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ORIGINAL ARTICLE

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Exploring gene-culture coevolution in humans by inferring neuroendophenotypes: A case study of the oxytocin receptor gene and cultural tightness

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Abstract

The gene-culture coevolution (GCC) framework has gained increasing prominence in the social and biological sciences. While most studies on human GCC concern the evolution of low-level physiological traits, attempts have also been made to apply GCC to complex human traits, including social behavior and cognition. One major methodological challenge in this endeavor is to reconstruct a specific biological pathway between the implicated genes and their distal phenotypes. Here, we introduce a novel approach that combines data on population genetics and expression quantitative trait loci to infer the specific intermediate phenotypes of genes in the brain. We suggest that such "neuroendophenotypes" will provide more detailed mechanistic insights into the GCC process. We present a case study where we explored a GCC dynamics between the oxytocin receptor gene (*OXTR*) and cultural tightness–looseness. By combining data from the 1000 Genomes project and the Gene-Tissue-Expression project (GTEx), we estimated and compared *OXTR* expression in 10 brain regions across five human superpopulations. We found that *OXTR* expression in the anterior cingulate cortex (ACC) was highly variable across populations, and this variation correlated with cultural tightness and socio-ecological threats worldwide. The mediation models also suggested possible GCC dynamics where the increased *OXTR* expression in the ACC mediates or emerges from the tight culture and higher socio-ecological threats. Formal selection scans further confirmed that *OXTR* alleles linked to enhanced receptor expression in the ACC underwent positive selection in East Asian countries with tighter social norms. We discuss the implications of our method in human GCC research.

KEYWORDS
anterior cingulate cortex, coevolution, cultural tightness–looseness, OXTR, social norms

1 | INTRODUCTION

Human diversity emerges at the confluence of culture and genes. Of many endeavors to understand the interaction between the two systems, the gene-culture coevolution framework (GCC) has gained increasing attention in both social- and natural sciences.[1] Unlike traditional theories in evolutionary biology that focused on the external environments beyond human control as the primary selection

pressure, GCC theories posit that human culture can also shape gene pools by altering the environments within which selection operates. As a result, specific genetic traits that are adaptive in particular social environments[2,3] may proliferate, sometimes rapidly over the course of a few hundred generations.[2] Based on a strong theoretical foundation[1,4] and empirical evidence accumulated across disciplines,[2,3,5] GCC is now believed to be one of the prominent modes of human adaptation in the recent past.[2,6]

Much of GCC research in humans has centered on the evolution of lower-level physiological or morphological traits,[2] with the best-known case being the coevolution of dairy farming and lactose tolerance in human adults.[7] Importantly, however, effort has also been devoted to studying the emergence of complex social behaviors and cognition in humans with respect to the GCC process.[5,8] For example, a pioneering study by Chiao and Blizinsky found a robust statistical association between allelic frequency of the serotonin transporter polymorphisms (i.e., 5-*HTTLPR*) and prevalence of a cultural value (i.e., individualism–collectivism) across the globe, which might reflect a GCC process.[9] Others have also looked for the geographical overlap between a genetic and cultural cline, confirming similar results with different genes and macro-level cultural patterns.[10–12]

Despite the fruitful results, these studies on GCC of high-level social traits in humans still suffer critical methodological limitations. The first limitation is insufficient or total lack of mechanistic explanations as to how variation within a specific gene leads to phenotypic variation.[13] Unlike the physiological or morphological traits for which genetic mechanisms can be traced with greater precision,[14] the pathways from genes to social cognition and behaviors are often complex and likely involve many intermediate mechanisms, most importantly, the brain.[15] The human brain is a critical node in a feedback loop where inputs from an ecological and cultural environment influence cognitive processes and behaviors of individuals, which can, in turn, shape the environments.[16] Therefore, understanding the intermediate phenotypes of genes in the brain, or neuroendophenotypes, is necessary for adequately tracing the connection between genes and their psychological, cognitive and behavioral correlates and also for inferring the adaptive significance of these phenotypes.[17] However, previous research on GCC of complex human social traits has not fully investigated the specific neuroendophenotypes of target genes.[13] Instead, studied have relied heavily on the associations between the genes and their distal phenotypes without elucidating the biological pathway linking them.

Another important limitation is that many prior studies on the GCC of human social traits have not presented any direct evidence of natural selection on the implicated genes. A mere difference in allele frequency across human populations, however, does not necessarily indicate selection and could be the result of neutral evolutionary processes such as genetic drift or founder effect.[18] Not testing the proposed GCC model against the null hypothesis of no selection can weaken the conclusion that GCC drives the cross-population variation in allele frequency.[18]

The main goal of this study is to propose a novel method by which researchers can explore a GCC of complex social traits in humans while addressing the key limitations of previous studies mentioned above. Central to our approach is to estimate specific neuroendophenotype of a target gene at the level of individuals and also populations. This is achieved by combining data on allele frequency distribution and expression quantitative trait loci (eQTL) of that gene. The resulting index of neuroendophenotype is then used to build and test the statistical models that may capture possible GCC dynamics involving the genetic- and cultural variables. Last, we also perform formal selection scans to determine if the results favor our GCC models over neutral evolutionary processes.

To empirically show the utility of our approach in studying GCC of complex social traits in human, we conducted a case study where we defined and tested a novel GCC model that centers on the following variables: cultural tightness–looseness (CTL) and the oxytocin receptor genes (*OXTR*).

CTL is an emerging theoretical framework that concerns the evolution of social norms in humans.[19] Social norms, or standards of behaviors and beliefs shared within a group,[20] are a uniquely human construct that allows us to coordinate more effectively with one another and punish those who undermine in-group cohesion. Importantly, studies have found that some human groups endorse stronger social norms and punishment than others,[19,21] and this cross-cultural variation in the strengths of social norms is what modern CTL theories seek to explain.

The central claim of CTL theories is that social norms are a cultural adaptation to various socio-ecological conditions that select for coordination and cooperation among group members. Specifically, factors such as harsh environments and intergroup conflicts can give rise to tight cultures with strong norms and sanctions to ensure stability and cohesion.[22] The theory also makes predictions for how CTL can shape individuals' psychological and behavioral traits over time.[23] That is, individuals in a tighter cultural environment are predicted to be more sensitive to norm violation, conformity pressure and show an overall preference toward social cohesion.[22] For several decades, the CTL framework has been widely applied to explain variations in social norms across human groups and gained empirical support.[19,23–25]

CTL offers a great avenue for exploring possible GCC dynamics as it concerns human adaptation[22] and has a theoretical structure with clearly defined causal relationships between its antecedent conditions (e.g., socio-ecological threats) and downstream phenotypic effects[22] (e.g., sensitivity to norm violation and preference for social cohesion). In fact, one study has recently applied a GCC framework on CTL and showed that allelic frequency of the serotonin transporter gene polymorphism (i.e., 5-*HTTLPR*) is associated with CTL and ecological threats across the globe.[10] However, like other previous studies, the specific mechanisms through which the short allele (i.e., S allele) of the *5-HTTLPR* could confer evolutionary advantages on its carriers in an environment with elevated threats were not discussed in relation to concrete physiological or neural mechanisms.[10]

In this case study, we examine the *OXTR*, which regulates the level of oxytocin (OT) receptor expression in the brain, as a possible genetic correlate of CTL. It should be noted that CTL is a complex construct and that its individual-level phenotypes are likely to be

subserved by many genes. Yet, our exclusive focus on *OXTR* is based on the following three considerations.

First, genetic variation in *OXTR* and its neuroendophenotypes in mammalian brains (i.e., the region-specific receptor expression level), including humans',[26] have widely been studied and relatively well-characterized.[27,28] Second, only small variations in the promotor regions of *OXTR* are required to alter the receptor expression sites in the brain, which may suggest that *OXTR* can evolve rapidly[29] in response to environmental pressure.[26] As cultural environment is also thought to change fast,[30] variations in the *OXTR* could be one of the key genetic factors that may be widely involved in the GCC process. Last, the neuropeptide OT is referred to as the "herding" or "binding" hormone because of its role in social alignment and cohesion.[31,32] For instance, intranasally administered OT (INOT) enhances cooperation,[33] behavioral coordination,[34] conformity,[32] interpersonal synchrony[35] and social learning in humans.[36] This points to the possibility that OT signaling in the brain can influence individuals' psychological and cognitive traits that could have direct adaptive significance in environments varying in CTL. Specifically, those with increased *OXTR* expression, or enhanced OT signaling in the brain, would be more likely to exhibit phenotypes that are adaptive in tight cultures (e.g., preference for social conformity, cohesion and sensitivity toward behavioral coordination).

If enhanced OT signaling in the brain indeed has differential fitness consequences in tight versus loose cultures, the allelic frequency of *OXTR* single nucleotide polymorphisms (SNPs) linked with higher receptor expression in the brain could also vary between tight versus loose cultures. While the cross-population difference in allele frequency of *OXTR* SNPs has been confirmed and studied,[11] it has not been linked with brain receptor expression, and the specific evolutionary mechanisms that could give rise to such variation have not yet been studied with respect to CTL and GCC.

In sum, CTL and *OXTR* are ideal candidates to be analyzed within a GCC framework. In our case study, we propose a hypothesis that variations in the *OXTR* SNPs leading to enhanced *OXTR* expression in the brain would be more prevalent in tighter cultures. We tested this hypothesis by applying the above-mentioned methodological proposal that focuses on neruoendophenoype.

2 | MATERIAL AND METHODS

The workflow of this study is summarized in Figure 1. First, we defined a quantitative index that captures the neuroendophenotype of *OXTR* SNPs (i.e., multi-locus profile score, MPS), and tested if significant variation exists in this measure across human populations worldwide. Second, we calculated a population-specific index for CTL and its various antecedent conditions (e.g., socio-ecological threats). Third, we tested the statistical relationships among our key variables (i.e., CTL, socio-ecological threats, and MPS) using correlation and mediation analyses and compare the results with what would be expected under the proposed GCC processes. Finally, we performed a formal selection scan using population branch statistic (PBS)[37] to confirm the evolutionary relationship suggested by the previous analyses. Each step will be described in detail below.

2.1 | Measurement and procedures

2.1.1 | Identifying *OXTR* SNPs that affect receptor expression in the human brain

First, we used the GTEx database (https://gtexportal.org; release V8) to obtain all *OXTR* SNPs ($N = 70$) that influence receptor expression in 10 brain regions of interest (ROIs): the anterior cingulate cortex (ACC), prefrontal cortex (BA9), frontal cortex, caudate nucleus, putamen, nucleus accumbens, hypothalamus, hippocampus, amygdala and substantia nigra. The complete list of the expressive variants for each ROI is provided in Table S1. Based on the eQTL, the allele associated with higher receptor expression within each ROI (i.e., high-expressing allele) and its respective effect size (i.e., normalized effect size, NES) were identified for each SNP.

2.1.2 | Allele frequency of *OXTR* SNPs across different human populations

Then, we downloaded the genetic information of 2504 individuals from 1000 Genomes Project Phase III database.[38] The raw DNA samples were obtained from five continental superpopulations (i.e., Africa, America, Europe, East Asia and South Asia), which were subdivided into 26 ethnic samples (Table S2). Recent evidence showed that the ancestry maps for these subpopulations can be reliably classified using a machine learning algorithm,[39] which suggests that our data can be less susceptible to the issue of genetic non-independence between study populations.[39] For each individual, we extracted the genotypes for all target *OXTR* SNPs identified via GTEx.

2.1.3 | Estimating endogenous OT signaling: *OXTR* multi-locus profile scores

To estimate the level of *OXTR* expression in each ROI, we computed *OXTR* multi-locus profile scores (MPS_{ROI}). MPS has widely been used in behavioral- and imaging genetics studies to model the effects of multiple SNPs or genes on complex behavioral traits[40] and brain functions.[41] Yet, unlike previous studies where MPS was defined solely based on the psychological or behavioral phenotypes of genes (e.g., the number of autism risk alleles[42]), MPS in our study instead captures the effects of *OXTR* SNPs on receptor expression in specific brain ROIs. As genetic variations in the *OXTR* influence social behaviors via receptor mRNA expression as well as the receptor density in the brain,[43] our approach could illuminate specific intermediate mechanisms that mediate the relationship between a genetic trait and its distant higher-level phenotypes that are under selection.

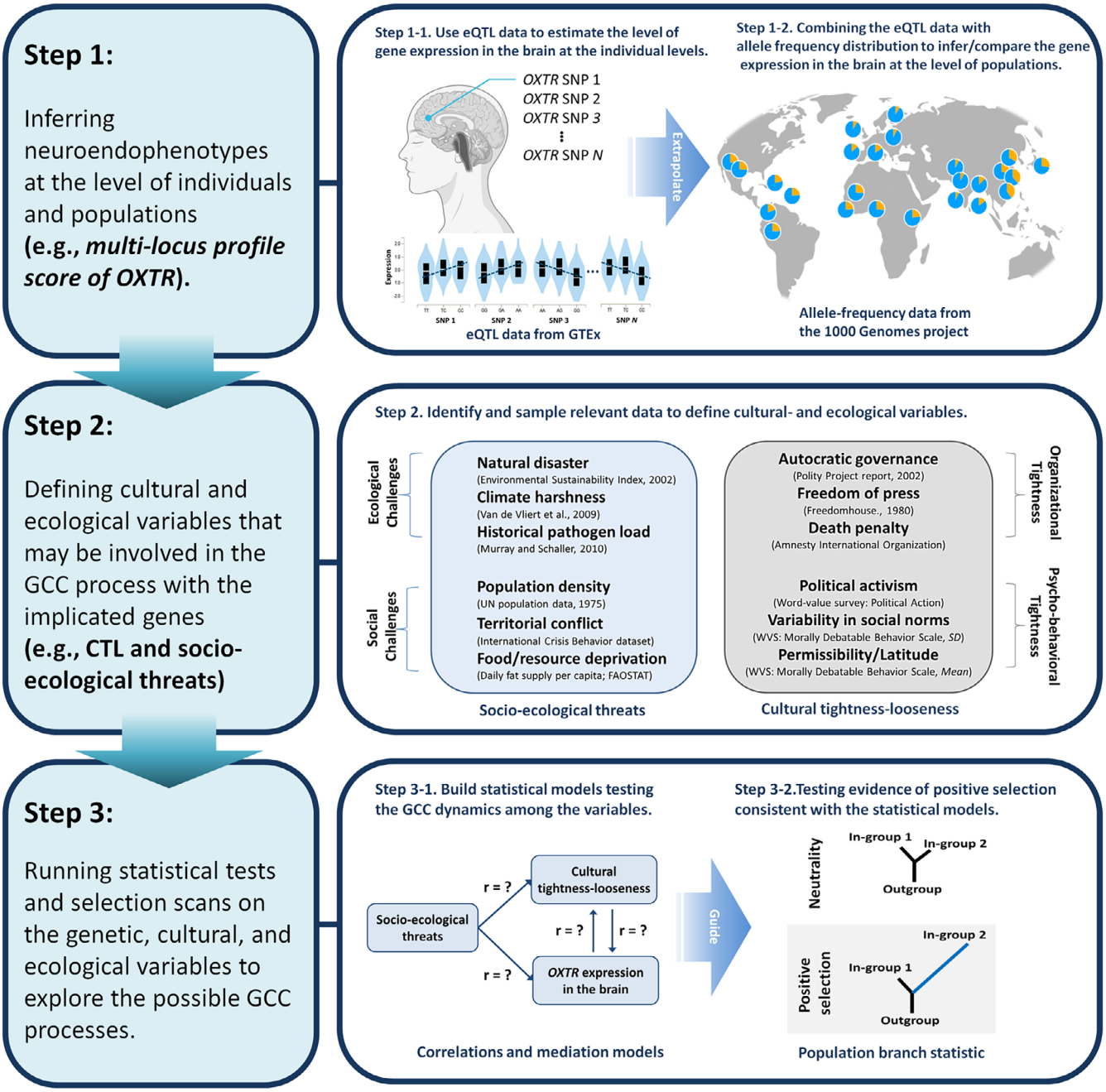

FIGURE 1 Flowchart showing the method and workflow of this study

Within individuals, we first calculated the number of high-expressing allele for each *OXTR* SNP (i.e., 0, 1 or 2) that affects each brain ROI ($N = 10$). The raw allele counts were then weighted by the SNP-specific NES. The raw NES values can be positive or negative as they represent the relative effects of alternative versus reference alleles on the receptor expressions. We only used the absolute NES values because high-expressing alleles could be either alternative- or reference alleles. The final MPS values were calculated by summing the weighted allele counts across all SNPs that regulate receptor expression in each ROI.

Here, it is important to point out that eQTL results and specific NES values, although highly conserved,[44] may not be identical across populations. As large proportion of the GTEx tissue samples were obtained from donors of European ancestry, such variability in eQTL and NES data raises the possibility that our MPS estimation process may not yield the most accurate result for the populations that are underrepresented in GTEx. To address this caveat, we repeated our key analyses using only the populations represented in GTEx (i.e., European, African and East/South Asians). The outcomes of these analyses largely replicated the main findings. The relevant information is reported in Figure S10.

2.1.4 | Cultural tightness–looseness

Next, we calculated the CTL index for the geopolitical entities (i.e., countries) that correspond to the subpopulations represented in 1000 Genomes database.

CTL is usually measured either via (1) a self-report questionnaire that asks respondents' subjective perception of social norms,[19] or through (2) societal- and individual-level indicators that are nomologically linked to the construct.[22,23,25] Although these two approaches have shown convergent validity,[22] the latter is often favored in the related literature,[10,23,25] as it captures various facets of CTL (e.g., socio-political contexts and organizational practices) that may not be adequately measured by the survey-based method.[45] It also allows researchers to study populations for which self-report data are not available.[25] Therefore, we also defined the index of CTL based on the organizational/institutional- and behavioral/psychological traits that have been shown to comprise the nomological network of the construct.

We specifically selected the following indices as proxies for tighter culture: authoritarian governance style, media freedom, retention of the death penalty, sociopolitical activism, public attitudes toward norm deviation (i.e., latitude/permissiveness) and overall variability in normative judgments. The first five variables were adopted from Gelfand et al., where the authors identified various socio-political correlates of CTL based on data from 33 nations.[19] The sixth variable was from Uz et al., which analyzed data from 68 countries to assess a dimension of CTL (e.g., behavioral variability) that was not measured in Gelfand et al. (2011) despite its theoretical importance.[22,45] By combining multiple, mutually complementing indicators of CTL, we aimed to better capture the multifaceted nature of the construct.[22] Unless otherwise noted, we used the oldest data available for each index to model historical conditions that can represent GCC processes. Data spanned the years 1800–2005. Possible effects of the wide time range represented in our CTL index are explored and discussed in Figure 11A,B.

The first three variables correspond the organizational and institutional correlates of strong social norms and norm enforcement, the two key hallmarks of CTL.[22] Data on authoritarian governance style were obtained from the Polity Project report 2005 (https://www.systemicpeace.org), which evaluated autocratic qualities in governing institutions worldwide. Media freedom was assessed using the Freedom of the Press public report compiling data between 1980 and 2017 across the globe ("Free" = 0, "Partly Free" = 1, "Not Free" = 2) (https://freedomhouse.org). We calculated the composite media freedom index by summing these scores for print and broadcast media. Last, the legality of the death penalty was coded based on Amnesty International report ("Retentionist" = 3, "Abolitionist for Ordinary Crimes" = 2, "Abolitionist for All Crime" = 1) (https://www.amnesty.org) as culturally tight societies tend to have stricter criminal justice systems.[19]

The last three variables tap into the behavioral and attitudinal phenotypes of CTL at the level of individuals.[22,45] Sociopolitical activism was measured with the "Political Action" questionnaire of the World Value Survey (WVS), where respondents' indicated their willingness to engage in collective actions against existing social orders.[19] One of five items (i.e., Occupying buildings or factories) from WVS was excluded due to limited data availability. We reverse-coded and averaged the raw ratings across the remaining four survey items so that the higher scores stood for stronger adherence to the existing social norms. Latitude/permissiveness and the variability in normative judgments were assessed with Morally Debatable Behavioral Scales (MBS) from WVS.[46] The survey measured the perceived justifiability of various topics that can be deemed deviant or contentious (e.g., Bribing, abortion, cheating on taxes, divorce, etc.). For latitude/permissiveness, we used the average justifiability ratings for 10 MBS items consistently measured across our cross-national samples.[10] The average scores were then reverse-coded so that the higher scores meant less permissiveness toward norm deviations. The overall variability in normative judgments was defined as the average SDs of the responses made for the 10 MBS items.[45]

Of 26 populations represented in the 1000 Genomes database, a total of 14 countries were selected for analyses based on data availability. For each national sample included, the final CTL index was calculated by averaging the Z-scores of all six scores discussed above. These indicators were internally consistent (Cronbach's $\alpha = 0.71$) and loaded onto a single factor that explained 57.9% of the sample variance (Figure S1). Our CTL index also significantly correlated with the two existing indices for CTL reported in Gelfand et al. (2011) [$r_{(8)} = 0.743$, $p = 0.03$] and Uz et al. (2015)) [$r_{(12)} = -0.724$, $p = 0.008$], where the authors calculated CTL scores for the cross-national samples that partially overlapped with ours.

2.1.5 | Socio-ecological threats

Last, we compiled multiple sources of social- and ecological threats that have been theorized and shown to correlate with various aspects of CTL: historical pathogen prevalence, disaster vulnerability, climatic harshness, population density, resource scarcity and territorial threats.[19,24,25,47,48]

The first three variables capture threats from the natural environment. Data on historical pathogen prevalence were taken from the epidemiological records of seven infectious diseases (i.e., leishmanias, schistosomes, trypanosomes, malaria, typhus, filariae and dengue), with the higher scores indicating increased risk.[49] Disaster vulnerability was assessed based on the Environmental Sustainability Index (ESI) codebook (i.e., Item 14, "Reducing Environment-Related Natural Disaster Vulnerability") (https://sedac.ciesin.columbia.edu/data/collection/esi/sets/browse). The original scores reflect the frequency of natural disasters and its resulting deaths between the years 1980 and 2000. Following a previous study,[47] we reverse-coded the raw index such that higher values denote increased susceptibility to natural disasters. Climatic demands are the variations in temperature from the optimal climatic livability. The index was defined for each country by the sum of the absolute deviations from 72 °F/22°C for the average lowest and highest temperatures in the coldest and in the hottest months.[48] We averaged the data for the coldest and the hottest months to create the combined index of climatic demands.

The next three variables depict the threats linked to human subsistence and intergroup conflicts. Data on population density (i.e., Population per km^2) in the year 1975 were obtained from the United Nations World Population database (https://population.un.org/wpp/). The year 1975 was selected from McEvedy and Jones

where the authors documented the growth trends of world populations between BC 400 and AD 1975.[50] We used modern population density because country-level statistics for historical population density were not available for many of our samples. Note that the modern population density in 1975 nevertheless correlated with historical population density in AD 1500 ($r_{[13]}$=0.82, $p = 0.001$). Resource scarcity was defined as a relative deprivation of high-energy food.[47] The index was calculated by reverse-coding the total dietary fat supply in 1961 based on the Food and Agriculture Organization of the United Nations (FAOSTAT) database (http://www.fao.org/faostat/en/#data/CC). Last, to code for intergroup conflict, we used the International Crisis Behavior (ICB) Project data (https://sites.duke.edu/icbdata/) which reported the number of territorial threats across nations between 1918 and 2013.[47] Each of these indices were Z-transformed and averaged across all available cross-national samples, yielding a single composite measure of socio-ecological threat. Consistent with the data inclusion criterion used for the CTL index, only the countries with data for all six variables for socio-ecological threats were considered for analyses ($N = 18$).

Worth noting is that socio-ecological threats may not be the only factor that selects for social coordination and cooperation, and thus the rise of tighter cultures. For example, differential subsistence style (i.e., farming vs. herding) is known to contribute to CTL partially independently of socio-ecological threats.[24] Therefore, while our main analyses focused on socio-ecological threats, we also explored an alternative, yet not mutually exclusive scenario of GCC centering on subsistence style. Converging results from this analysis will add to the validity of our proposed GCC process involving *OXTR* and CTL. The relevant procedures and findings are reported in Figures S3 and S4.

2.2 | Statistical analysis

Statistical analyses were performed using Statistical Software for Social Science (SPSS, version 26 and 28, Armonk, NY: IBM Corp), with the type-one error rate set to $\alpha = 0.05$ (two-tailed).

2.2.1 | Comparing *OXTR* expression profile in the brain ROIs across human populations

A series of linear mixed models (LMM) were used to test cross-population difference in *OXTR* expression in all target ROIs. The model included the five continental superpopulations as a fixed factor and 26 subpopulations and individual genetic samples as random factors with the unstructured covariance matrix. Restricted maximum-likelihood estimation was used with 100 iterations to reduce bias in random effect variance estimation. Bayesian information criterion (BIC) was used as a measure of model fit. We also explored the association between sex and *OXTR* expression in the brain based on previous findings that reported sex-specific OT signaling in the brain and its effects on social behaviors.[51] Accordingly, a separate LMM was defined for each ROI with sex as an additional fixed factor.

2.2.2 | Testing the associations among the *OXTR* MPS, CTL and socio-ecological threat

Pearson's correlation analyses were used to test the associations between (1) the ROI-specific average MPS values and the CTL indices obtained for each of our cross-national samples ($N = 14$), (2) the socio-ecological threat and the CTL indices ($N = 14$) and (3) the average MPS and the socio-ecological threat index ($N = 18$). The MPS values for three subpopulations (i.e., African ancestry in the southwest United States, ASW; African ancestry in Barbados, ACB; Utah residence with East and West European Ancestry, CEU) were excluded from these analyses because there were no specific geopolitical entities that corresponded to the ancestry labels. A single average MPS value was calculated and used for the countries that included multiple ethnic subpopulations (i.e., China: CDX, CHS and CHB; Nigeria: ESN, and YRI; India: GIH and ITU).

2.2.3 | Mediation analyses using *OXTR* MPS, CTL and socio-ecological threat

Next, we built and tested mediation models to further explore possible GCC dynamics involving socio-ecological threat, cultural tightness and *OXTR* MPS (i.e., MPS_{ACC}, See Section 3.1). It should be noted that mediation analysis on observational data is rarely sufficient to prove actual causal paths among the variables. Our use of a mediation model, as in other previous GCC studies,[9–11] was intended to explore if the relationships among MPS_{ACC}, CTL and socio-ecological threats are consistent with what would be expected under a GCC process, which posits a reciprocal mediation between genetic and cultural selection.[52]

Model 1 tested whether CTL could mediate the relationship between socio-ecological threats and MPS_{ACC}. In Model 2, MPS_{ACC} was entered as a mediator between socio-ecological threats and CTL. Inclusion of these two models was to explore the possibility that cultural- and genetic traits can form a mutually-reinforcing positive feedback loop.[53] The log-transformed gross domestic product (GDP) for each national sample in the year 1961 (https://data.worldbank.org) was entered as a covariate, following previous findings that socio-economic factors (e.g., modernization) may influence CTL irrespectively of socio-ecological threats.[10,24] The year 1961 was selected here as the oldest data point available from the source. The mediation effects were analyzed using a bootstrap estimation implemented in PROCESS macro on SPSS with 5000 bootstrap samples.[54]

2.2.4 | Test of positive selection for *OXTR* MPS across human populations

Last, to test the evidence of positive selection for enhanced OT signaling in the ACC, we used PBS.[37] PBS is a summary statistic that compares the pairwise F_{ST} values between three populations including

a genetically distant outgroup. F_{ST} stands for the proportion of the total genetic variance contained in a subpopulation, relative to the total genetic variance. A PBS value for a population at the given locus indicates the magnitude of population-specific sequence differentiation from the other two populations, which would indicate positive selection.

We targeted East Asians and Europeans as they represented the higher and lower side of CTL. Our choice of the ingroups also took into account the variance in the average MPS_{ACC}, which was the lowest and highest for the East Asian and European samples. This suggests that the difference in selection pressure imposed by CTL might have been most pronounced for East Asians versus Europeans. Africans were not considered for the PBS analysis because the country-level data on CTL were not available for most subpopulations except for Nigeria. For the genetically distant outgroup, we chose 25 unadmixed individuals from Peru (PEL), which allows for detection of positive selection between the East Asian and European populations as their divergence from populations from the Americas.

To detect regions under positive selection in the East Asian populations, the PBS employed a set of three populations (*X*, *Y* and *Z*), and assumed a rooted relationship ([X,Y],Z). We placed *X* as an East Asian population, *Y* as a European population and *Z* as the outgroup. All populations used in the selection scan are from the 1000 Genomes Project. We therefore were interested in computing:

$$PBS_{\text{East Asia}} = \frac{T_{\text{East Asia,Europe}} + T_{\text{East Asia,Peru}} - T_{\text{Europe,Peru}}}{2}$$

F_{ST} was estimated on a per SNP basis using the Weir and Cockerham calculation,[55] before proceeding with the PBS, which was confined to Chromosome 3 to focus the scan to the location of the *OXTR*. The scan was run with three European populations (i.e., IBS, GBR and FIN) versus East Asian populations (i.e., CHS, JPN and KHV). Among three subpopulations sampled within China, we chose CHS as southern Chinese provinces are known to have tighter social norms.[24] *OXTR* SNPs from the top 1% of the selection scans were tested for linkage disequilibrium (LD) with relevant SNPs from the expression analysis using the NCBI LDpair Tool. The relevant 1000 Genomes populations were selected for the LD test (i.e., CHS, JPT, or KHV).

3 | RESULTS

3.1 | Is *OXTR* differentially expressed in the brain across human populations?

Human groups worldwide show differential *OXTR* expression profiles in the brain, as indicated by a significant main effect of population on the average MPS for each of 10 ROIs (All *ps* < 0.001, Bonferroni corrected for the total number of ROIs). No main effect or interaction involving sex was identified (All *ps* > 0.262).

In this study, we focused on the *OXTR* expression in the ACC (i.e., MPS_{ACC}) as our post-hoc analyses showed the most consistent intergroup difference in the average MPS_{ACC}. That is, nine out of 10 pairwise comparisons yielded either a significant or marginally significant effect (Figure 2). Descriptive statistics and the summary of post hoc analyses for all ROIs are presented in Tables S3 and S4 and Figure S9.

It should be made clear that we did not have a priori hypotheses concerning a specific brain region. Yet, our decision to focus on MPS_{ACC} as the primary target of analysis was further guided by the finding that the ACC was one of two ROIs for which the average *OXTR* expression significantly correlated with both CTL and socio-ecological threats (See Section 3.2, and Table S8). The other ROI, the hippocampus, yielded a poorer model fit in the LMM analyses (BIC for $MPS_{ACC} = 13036.262$; $MPS_{Hippocampus} = 15357.781$). See Figures S6–S9 for the exploratory analyses and discussion on other ROIs, including the hippocampus.

3.2 | Are MPS_{ACC}, socio-ecological threat and cultural tightness correlated?

Across human populations, the average MPS_{ACC} values significantly correlated with both socio-ecological threat [$r_{(18)} = 0.792$, $p < 0.001$]

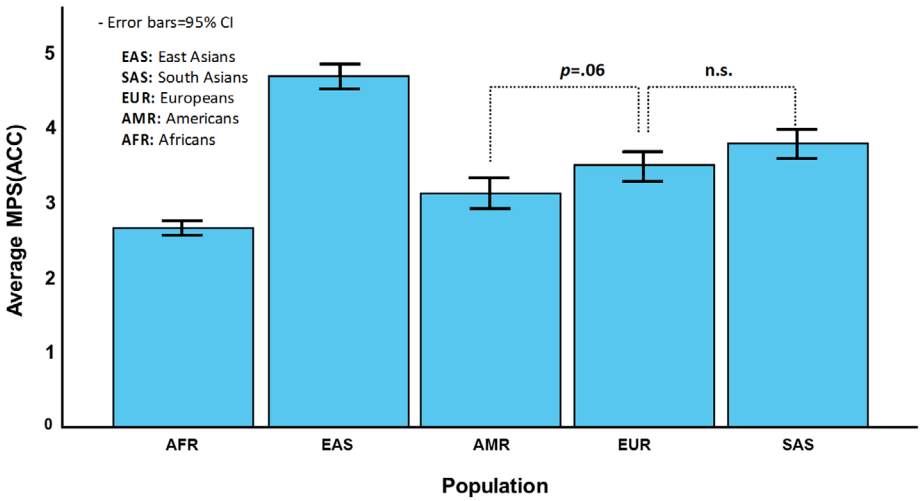

FIGURE 2 Cross-population difference in MPS_{ACC}. The average MPS_{ACC} denotes the estimated *OXTR* expression level in the ACC, with the higher values indicating more expression. All pairwise comparisons were significant at $p < 0.01$ (Bonferroni corrected for multiple comparisons between subpopulations) unless otherwise noted. (ACC, anterior cingulate cortex; MPS, *OXTR* multi-locus profile score)

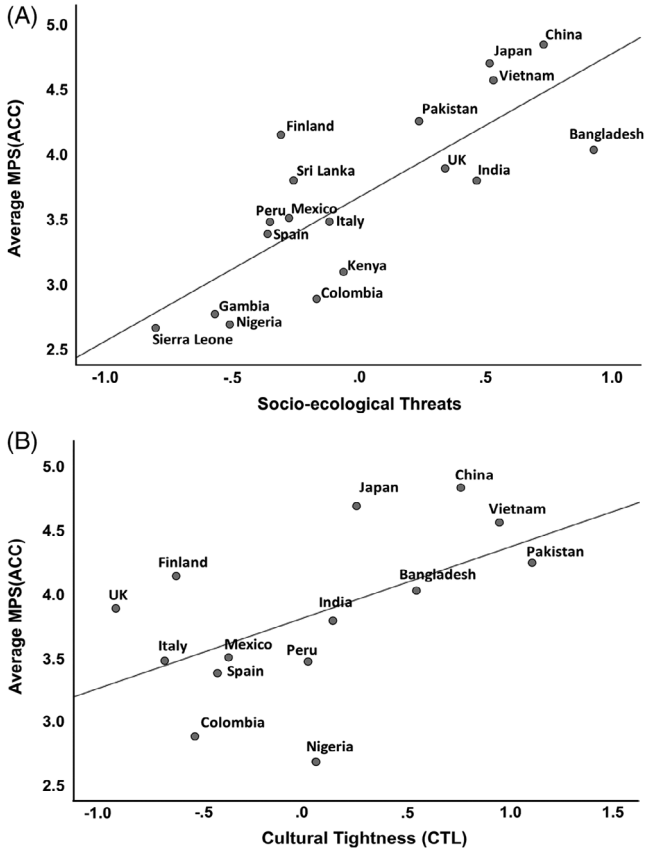

FIGURE 3 The associations between MPS_{ACC}, socio-ecological threats and CTL: The average MPS_{ACC} significantly correlated with socio-ecological threats (A) and CTL (B). (CTL, cultural tightness–looseness; MPS_{ACC}, MPS, *OXTR* multi-locus profile score)

and CTL [$r_{(14)} = 0.567$, $p = 0.034$] (Figure 3A,B). An exploratory analysis showed that MPS_{ACC} was associated more strongly with social threats [$r_{(18)} = 0.798$, $p < 0.001$] than with ecological threats [$r_{(18)} = 0.503$, $p = 0.034$]. Last, consistent with previous findings,[19,23] the CTL index positively correlated with socio-ecological threats [$r_{(14)} = 0.576$, $p = 0.031$]. The overall patterns of associations between CTL, socio-ecological threats and MPS_{ACC} remained consistent after controlling for the effect of GDP (Table S5).

3.3 | Does MPS_{ACC} mediate the relationship between socio-ecological threats and CTL?

Model 1 showed a statistically significant mediation effect, with the bootstrapped unstandardized indirect effect of 0.792 (Bootstrapped 95% CI [0.347, 1.545]). The direct effect was non-significant ($p = 0.253$) suggesting that socio-ecological threats promote CTL via its effects on the *OXTR* expression in the ACC (Figure 4A).

Model 2 also turned out significant, with the bootstrapped unstandardized indirect effect was 0.453 (bootstrapped 95% CI [0.0774, 1.1131]). Notably, the direct path remained significant ($p = 0.007$). This result indicates that socio-ecological threats influence the *OXTR* expression in the ACC both through and independently of CTL (Figure 4B).

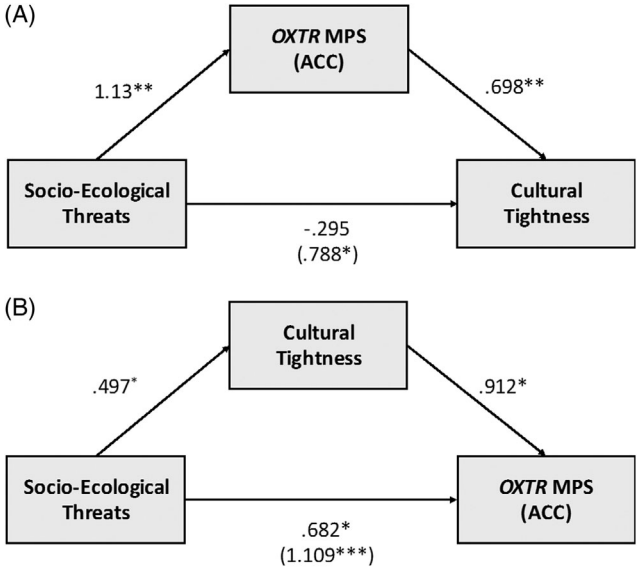

FIGURE 4 Mediation analyses showing the relationships between socio-ecological threat, MPS_{ACC}, and CTL, controlling for GDP. The average MPS_{ACC} was included as either an a mediator (A) or an outcome variable (B) ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{+}p = 0.06$). The direct effects (c') are shown inside the parentheses (*OXTR*, the oxytocin receptor gene; MPS, *OXTR* multi-locus profile score)

3.4 | Is there evidence of positive selection for MPS_{ACC} in societies with higher culture tightness and socio-ecological threats?

Our selection scans showed significant evidence of positive selection for multiple *OXTR* SNPs in East Asians (i.e., CHS, JPN and KHV) compared with IBS, with PEL included as an outgroup. The results of selection scans are summarized in Table S6. Notably, the C allele of *OXTR* rs9840864 showed elevated frequency in all three East Asian populations. Our follow-up analyses and discussion thus centered on rs9840864. The cross-population allelic frequency of *OXTR* rs9840864 and the sequence differentiation among East Asians, Iberians and Peruvians are illustrated in Figure 5.

While *OXTR* rs9840864 does not regulate receptor expression in the brain (i.e., NES = 0.03, $p = 0.7$), our follow-up LD analyses showed significant linkage between rs9840864 and two *OXTR* SNPs associated with receptor expression in the ACC (Figure S2). Specifically, for JPN and KHV, the C allele of rs9840864 is correlated with the T allele of rs151463 and the A allele of rs237893, both of which are linked to higher receptor expression ($D' = 0.38$, $R^2 = 0.11$). While the LD is not very strong, we did find that 60.6% of the relevant haplotypes in the JPT and KHV populations linking rs9840864(C) with rs151463(T). We found the same result for CHS ($D' = 0.38$, $R^2 = 0.13$), with rs9840864(C) linked to rs151463(T) in 62.9% of the CHS haplotypes.

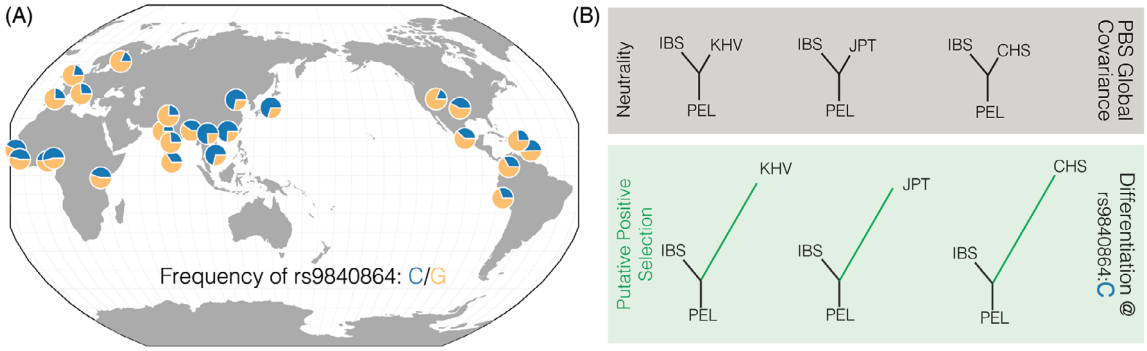

FIGURE 5 Putative evidence for selection on an *OXTR* allele in East Asian populations. Frequency of the rs9840864 variants from the 1000 Genomes Project, visualized with GGV[56] (A). Rooted trees showing the PBS covariance between populations, as expected under neutrality (top), and the differentiation exhibited at the rs9840864 locus (bottom) (B). Trees are drawn to scale. The 1000 Genomes populations utilized were 25 un-admixed individuals from PEL, 106 individuals from IBS, 98 individuals from KHV, 103 individuals from CHS and 103 individuals form JPT. (CHS, Southern Han Chinese; IBS, Iberian population in Spain; JPT, Japanese in Tokyo; KHV, Kinh in Ho Chi Mihn city; PBS, population branch statistic; PEL, Peruvians from Lima)

4 | DISCUSSION

Studies applying a GCC framework to study complex human traits have often suffered from lack of mechanistic specificity, which prevents researchers from understanding how specific genetic traits are linked to high-level phenotypes such as behaviors or cognition. In this study, we introduced a novel approach that can be used to infer the intermediate phenotypes of genetic traits in the brain (i.e., neuroendophenotpe). While restricted by the small sample size and cross-sectional data, our analyses yielded the findings in support of a GCC model where cultural tightness (CTL) across populations coevolves with OXTR polymorphisms that regulate OT signaling in the brain. Especially, increased CTL and socio-ecological threats were positively associated with *OXTR* expression in the ACC. The selection scans using PBS also found a result consistent with this putative GCC model of CTL and *OXTR* in three of the Asian populations that endorse tighter social norms.

4.1 | Quantifying *OXTR* expression in the brain: From allele frequency to neuroendophenotype

We combined the 1000 Genomes project database and the GTEx database to convert allele frequency data to the average *OXTR* expression levels in the human brain (i.e., MPS). This novel approach addresses the lack of mechanistic specificity found in the GCC literature by showing how genetic variation in *OXTR* is represented in specific brain regions of which the functions are relatively well-defined vis-à-vis social cognition and behaviors.[17] Also, we derived the MPS values from multiple *OXTR* SNPs and their ROI-specific effect sizes. Therefore, the MPS used in this study can serve as a more sensitive index than a single genetic marker for modeling the evolution of complex social cognition and behaviors in humans.

One important question is whether and to what extent the level of mRNA expression in the specific brain areas reflects the receptor density in those regions. For instance, mRNA can travel to distant sites via axons and be translated there. OT receptors can also be internalized and may not be available for surface signaling.[57] However, our approach is supported by a recent animal study showing that an *OXTR* SNP (i.e., NT213739) can regulate region-specific mRNA expression in the brain (i.e., the nucleus accumbens but not in insula).[43] Importantly, the level of mRNA expression in the nucleus accumbens showed a strong linear association with the local receptor protein binding density, which, in turn, predicted relevant social behaviors such as mating preference.[43] Overlap between *OXTR* mRNA expression and receptor density has also been found in primate species such as titi monkey[58] and rhesus macaque.[59] These results suggest that *OXTR* SNPs and their corresponding *OXTR* expression levels in the brain could be used as an index of localized, functional and quantifiable effects of OT in the brain. In all, while further investigation would be necessary to directly validate the relationship between *OXTR* mRNA expression, receptor density and its behavioral/cognitive effects in humans, our analytic strategy that focuses on neuroendophenotypes holds promise for elucidating the mechanisms through which genes and their polymorphisms could lead to adaptive phenotypes in a given evolutionary environment.

4.2 | Socio-ecological threats and *OXTR* in the ACC

Our result in the case study successfully replicated a well-established link between tighter cultures and socio-ecological threats, which are theorized to impose pressure for social coordination and cooperation.[22] Importantly, using the MPS values, we showed that *OXTR* expression in the ACC may also be implicated in the link between socio-ecological threats and CTL.

Both of our mediation models supported an evolutionary scenario where socio-ecological threats promote enhanced OT signaling in the ACC. Then, what might be the function of OT binding in the ACC? The ACC is a major node in the salience network of the human

brain.[60] The salience network is implicated in detecting important external events such as violation of expectations or errors.[61] This mechanism is also recruited during social interaction, especially in response to social misalignment.[31] For example, the dorsal ACC (i.e., dACC) shows increased activations when a mismatch between beliefs,[62] normative expectation,[63] esthetic preference,[64] or motor movement[65] is found between self and others. These activations within the dACC not only track the magnitude of the gap but also reflect negative emotional reactions to the misalignment.[66] These affective signals can, in turn, trigger context-specific behavioral adaptations such as conformity or punishment of norm violators.[31] The dACC is also robustly implicated in empathy which allows individuals to synchronize with others' internal states such as pain[67] or reward.[68] The neural signatures of such affective synchrony in the ACC are also known to predict altruism,[69] especially toward in-group members.[70]

To date, animal studies have provided the strongest causal evidence that OT can facilitate the ACC's role in social alignment and prosociality. Direct injection of OT into the ACC promotes behavioral coordination in macaque monkeys.[71] In rodents, the empathic response toward distressed, familiar conspecifics are mediated by *OXTR* in the ACC.[72] This effect was abolished when an *OXTR* antagonist was administered into the region.

Results from human studies also parallel the findings from animal literature. For instance, INOT increases cooperation,[33] conformity,[32] behavioral synchrony[34] and empathy.[73] Again, these effects are known to be stronger for in-group versus outgroup members.[33] Accumulating evidence also suggests that the ACC is implicated in the facilitative effects of the OT on social alignment and prosociality in humans. A recent meta-analysis showed that the ACC is one of the brain structures where the modulatory effects of INOT are most robustly identified.[74] Available evidence also indicates that INOT can enhance the neural representation of evaluative social feedback,[75] affective empathy,[76] reciprocated cooperation,[77] social rejection[78] and interpersonal synchrony[35] at least partially through different subregions of the ACC.

These findings suggest that increased *OXTR* expression in the ACC may be adaptive in conditions of elevated threats due to its function in promoting social alignment and cohesion among group members. As *OXTR* MPS values correlated more strongly with the composite measure of social threats than with ecological threats, it is possible that enhanced OT signaling in the ACC was driven by coordination problems of mostly social origins, such as intergroup conflict (i.e., warfare) and subsistence strategy (i.e., hunting and agriculture).[79,80] Future studies will be necessary for fine delineation of the paths linking different types of threats and the evolution of *OXTR* expression in the human brain.

4.3 | Possible GCC of CTL and *OXTR* in the ACC

Across populations, we found a significant positive association between MPS_{ACC} and the CTL index. As tighter culture has been theorized to be an adaptation to elevated socio-ecological threats, it is expected to find concordance between the relative prevalence of phenotypes associated with enhanced OT signaling in the ACC and tight culture. In fact, cross-cultural studies have also shown that individuals in tighter cultures tend to be more vigilant toward in-group deviants,[81] prone to conformity pressure[82] and showed a greater event-related potential (ERP) associated with error-processing when exposed to social norm violations.[83]

Our mediation models pointed to two possible scenarios that could give rise to this association. Model 1 indicated that tighter cultures emerged as the MPS_{ACC} values increased. One possibility is that the higher *OXTR* expression in the ACC is linked with cognitive- and psychological phenotypes of individuals that, when facing socio-ecological threats, may promote the rise of tight cultural institutions and practices at the level of populations.[84] In other words, those with a heightened sensitivity toward social alignment may develop strong norms and punishment to better achieve coordination and cohesion within society.

According to Model 2, the selection for high OT signaling in the ACC can be driven in part by CTL. This is consistent with a key tenet of the GCC framework that culturally transmitted norms and values can form a stable social environment and influence individuals' fitness, at least partially independently of its surrounding ecology.[5] Our finding thus points to the possibility that the selection for higher MPS_{ACC} may be in part mediated by social institutions that enforce the norms of social coordination and cohesion within society.

The results from the two mediation models may not be mutually exclusive. Instead, they may represent a GCC relationship where OT signaling in the ACC and CTL form a positive feedback loop in response to socio-ecological threats.[53] For example, (1) socio-ecological threats could initially select for high MPS_{ACC}, as those with high MPS_{ACC} will show a better propensity for coordination and cooperation. (2) Individuals with enhanced OT signaling in the ACC could then promote the emergence of tight cultures to better cope with the socio-ecological threats, which may, in turn, create selection pressure and further increase the prevalence of high expression *OXTR* SNPs within a society.

It should be made clear that the GCC scenario described above is highly speculative: the mediation analyses employed in a cross-sectional design, although significant, do not permit us to make a strong causal claim. Future studies using longitudinal data might shed more light on this proposed GCC dynamic between OXTR and CTL.

4.4 | Multiple routes to the coevolution of CTL and *OXTR* in the ACC

While most studies on CTL[19,22,23,25] posited socio-ecological threats as the key antecedent to tight cultures, they need not be the only condition that selects for increased social coordination and cooperation. In fact, we found a very similar coevolutionary relationship between cultural tightness and MPS_{ACC} using an index of

interdependent subsistence style, or the relative prevalence of farming versus herding[24,47] (Figures S3 and S4). This is consistent with an emerging view that subsistence culture that necessitates intense labor and resource control selects for strong social coordination, and thus leads to tighter cultures.[24]

While we are underpowered to directly pit one theory against another, evidence exists that subsistence styles and socio-ecological threats can influence CTL via partially independent pathways.[24] Therefore, our results suggest that the increased OT signaling in the ACC may evolve as a general adaption that support social coordination and cooperation, irrespective of the specific sources of selection pressure.

4.5 | Evidence of selection for enhanced *OXTR* in the ACC

Previous GCC studies on complex human social traits[9–11] have been criticized for not eliminating the possibility that the observed allele frequency difference across populations result from neutral evolutionary processes such as genetic drift or founder effects.[18] Addressing this limitation, we used PBS which tests the sequence differentiation between three human groups against the data generated by neutral simulations.[37]

The results of our selection scans were consistent with what would be expected under the GCC process depicted in the mediation models. Our PBS results, along with the LD analyses, showed the evidence of positive selection for enhanced OT signaling in the ACC among all East Asian samples compared with IBS. We also found the similar, yet subtler, evidence of selection with other European populations (i.e., GBR and FIN) (Table S6). What contributes to the differential selection signal is unclear, except that IBS had lower socio-ecological threats than other European samples considered in this study. Additional data and analyses will be necessary to determine the specific sources of differential selection pressure between various European populations versus East Asians.

Markedly, the C allele of *OXTR* rs9840864 showed elevated frequency in all East Asian samples and was in LD with two *OXTR* high expressing alleles (i.e., rs151463 and rs237893) that affect receptor expression in the ACC. By contrast, rs9840864 was not in LD with these two expressive variants in European populations. These results suggest that rs9840864, rs151463 and rs237893 may be traveling together on the same haplotype in Asian populations, potentially reflecting a common selection pressure imposed upon the three Asian populations.

The fact that we found evidence of selection does not necessarily imply that selection was driven by CTL and its antecedent conditions. However, the average MPS_{ACC} values calculated using rs151463 and rs237893 still showed a strong positive correlation with CTL, and replicated a significant mediation effect (Figure S5). It is thus plausible that the observed evidence of positive selection for high MPS_{ACC} in East Asian populations may at least partially reflect the specific co-evolutionary dynamics proposed in this study.

4.6 | Limitations

It should be noted that our population-level analyses were carried out with a relatively small sample sizes. This was unavoidable to a degree, as our choice of study populations was restricted by the combined availability of genetic data from the 1000 Genomes project and socio-cultural variables. Still, as a small sample can inflate the estimated effect size, the associations found in this study should be interpreted with caution until they are replicated with a larger dataset. The second limitation concerns the cross-sectional design of the study. Although we incorporated historical data to capture the evolutionary dynamics between CTL, socio-ecological threats and the *OXTR* expression in the brain, the results of our mediation models will ultimately remain correlational, unless they are confirmed in a longitudinal design that tracks actual changes in the allele frequency, socio-ecological threats and CTL through time. The rapid accumulation of ancient DNA data holds promise in this regard as it allows us to compare the genomic structure of prehistoric versus contemporary populations of modern humans.[85,86] In the context of CTL, for example, it may be possible to test whether the *OXTR* SNPs associated with higher receptor expression in the ACC exhibit an allele frequency trajectory consistent with positive selection by examining samples through time and whether such change could be attributed to population-specific historical conditions associated with CTL (e.g., the spread of rice farming in China and worldwide[24,87]). Last, as noted earlier, the tissue samples used for eQTL analysis in GTEx were obtained mostly from Caucasian individuals between the age of 50–70 ($N = 147$ for the ACC tissues). The limited ethnic representation in GTEx, coupled with the relatively small sample size, raises the possibility that the effect size of *OXTR* SNPs used in this study may vary depending on the specific demographics of the tissue donors in the database. Inclusion of additional biological samples, ideally from genetically and culturally diverse populations, will be important for addressing these limitations.

4.7 | Implication and future direction

Our approach has an important methodological implication for human GCC studies. First, characterizing neuroendophenotypes of a gene can show an additional layer of human variations embedded in allele-frequency data: the intermediate mechanisms through which a gene affects other higher-level phenotypes. This will also allow researchers to infer the adaptive significance of the implicated genes based on their neurological effects instead of more distal phenotypes. With regards to the specific findings of the study, one important future direction is to explore how *OXTR* expression outside the ACC is involved in the proposed co-evolutionary process. As mentioned earlier, CTL is a multi-faceted construct that influences a wide array of individual-level phenotypes.[22,25] In other words, the sensitivity toward social alignment and cohesion, although highlighted in this study, does not represent the full breadth of the possible phenotypes associated with CTL.[22] It could thus be fruitful to investigate whether

OXTR SNPs expressed elsewhere in the brain (e.g., rs53576) are linked with other aspects of CTL or even other closely related macro-level cultural traits (e.g., individualism–collectivism).[11] In a similar vein, examining other genes that influence OT signaling in the brain,[88,89] such as the OT gene itself and CD38, which is involved in OT secretion, will be important for more accurate characterization of neuroendophenotypes. Genes that regulate the serotonergic (e.g., 5-*HTTLPR*) and dopaminergic signaling (e.g., Dopamine D4 receptor, *DRD4*) also deserve attention, as they have been implicated in GCC of human social cognition[9,12] and known to interact with OT signaling in the brain.[90] Finally, a more thorough theorization and empirical demonstration of how macro-level cultural patterns arise and change over time would be imperative to further support our model findings, and also to extend the scope of this research beyond CTL and *OXTR*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The sources of raw data are reported in the manuscript. Processed data used for the analyses can be found at: https://osf.io/sbemd/?view_only=b623455053c84d0ea60564aface5f4f0

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