in nonhuman primates that encapsulates both social interest and engagement[45,46,47].

Statistical Analyses

For each observation, 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:

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

A Kruskal-Wallis H test and a Wilcoxon rank test were utilized to assess genetic differences in social behavior.

Results

Genetic Variation

129 Of the 29 biological samples, 13 were of sufficient quality for Sanger sequencing
130 (whole blood n=7, buccal swab n=6). See Supplementary Material 2 for the coefficients
131 of relatedness between each subject. Analyses revealed a novel SNP in bonobo
132 *FOXP2*, 75bp to the right of *FOXP2* rs6980093 (human chr7:114522685; A/G). Three
133 bonobos were heterozygous at this location (G/A), while the rest were homozygous
134 (A/A; bonobo 5' flanking sequence CACTCGTATCACATTATAAT A/G; Figure 1). Both
135 genotypes differ from the bonobo reference genome (G/G).
136 In addition, analyses revealed genetic variation in bonobo OXTR at four novel
137 loci (Figure 2A). Specifically, we identified a novel SNP 78bp to the left of rs237877
138 (chr3:8741201; C/T) and 184bp left of rs237878 (chr3:8741312; T/A/C); 12 of the 13
139 bonobos were homozygous (T/T) and 1 individual was heterozygous (T/G; 5' flanking
140 sequence TTGCAGCTATCACCTCATTT T/G). We also identified a novel SNP 19bp to
141 the right of human SNP rs35062132 (chr3:8753201; G/A/C). In our sample, 11 bonobos
142 were homozygous (G/G), and 2 bonobos were heterozygous (G/A; 5' flanking sequence
143 CGATGGCTCAGGACAAAGGA G/A). In addition, a novel SNP was observed 17bp to
144 the left of rs2254295 (chr3:8760606; T/C; Figure 2B-D) and adjacent to rs2254298
145 (chr3:8760542; G/A). Ten of the 13 bonobos in our sample were homozygous (T/T) and
146 3 bonobos were heterozygous (T/C; 5' flanking sequence
147 GGCACTGGATGAGGCTGCC T/C). The fourth novel SNP we observed in bonobo
148 *OXTR* was 19bp to the left of rs237900 (chr3:8767010; G/A). Analyses revealed 3
149 genotypes at this locus; 2 bonobos were homozygous (A/A), 2 bonobos were
150 heterozygous (C/A), and 9 bonobos were homozygous (C/C; 5' flanking sequence
151 GCCCAAGGACTGTGCTAAGG A/C). Collectively, we observed the same allelic

152 combination across the 4 novel *OXTR* SNPs (homozygous TGTC) in 6 of the 13

153 bonobos (Figure 2E). See Table 2 for a complete list of allele types and frequencies.

154 Table 2: Individual Sequencing Data

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	G/A	A/A	A/A	G/A	G/A	G/A	G/A	A/A
Novel SNP OXTR #1	T/T	T/T	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	C/T	T/T	T/T	T/T	T/T	C/T	T/T	T/T	C/T	T/T	T/T	T/T	T/T
Novel SNP OXTR #4	C/C	A/A	C/C	C/C	C/C	C/C	A/A	C/A	C/C	C/A	C/C	C/C	C/C
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

155 Table 2: Individual sequencing data for the novel SNPs identified in bonobo FOXP2 and
156 OXTR and for the selected human loci.

157 Behavioral Differences

158 For *OXTR*, individual bonobos were grouped based on the number of *OXTR*
159 TGTC copies (Figure 2E) – zero (n=2), one (n=4), or two (n=6). Kruskal-Wallis results
160 indicated a significant *OXTR* group difference in social proximity score (H (2) = 7.2991,
161 p = 0.026; Figure 3; Table 3). Bonobos with zero copies of TGTC were less social (*Mdn*
162 = 1.45) than individuals with one copy of TGTC (*Mdn* = 2.50) and individuals with two
163 copies of TGTC (*Mdn* = 2.00). A post-hoc Wilcoxon rank test with Benjamini-Hochberg
164 adjustment revealed a significant difference in social proximity scores between bonobos
165 with zero TGTC copies and bonobos with one TGTC copy (p = 0.049), but not between
166 bonobos with zero copies and two copies (p = 0.339) or between one copy and two
167 copies (p = 0.510). For *FOXP2*, individuals were grouped based on whether they were
168 homozygous (A/A) or heterozygous (G/A) at the *FOXP2* SNP locus. Wilcoxon rank test
169 results indicated there was no significant *FOXP2* group difference in social proximity
170 score (W = 1164.5, p = 0.275).

171 Table 3: Social Proximity Scores

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

Table 3: Social proximity scores for each of the bonobo's focal observations.

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* and *OXTR* 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 social behavior differences based on the bonobos' *FOXP* 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.

192 The human *FOXP2* SNP rs6980093 is an intronic polymorphism, suggesting it
193 may be involved in regulating *FOXP2* expression[7,48]. Several studies have identified
194 relations between *FOXP2* rs6980093 variation and speech production[49], cortical
195 activation in language-related regions[50], as well as speech and language learning
196 abilities[7,51] The identification of variation in bonobo *FOXP2* is a foundational step in
197 understanding the impact of *FOXP2* on social communication in humans and our
198 closest living relatives. Bonobos have the largest vocal repertoire size of the nonhuman
199 great apes[52], and modify their communicative signals depending on social
200 context[53,54,55]. In addition, multiple reports in a single bonobo support the notion that
201 bonobos are able to understand spoken English words and sentences[56,57,58], as well as
202 degraded and computer-generated speech[59]. Thus, it is possible that *FOXP2* alleles
203 modulate *FOXP2* expression in bonobos and that differential *FOXP2* regulation impacts
204 individual variability in bonobo vocal communication.
205 Specific *OXTR* variants have been linked to social functioning[60] and symptom
206 severity[14,15] in autism. In our sample of bonobos, we identified a novel SNP between
207 human *OXTR* rs237877 and rs237878. Variants at these loci have been linked to level
208 of extraversion[8] and reward responsiveness[61] in typically developed adults, as well as
209 neurological responses to oxytocin in autistic adults[62]. In addition, we identified a novel
210 SNP in bonobo *OXTR* close to the human SNP rs35062132. Eleven of the 13 bonobos
211 in our sample exhibited the G/G genotype. In humans, the C/G genotype of *OXTR*
212 rs35062132 was associated with an increased risk of ASD and proposed to be a
213 biological basis for individual differences in social behavior[63]. Although they identified
214 three genotypes in their sample, Egawa and colleagues did not find a relation between

215 these genetic variants and ASD[64]. Hence, further investigations are needed to
216 understand the role rs35062132 variation plays in social communication development
217 and to determine if the rs35062132 G allele is a risk factor for autism[63,64].
218 We also found novel SNPs in bonobo *OXTR* between rs2254295 and rs2254298
219 and adjacent to rs237900. Links between these genetic variants and social functioning
220 are a well-established finding, particularly for rs2254298[65,66,67,68]. For example,
221 rs2254295 and rs2254298 variants were associated with nonverbal communication
222 scores in Japanese adult males diagnosed with ASD[11]. Yang and colleagues found
223 differences in serum oxytocin levels based on rs2254298 genotype[11]. However, the role
224 that specific rs2254298 variants play in autism remains unclear. Specifically, results
225 differ among human populations; the "A" allele was associated with autism in
226 Japanese[69] and Chinese[70] populations, whereas the "G" allele was considered a risk
227 factor in a Caucasian population of autistic children and adolescents[71]. Parker and
228 colleagues also identified links between rs2254298 variants and social impairments in
229 children with and without ASD[72]. In both groups, individuals with the "A" allele exhibited
230 greater social impairments than individuals without the "A" allele. Collectively, these
231 findings suggest that specific *OXTR* variants might be promising biomarkers for social
232 communication dysfunction in humans and highlight the need for alternative approaches
233 to assessing the impact *OXTR* variants have on complex behavioral phenotypes, like
234 those observed in ASD[73,74].
235 Most notably, we observed the same allelic combination across the four novel
236 *OXTR* SNPs (homozygous TGTC) in six of the 13 bonobos – demonstrating linkage
237 between these *OXTR* variants. Although we did not find variability at the selected

238 human loci, the high prevalence of the homozygous TGTC genotype suggests that
239 these related variants influence individual-level social communication in bonobos.
240 Linked *OXTR* variants have also been found in children, adolescents, and young adults
241 diagnosed with ASD[12]. In addition, Wu and colleagues identified linkage among two
242 *OXTR* variants in autistic people from the Chinese Han population[70]. Thus, we
243 encourage researchers interested in the biomarkers of human social communication
244 disorders to consider the relative influence of individual SNPs and their collective
245 contribution to complex behavioral phenotypes.

246 If the observed TGTC combination was selected for in bonobos, we would expect
247 to see behavioral differences based on these genotypes. To test this hypothesis, we
248 utilized previously collected observational data that were available for 12 of the bonobos
249 in our sample. By grouping bonobos based on the number of TGTC copies (zero, one,
250 or two), we were able to investigate genetic differences in social behavior. Interestingly,
251 bonobos with zero copies of the TGTC combination had lower social proximity scores
252 (i.e., they spent less time in close proximity to conspecific social partners) than bonobos
253 with one copy of the TGTC combination. This result supports previous conclusions that
254 *OXTR* plays a pivotal role in bonobo social behavior[9,23,38] and is consistent with findings
255 in humans that indicate that linked *OXTR* variants are associated with greater
256 impairments on the social responsiveness and repetitive behavior scales in autistic
257 children[75]. Our results are also similar to data collected from children, adolescents, and
258 young adults diagnosed with ASD; a haplotype comprised of four *OXTR* loci was
259 associated with greater impairments in social interaction and communication in autistic
260 individuals[12]. These collective findings highlight the importance of considering multiple

genetic variants in a given study and the benefits of multi-loci investigations in nonhuman great apes. All told, our results suggest that the *OXTR* TGTC combination was selected for in bonobos.

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[76] and that oxytocin infusions can reduce RRBs in autistic adults[62,77]. 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[76]. Along with previous evidence of a low social, high RRB phenotype in bonobos[34], 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[62,76].

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 the complex phenotype of ASD[26,28,30]. In this study, we identified a naturally occurring linkage among 4 novel *OXTR* variants and documented differences in bonobo social behavior based on this combined *OXTR* genotype. We also demonstrated that it is possible to detect genetic variability, variant linkage, and behavioral differences in even

284 small samples of nonhuman great apes. Thus, we encourage the incorporation of
285 bonobos in future investigations and emphasize the need for a publicly accessible
286 database to report SNPs identified in nonhuman primates.

287 Here, we are the first to report a SNP in bonobo *FOXP2* – a gene necessary for
288 typical linguistic development in humans. We also identified four novel SNPs in bonobo
289 *OXTR* and demonstrated linkage among these *OXTR* variants. Our results indicate that
290 individuals without the *OXTR* TGTC combination are less social than individuals with
291 one copy of the TGTC combination. Our collective findings suggest that these *OXTR*
292 variants influence individual-level social communication in bonobos and support the
293 notion that linked *OXTR* variants could be promising biological factors for identifying
294 humans at risk of developing social communication deficits. This study also highlights
295 the advantages of studying biobehavioral mechanisms in the species most closely
296 related to humans and indicates that bonobos are a suitable model for testing
297 hypotheses about the etiology of ASD and other human neurodevelopmental disorders.
298

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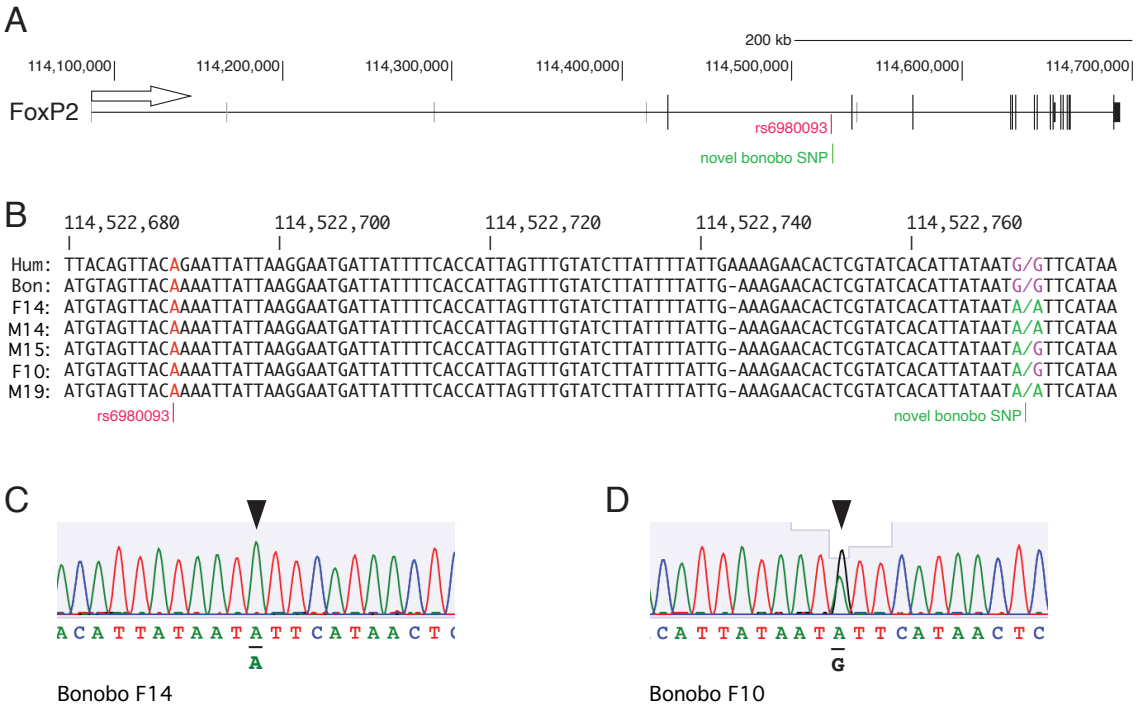
307 endangered species, with human activity as the greatest threat to their survival.

308

309 **Figures**

310 Figure 1



311

312 Figure 1: Identification of a single-nucleotide polymorphism in bonobo *FOXP2* (A)

313 Diagram of the human *FOXP2* gene showing relative location of the SNP rs6980093.

314 Arrow shows direction of gene transcription. Short vertical bars (grey) show

315 transcriptional start sites. Black vertical bars show exons. (B) Alignment of the human

316 and bonobo references genomes, along with representative sequencing data from

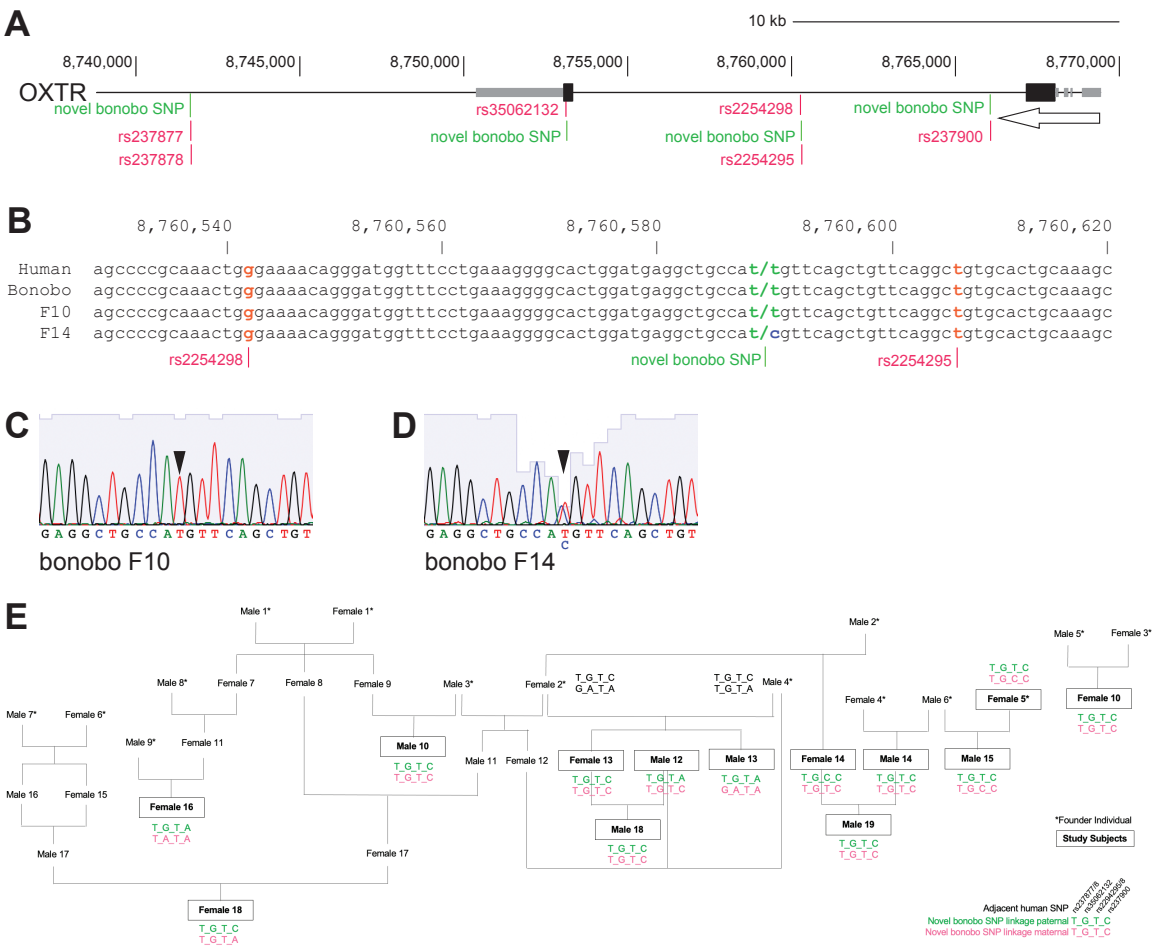
317 subjects in this study. Numbers refer to the human reference genome location on

318 chromosome seven. (C, D) Representative Sanger sequencing chromatograms across

319 the SNP, showing (C) a homozygous sample (Female 14), and (D) a heterozygous

320 sample (Female 10).

321 Figure 2



322

323 Figure 2: Identification of single-nucleotide polymorphisms in bonobo *OXTR* (A)

324 Diagram of the human *OXTR* gene showing relative location of SNPs rs237877,

325 rs237878, rs35062132, rs2254298, rs2254295, and rs237900, as well as the novel

326 bonobo SNPs. Arrow shows direction of gene transcription. Untranslated regions are

327 shown in grey. Black oblongs show exons. (B) Alignment of the human and bonobo

328 references genomes, along with representative sequencing data from subjects in this

study. Numbers refer to the human reference genome location on chromosome three. (C, D) Representative Sanger sequencing chromatograms across the novel SNP, showing (C) a homozygous sample (Female 10), and (D) a heterozygous sample (Female 14). (E) Pedigree of the 13 subjects, with corresponding *OXTR* genotypes at the four novel SNPs in bonobo *OXTR*.

Figure 3

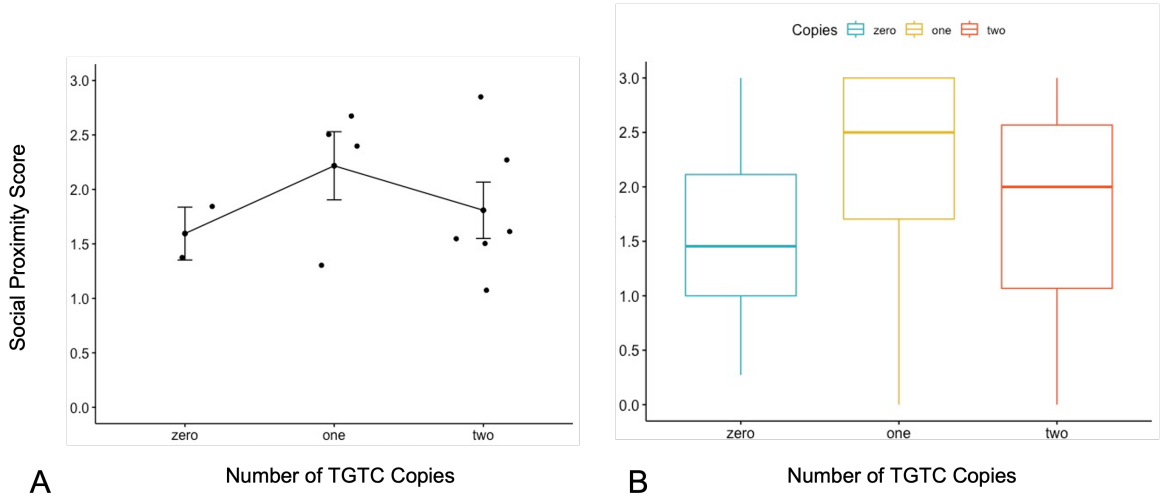


Figure 3: Observed group differences in social behavior based on the number of *OXTR* TGTC copies. Average social proximity scores with corresponding standard error bars (A) and raw social proximity scores (B) for bonobos with zero, one, and two copies of the TGTC combination.

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664 **Supplementary Material 1: Genetic Analyses**
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666 Bonobo DNA extraction was completed using the PureLink® Genomic DNA Mini Kit. For
667 whole blood samples, 3 ml of blood was collected from each bonobo and stored in a
668 freezer at -20 degrees Celsius. The blood lysate started as a combination of 200 µl of
669 frozen whole blood samples, 20 µl Proteinase K, and 20 µl RNase A in a 1.5 ml
670 microcentrifuge tube. The tube was then vortexed briefly and incubated at room
671 temperature for 2 minutes. Next, 200 µl PureLink® Genomic Lysis /Binding Buffer was
672 added to the lysate, and the lysate was vortexed until homogeneity. Once the lysate
673 was homogenous, it was incubated at 55 degrees Celsius for 10 minutes in a hot bead
674 bath to promote protein digestion. Then 200 µl 96-100% ethanol was added to the
675 lysate, and vortexed by 5 seconds to yield a homogenous solution. To ensure DNA
676 binding conditions were still met while removing salt and protein contaminants, 96-100%
677 ethanol was added to PureLink® Genomic Wash Buffer 1 and 2. Next, the lysate
678 prepared with the genomic lysis/binding buffer and ethanol (~ 640 µl) was transferred to
679 a PureLink® Spin Column in a collection tube. The column was then centrifuged at
680 10,000 x g for 1 minute at room temperature. The collection tube was discarded, and
681 the spin column was placed in a new sterile collection tube.
682
683 For buccal swab samples, the Qiagen Omni Swab tips were ejected and placed in their
684 own 2 ml microcentrifuge tube, and then 600 µl of phosphate buffered saline (PBS) was
685 added to the tube. Next, 20 µl of Proteinase K was added to a large 15 ml centrifuge
686 tube, as well as 600 µl of swab lysate, which were mixed well via pipetting. Afterwards,
687 600 µl of PureLink® Genomic Lysis/Binding Buffer was added to the lysate and mixed
688 by vortexing. The lysate was incubated at 55 degrees Celsius in a hot bead bath for 15
689 minutes. The tube was centrifuged to collect any leftover lysate that may be on the cap,
690 and then 200 µl of 96-100% ethanol was added to the lysate and vortexed for 5 seconds
691 to get a homogenous solution.
692
693 For DNA washing, 500 µl of ethanol prepared wash buffer 1 was added to the spin
694 column. Then the column was centrifuged at 10,000 x g at room temperature for 1
695 minute. The collection tube was discarded afterwards and replaced with a new sterile
696 tube. The DNA was washed a second time with ethanol prepared wash buffer 2 and
697 centrifuged at maximum speed, 15,000 x g, for 3 minutes at room temperature, and
698 then the collection tube was discarded. To elute the DNA, the spin column was placed
699 in a 1.5 ml microcentrifuge tube. Then 100 µl of PureLink® Genomic Elution Buffer was
700 added to the column and incubated at room temperature for 1 minute before
701 centrifugation at maximum speed. After centrifugation, the 1.5 ml microcentrifuge tube
702 contained purified genomic DNA. At the end, the DNA concentrations extracted from
703 blood were checked with a nanodrop.
704
705 Conventional polymerase chain reaction (PCR; PCR Master Mix (2X) from Thermo
706 Scientific) was used to amplify the target DNA sequence. A combination of 25 µl of PCR
707 Master Mix, 1 µM of forward primer, 1 µM of reverse primer, and 1 µg of template DNA
708 was then added to PCR tube kept on ice. Next, 22 µL of water was added to the mix
709 making it a total of 50 µL. The mix ratio was repeated for each bonobo DNA sample and

placed into the thermal cycler upon completion. The thermocycler conditions were set as follows:

1. 1.) Initial denaturation - temperature: 94 degrees Celsius, time: 1 minute
2. 2.) Denaturation – temperature: 94 degrees Celsius, time: 20 seconds
3. 3.) Annealing – temperature: 55 degrees Celsius, time: 20 seconds
4. 4.) Extension – temperature: 72 degrees Celsius, time: 20 seconds
5. 5.) Final extension – 72 degrees Celsius, time: hold
6.

The initial denaturation and final extension phases ran for one cycle each while the annealing and extension phases ran for a total of 35 cycles. Once the thermocycler completed the protocol, the PCR products were loaded onto 1.3% mini agarose gels for visualization. The visualized images served as a check to be sure that the PCR reaction was successful and produced the target amplicon. The gel was made of 1.3 g of agarose, 100 ml of TAE buffer, and 2 µl of ethidium bromide. The TAE buffer was a combination of 20 ml TAE buffer, 980 ml of dH2O, and 2 µl of ethidium bromide. 250 ml of TAE buffer and 2.5 µl of ethidium bromide was added to the electrophoresis cell. For each well, the PCR product and purple loading dye was loaded with a ratio of 10:2 as suggested by the Quick-Load Purple 100 bp DNA ladder guide. The cells ran at 90 volts and 400 amps for 55 minutes. Afterwards the gels were placed in a Bio-Rad ChemiDoc XRS+ System.

The third objective, gel purification, was conducted using a ZymocleanTM Gel DNA Recovery kit. The gels were placed on a UV transilluminator, and then each band was excised using a scalpel and transferred to a 1.5 ml microcentrifuge tubes. The microcentrifuge tubes' mass was 1.00 gram each. The total mass of the gel was calculated by taking the mass of the gel piece inside the microcentrifuge tube and subtracting the microcentrifuge tubes' mass. The mass number was then multiplied by 3 to figure out how many volumes (µl) of agarose dissolving buffer (ADB) to add to the microcentrifuge tube. Once ADB was added to the microcentrifuge tubes, they were incubated in a 55 degrees Celsius hot bead bath until the gel piece was completely dissolved and the solution was homogenous. Afterwards, the melted agarose solution was transferred to a Zymo-SpinTM Column in a collection tube and centrifuged for 60 seconds at 15,000 x g. The flow through was discarded from the collection tube so that it could be used again. For DNA washing, 24 ml of 96-100% ethanol was added to the 6 ml DNA wash buffer and 96 ml of 96-100% ethanol was added to the 24 ml DNA wash buffer. Next, 200 µl of DNA wash buffer was added to the column, and then the column was spun again for 30 seconds 15,000 x g. The flow through was discarded and the washing step was repeated. The final steps included placing the spin column into a 1.5 ml microcentrifuge tube, adding 10 µl of DNA elution Buffer directly to the center of the spin column, and centrifuging for 60 seconds. The final mass of the purified DNA products was determined using both gel visualization (using 1 µl of DNA mixed with 2 µl dye and 3 µl water for clarity), and a nanodrop machine. The remaining extracted DNA from the gels were sent to GENEWHIZ for Sanger sequencing.

Supplementary Material 2: Kinship Coefficients

Bonobo ID	F14	F16	M14	F13	M10	F05	M13	F18	M15	M12	M18	F10	M19
F14	1	0	0	0.125	0	0	0.125	0.031	0	0.063	0.094	0	0.25
F16	0	1	0	0	0.031	0	0	0.016	0	0	0	0	0
M14	0	0	1	0	0	0	0	0	0.125	0	0	0	0.25
F13	0.125	0	0	1	0	0	0.25	0.031	0	0.188	0.344	0	0.063
M10	0	0.031	0	0	1	0	0	0.063	0	0.063	0.031	0	0
F05	0	0	0	0	0	1	0	0	0.25	0	0	0	0
M13	0.125	0	0	0.25	0	0	1	0.031	0	0.188	0.219	0	0.063
F18	0.031	0.016	0	0.031	0.063	0	0.031	1	0	0.031	0.031	0	0.016
M15	0	0	0.125	0	0	0.25	0	0	1	0	0	0	0.063
M12	0.063	0	0	0.188	0.063	0	0.188	0.031	0	1	0.344	0	0.031
M18	0.094	0	0	0.344	0.031	0	0.219	0.031	0	0.344	1	0	0.047
F10	0	0	0	0	0	0	0	0	0	0	0	1	0
M19	0.25	0	0.25	0.063	0	0	0.063	0.016	0.063	0.031	0.047	0	1

Supplementary Material 2: Cell value is the kinship coefficient between the corresponding individuals. Each individual has a kinship coefficient of 1 with themselves.