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Recalled parental bonding interacts with oxytocin receptor gene polymorphism in modulating anxiety and avoidance in adult relationships

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ABSTRACT

Early interactions with parents affect subsequent social experience throughout the course of a lifetime, as a repeated and prolonged perception of different levels of care, independence or control influences the modulation of emotional regulatory processes. As many factors play a role in shaping the expectations and features of social interaction, in this study we consider the influence of reported parental bonding and allelic variation of oxytocin receptor gene polymorphism (rs53576) over relationship levels of experienced anxiety and avoidance in 309 young adults belonging to two different cultural contexts, namely Italy and Singapore. Results highlight a major effect of maternal characteristics, care and overprotection, with differences between the two cultural groups. Additionally, the interaction between rs53576 and maternal overprotection suggest different environmental susceptibility in the Italian sample and the Singaporean one. Implication in clinical work and future steps are described in the conclusion.

Introduction

Several studies in developmental science have shown that early social interactions with parents (and caregivers in general) influence different aspects of child development, such as social relationships[1–3], academic performance[4], response to stress[5,6], individual well-being and risk for psychopathology[7–10]. In addition, emotion-related skills in children such as perception, regulation, and communication are built on the emotional and social relationships they share with their close ones[11]. Parental care in this case refers to the sensitivity with which parents attend to their child's needs (emotional, physical, psychological), while parental over-protection here refers to the excessive restrictions (emotional, physical, psychological) imposed on the child. Generally, the literature points towards higher care and lower over-protection as an ideal parental pattern[12], since parental care helps individuals to be empathetic in social interactions, while parental over-protection, perceived as control, reduces the possibility to develop a more adaptive way to regulate emotions and explore different social contexts[13]. Therefore, it is likely that perceiving positive parenting features, like higher levels of warmth, care and sensitivity, will result in more adaptive emotion-regulation skills[14]. Additionally, the quality of parental bonding influences the development of top-down processes of emotional regulation, which could potentially shape social interactions among individuals[15]. Consequently, adverse parenting practices are also relevant to the manifestation of social anxiety traits in individuals[16].

According to the original theories on attachment by Bowlby and Ainsworth[17,18], attachment-specific features were considered to extend beyond cultural boundaries or parental style. In other words, Bowlby's and Ainsworth's original theory was considered to be culturally universal in the sense that parental behavior that is sensitive to the child's needs will result in attachment security across a diverse array of cultural and ecological contexts[17]. Despite this assumption, the components of attachment belonging to the family environment have always been seen as an important factor that need to be taken into account, together with other features that might play a role in child rearing behavior, such as socioeconomic status or ethnicity. In fact, one of the major concerns in attachment-related research is the limitation due to results coming primarily from countries belonging to Western societies. With regards to cultural studies, Ainsworth's canonical work in Uganda and Baltimore underlined that processes related to maternal behavior reflect specific universal features that cross cultural, geographic and linguistic boundaries[19]. In their research based on a sample of Japanese mothers, Behrens and colleagues found that maternal attachment was predictive

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for the child's attachment classification with outcomes comparable to global results[20]. In a recent review on parental sensitivity in ethnic minority families, Mesman highlights the positive role of secure patterns of attachment on children's development[21]. A recent work on the link between perceived caregiver's warmth and levels of anxiety and avoidance in adult attachment in a Chinese population found that participants who were classified as "secure" were reporting more optimal parental patterns, with females resenting more of paternal care, but no differences were found between genders for higher levels of anxiety[22].

In light of these considerations, it appears clear that, to have a proper understanding of familial bonds, it is essential to take a wider look at the concept of familial nucleus according to the country where these family units are immersed. In the present study, we will focus on two countries, one belonging to a European-Western model (Italy) and one to an Asian model (Singapore)[23]. In Italy, which is configured within individualistic countries, family is seen as an expanded network of relationships and ties, where a lot of attention is given to the child. The relationship that forms between parent and child tends to be supportive and encouraging towards independence, presenting high levels of emotional bonding[24]. On the other hand, Singapore is described as a multicultural city, comprised of a majority of Chinese, Indian and Malay families[25]. In Singapore, which belongs to a collectivist culture[23], family is considered the first group a person joins in his/her life. The family framework belongs to a collectivist culture, like other Asian countries, where interdependent relationships occur on a social and familial level, especially for Chinese and Indian households. However, for all ethnicities, the extended family traditionally used to live with the original family and parental control over the children was extended until adulthood. With the sudden rise of technology and globalization, it is difficult for the Singaporeans to maintain a multigenerational structure in families[26], and many people decide either to live alone or not to have children[25]. With regards to both the countries, and to a worldwide trend, parental roles changed for those who decide to have children: if the hierarchy once used to be based on a patriarchal model, with the father having disciplinary power and the mother staying at home taking care of family duties, now both parents share the care of their children. Although evidence coming from different cultures and ethnic groups have been reported and discussed, concerns about the application of methodologies based on Western samples to non-Western environments still remains. Furthermore, while attachment dynamics have been profusely explored, little is known about the effect of processes that elapse between childhood and early adulthood, in the manifestation of dynamics in adult relationships. For these reasons, it is necessary to investigate factors belonging to both nature and nurture in order to provide a global representation of the dynamics of social development[12].

Considering the development of specific social characteristics is a result of the interaction between biological and environmental factors, it appears clear that behavioral facets might present a combination of genetic, environmental, and neurobiological factors. This requires the genetic regulation of neurotransmitters that are notably involved in the modulation of social functioning, such as oxytocin receptor gene (OXTr)[27–29]. Evidence in literature reports results about the involvement of oxytocin receptor gene SNPs in modulating the response to social stressors[30–32]. Within the polymorphic region of the OXTr, allelic variations of the single nucleotide polymorphism rs53576 have been shown to be associated with different social behaviors. Specifically, the G allele appears to be linked with optimal social development, which is described through higher levels of dispositional empathy[33,34], favourable prosocial features[29,33,35,36], and greater autonomic reactivity to social stressors[37]. On the other hand, A/A homozygotes have shown overall poorer social traits[38,39], reduced empathic accuracy[33], and positive affect[40], all representing a less flexible social development. Although many results highlight the association between specific variations of the genotype and their determined social features, there are still some discrepancies in literature[41–43]. Moreover, the gap in literature exploring the association between oxytocin receptor gene variants and environmental factors, such as early interaction and cultural environment, needs to be deepened.

Therefore, the first aim of our study is to deepen the understanding of how the interaction between individual genetic features and the perception of parental warmth during childhood affects levels of anxiety and avoidance in adult relationships. A second aim is to analyze the differences occurring in two countries, namely Italy and Singapore. The choice of these two contexts allows for a comparison of a Western country and a non-Western country, as environmental factors, such as the country one belongs to may affect on one hand the perception of parental features in childhood, and on the other hand the formation of behavioral constructs that drive social relationships. In order to explore parental transmitted behavior towards the child (assessed in terms of warmth/care and overprotection), we used the Parental Bonding Instrument by Parker and colleagues[2]. To measure levels of anxiety and avoidance in adult relationships, we employed the revised version of the Experience in Close Relationships[44]. For the Singaporean sample, we used the original versions in English, while for the Italian sample, participants completed the translated and validated Italian versions[45,46]. We hence set two hypotheses for our study, following an exploratory approach:

HP1: We predict a differential influence of perceived parental bonding (assessed with the PBI subscales) between the Italian and the Singaporean groups in explaining levels of anxiety and avoidance (measured using the ECR index);

HP2: We expect to find statistically significant effects of the interaction between OXTr polymorphism and parental bonding features (assessed using the PBI scales) over the main features of adult social relationships (measured with the ECR).

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Results

ANCOVA analyses of the ECR subscales have revealed significant interaction effects between the two cultural samples and PBI subscales, and between OXTr rs53576 and PBI subscales. As levels of Anxiety and Avoidance were analyzed separately with two ANCOVA tests, the alpha, corrected with the Bonferroni method, is 0.025. In the following subsections, only significant results that survived the Bonferroni correction are listed and discussed.

Interaction effects of country of belonging and recalled parental bonding

Results for the comparisons between the two cultural groups are displayed in Figure 1. A significant interaction result was seen for *ECR Anxiety* between the sample and Maternal Care on levels of anxiety in adult attachment (F_{Anx} (2,592) = 6.935, p= 0.0089). Post-hoc analysis revealed a significant correlation for the Italian group (r_{Anx_ITA}= -0.376) but not for the Singaporean one (r_{Anx_SNG}= -0.075) (see Figure 1A) with a significant difference between the two groups at high levels of Maternal Care (t(142)= -3.139, p= 0.002) (Figure 1B).

According to the results, *ECR Anxiety* is also affected by the interaction between culture and maternal overprotection (F_{Anx} (3,685) = 9.860, p= 0.002). Student's *t-test* highlighted a significant difference in Anxiety between the two samples at low levels of *Maternal Overprotection* (t(159)= -3.641, p < 0.001)(Figure 1D) and a significant correlation for the Italian group (r_{Anx_ITA}= 0.429) but not for the Singaporean one (r_{Anx_SNG}= 0.010) (Figure 1C).

With regards to *ECR Avoidance*, we found a significant interaction effect between OXTr rs53576 allelic variation and *Maternal Care* (F_{Avoid} (1,390) = 5.289, p= 0.022), but post-hoc analyses failed to find significant differences. The interaction between culture and *Maternal Overprotection* resulted to be significant in explaining relational avoidance in adulthood (F_{Avoid} (1,339) = 5.172, p= 0.024). Student's *t-test* highlighted that the difference is significant at low levels of *Maternal Overprotection* (t(159)= -2.949, p = 0.004)(see Figure 1F), with a weak correlation for the Italian sample (r_{Avoid_ITA}= 0.200) but not for the Singaporean one (r_{Avoid_SNG}= 0.047)(see Figure 1E).

Interaction effects of rs53576 allelic variations and recalled parental bonding

While analysing effects of *Avoidance* levels in the total sample, we found an interaction effect among the allelic variation of OXTr rs53576, culture and *Maternal Overprotection* (F_{Avoid} (1,352) = 5.145, p = 0.024). From the results of the Student's *t-test*, we found two significant outcomes. With regards to the first outcome, post-hoc analysis revealed that levels of Avoidance differ between Italy and Singapore for G-carriers only, with a history of low perceived Maternal Overprotection (t(102)= -2.791, p = 0.006) (see Figure 1H). Further analyses revealed a weak positive correlational effect for the Italian sample (r_{Avoid_ITA}= 0.180) and none for the Singaporean one (r_{Avoid_SNG}= 0.014)(see Figure 1G).

The second significant difference we found for this interaction applies only to the Italian sample, where rs53576 variants interacted significantly with *Maternal Overprotection* levels in describing *Avoidance* (t(48)= 2.121, p = 0.039). Specifically, A/A homozyogtes showed increased levels of avoidance for higher levels of maternal overprotection ($r_{ITA\_A/A}$= 0.476) compared to G-carriers (r_{ITA_G}= 0.180). Results are shown in Figure 2.

No significant effect was found for perceived Paternal Care nor Paternal Overprotection.

Discussion

The main purpose of this study is to examine the effects of different genetic susceptibility and early interactions on levels of anxiety and avoidance experienced in adult relationships. Specifically, we focused on recalled parental bonding, assessed in terms of parental care and overprotection. Additionally, we included in our analyses the effect of allelic variation of one OXTr gene polymorphism, namely rs53576. Taking the results together, both anxiety and avoidance appear to be modulated by the effect of recalled maternal features and culture. In fact, both maternal care and overprotection appear to explain levels of anxiety, with a negative correlation: the greater the care, the lower the anxiety. This result is in line with the theoretical framework of the development of a secure bonding[47] and, furthermore, it is also supported by other studies[22,48]. On the other hand, perceived maternal control during childhood, assessed in terms of overprotection, seems to be the construct that better explains levels of avoidance in adult relationships. This reflects the diversified, but equally important, contributions that appraised parental warmth produce in their offspring, which bring about different outcomes as a consequence. We observed that the effects on the two groups are different, especially in the relationship between maternal care and anxiety. As previously mentioned, care refers to the warmth and acceptance that parents have towards their child, so it appears that different parenting practices, due to environmental context such as culture, and maternal preoccupation affect the eventual level of anxiety experienced[49]. Although our results show differences between the two populations, Italians and Singaporeans, the correlational analysis revealed a stronger effect in the Italian group. This can be explained, to a certain extent, through the lens of the cultural context and the influence it exerts on the formation of familial bonds in the respective countries. As aforementioned, Italian families present stronger emotional connections[24,50] and commonly the mother represents the relational core of the family, especially during late childhood and adolescence[51], which corresponds to the period assessed by the Parental

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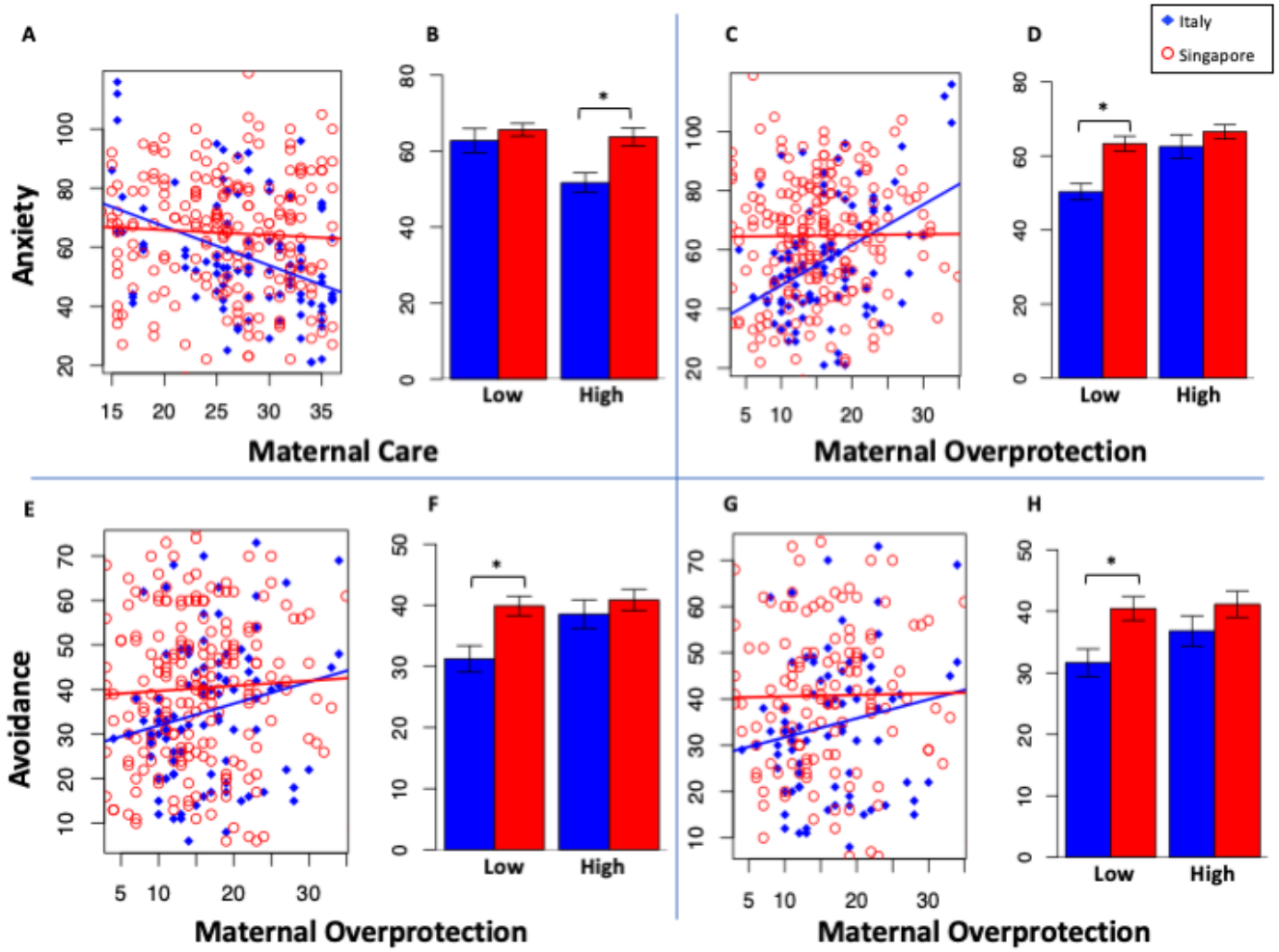

Figure 1. Interaction Effects of OXTr rs53576 allelic variation, perceived Maternal features and culture over the levels of experienced Anxiety and Avoidance in adult social relationships. *p<0.05. **(A)** The Italian sample, compared to the Singaporean one, shows a greater negative correlation between Maternal Care and levels of Anxiety. **(B)** The difference between Italy and Singapore in describing experienced Anxiety in adulthood is significant for high levels of Maternal Care. **(C)** The Italian sample, compared to the Singaporean one, shows a greater positive correlation between Maternal Overprotection and levels of Anxiety. **(D)** The difference between Italy and Singapore in describing experienced Anxiety in adulthood is significant for low levels of Maternal Overprotection. **(E)** The Italian sample, compared to the Singaporean one, shows a greater positive correlation between Maternal Overprotection and levels of Avoidance. **(F)** The difference between Italy and Singapore in describing experienced Avoidance in adulthood is significant for low levels of Maternal Overprotection. **(G)** With regard to OXTr rs53576 carriers of the G variation in the Italian sample, compared to the Singaporean one, G-allele carriers show a greater positive correlation between Maternal Overprotection and levels of Avoidance. **(H)** The difference between Italian G-carriers and Singaporean G-carriers in describing experienced Avoidance in adulthood is significant for low levels of Maternal Overprotection.

Bonding Instrument. As only the maternal dimensions differ between the two cultural samples, we can affirm that our first hypothesis is partially confirmed. Environmental sensitiveness due to rs53576 allelic variation partially contributed to the explanation of the differences between the two groups, described by the country of origin, but results have a stronger evidence only for the Italian sample. This might reflect the fact that, as aforementioned, Italian families tend to have greater levels of emotional bonding expressed[24]. In fact, evidence in literature reports that G-carriers for rs53576 are considered to be more susceptible to environmental factors[27,35]. At the same time, although we did not find statistically significant results for paternal characteristics, the correlations with recalled maternal characteristics followed the same direction of effect. This reflects to a certain extent the universality of attachment, as theorized by Ainsworth and Bolwby[18,52], which, in spite of everything, is still a

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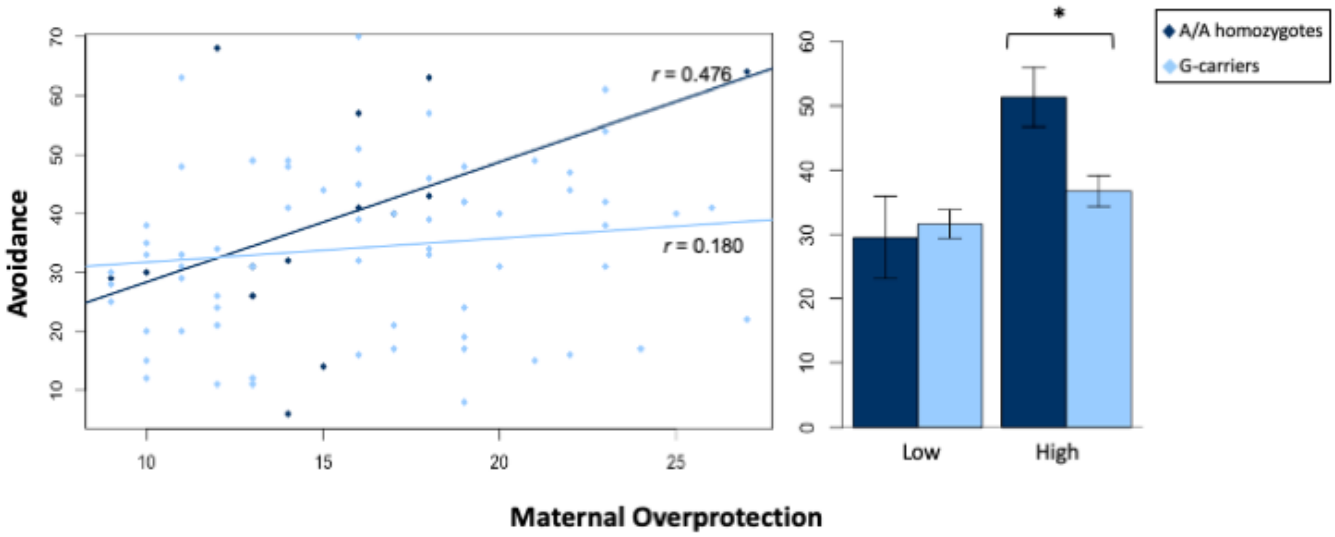

Figure 2. Interaction Effect of OXTr rs53576 allelic variation and Maternal Overprotection on Avoidance levels in Italian sample

very debated topic, particularly with regards to the ecosocial diversity among cultures[53]. One of the strengths of this study is to provide a further step in comparing two distinct environments, each consisting of different parenting strategies and family composition. Another important element considered is the distinction between maternal and paternal bonding. In fact, although nowadays the competence regarding child care is becoming more and more equal between the two parents, the paternal role is an area that still under-explored, especially in reference to the temporal dynamics of attachment in adulthood. With regards to the interaction between OXTr rs53576 and maternal overprotection in explaining avoidance levels in the Italian sample, the outcomes reflect the tendency in A/A homozygous individuals, as reported in other studies, to display less favourable social features when facing stressful situations[33,54], such as in deciding to act avoidantly when perceiving overprotection and control from other significant people. This result corroborates evidence in literature based on an Italian sample, where perceived overprotective parenting was found to be predictive of a more avoidant relational style[55]. This appears to be even more in reference to the maternal figure, as appraising excessive control and protection could cause in the offspring a pattern of non-satisfying relationships where the self is negatively represented while favouring a more positive view of the others, hence leading the person to avoid close relationships in order to feel less inadequate[56]. These mechanisms, according to our results, appear to be reinforced by the genetic allelic variation of the OXTr rs53576, that confers an increased vulnerability towards social behavior. Although our initial hypotheses have been confirmed by results and supported by previous evidence in literature, this study has limitations that are important to consider, starting from the cross-sectional approach that presents a greater reliance on retrospective reporting of parental bonding, compared to a longitudinal one. Another point is that, although we utilised original (for Singaporean group) and validated translated (for Italian group) versions of the questionnaires, some conceptual-understanding issues could have led to different interpretation of the items, hence to altered scoring that might have influenced the results. For instance, a new conceptualization to investigate adult attachment have been proposed in Asian countries, favouring a more articulated framework that goes beyond the only two dimensions of anxiety and avoidance[57], and that includes specific features that are context-dependent, like dialectical thinking, independence, interdependence, traditional romantic love-styles[58]. This could explain the weak correlation found for the Singaporean sample, suggesting that a more context-specific model should be adopted by researchers to address adult relationships. Additionally, the numerosity of the sample represents a challenge in gene-by-environment interaction studies. In fact, despite the adequate sample size and the percentage of allelic distributions comparable to the average frequency, the Italian sample (N=93) is smaller compared to the Singaporean one (N=216) and this unbalance might have affected the outcomes to a certain extent, either masking effects that did not survive the Bonferroni correction or by enhancing the results obtained. Finally, for both of the countries, the sample was composed by undergraduate and post-graduate students, mainly belonging to Social Science or Psychological fields, who happened to be familiar with the theories beneath the study. Overall, while this study represents a further step in understanding the development of bonding mechanisms in different cultural contexts, it is at the same time a starting point for further exploration where more modern approaches could be combined to overcome the use of self-report tools. By directly

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measuring diverse dimensions of social behaviors, it would be possible to have a more comprehensive understanding of the factors that might affect the dynamics of attachment, in both early stages and subsequent phases of life. Furthermore, the assessment should include eventual psychological mechanisms underlying social behavior and expectations, which may play a mediating role between childhood reminiscence of parental care and experienced anxiety or avoidance in adulthood. This would provide practitioners in psychological and clinical field with a more complete view of the individual, and therefore allow more focused work on targeted processes by making use of information about interaction and relationships derived from social data about how people perceive social relationships in general and the mechanisms that drive his/her subjective case.

Methods

The research was approved by the Ethical Committee of University of Trento (Prot-2017-019) and of Nanyang Technological University (IRB-2015-08-020-01). Data of the present study are available at the Nanyang Technological University Data Repository https://doi.org/10.21979/N9/MTVUCX. Informed consent was obtained from all participants, and the study was conducted following the Declaration of Helsinki. Participants were recruited through social media platforms (students' Facebook groups, for the Italian sample) and web-based software research study participation and management systems (SONA System, for the Singaporean sample) among non-parent students of University of Trento, Italy, and Nanyang Technological University, Singapore. After informed consent was given, the online form of the questionnaires were sent through email to the participants (links to Google Modules for the Italian sample, links to Qualtrics for the Singaporean sample). Participants who gave consent for the genetic part of the experiment were contacted to set an appointment to obtain a buccal mucosa sample for genetic information. The genetic assessment was conducted on anonymized biosamples at the Nanyang Technological University (Singapore) and at the Department of Neurobiology and Behavior at Nagasaki University (Japan). Results from questionnaires were anonymized at the beginning of data collection. The final group consisted of 309 participants that fully completed the two questionnaires and provided the experimenters with DNA sample (Italian sample N = 93 (M = 37; F = 56); Mean age = 23.08 (3.87) [range 18 35]; Singaporean sample N = 216; M = 73; F = 143; Mean age = 21.51 (1.83) [range 18 31]).

Questionnaires

Parental Bonding Instrument. The Parental Bonding Instrument is a 50-item self-report questionnaire that investigates participants' perception of both maternal (25 items) and paternal (25 items) care and protection in their first 16 years of life. It was developed by Parker, Tupling and Brown in 1979 using factor analyses from parents self-reporting their childhood experiences, the results of which yielded two factors: warmth/care (hereafter referred to as care) and overprotection[2]. The care scale (PBI_Care) measures the degree to which a mother or a father was empathetic and caring versus cold and indifferent. The overprotection scale (PBI_OverP) measures the extent to which a parent was intrusive or, conversely, fostered independence in the subject. The measure has been shown to have high reliability, stability over time and no association with social desirability, Neuroticism or Extroversion[59].

Experience in Close Relationships - Revised. In order to evaluate anxiety and avoidance levels in close relationships, the revised form of Experience in Close Relationships-Revised was employed (ECR-R[44,46]). The 36-item self-report questionnaire assessing two major dimensions of an individual's attachment style (anxiety and avoidance) in a sentimental relationship was employed. Both the anxiety and avoidance subscales consisted of 18 items each, rated on a 7-point Likert scale ranging from: 1 (Strongly disagree) to 7 (Strongly agree). The anxiety dimension measures insecurity, jealousy and fear of abandonment as opposed to feeling secure about the availability and responsiveness of romantic partners. The avoidance dimension measures the feeling of discomfort being close to others and tendency to refrain from attachment. Instead of assigning participants to an attachment style category, this scale yields two separate dimension scores for each participant.

Preliminary Analyses

Before proceeding with the data analysis, we tested the internal validity of each questionnaire in both groups (Italy, Singapore). The Cronbach's alpha coefficients ranged from 0.83 to 0.94, suggesting an overall very good internal consistency. In Table 1, all the Cronbach's alpha values are listed, together with the main descriptive statistics of the sample. Psychometric features of the two instruments are compatible with those reported in a recent review on adult attachment assessment tools[60].

Preliminary Chi Square tests were made in each population group to exclude the possibility of any significant effect on ECR subscales could not be attributed to participants' gender. None of the analyses yielded significant results ($X^2_{\text{ITA\_Anxiety}}(49)= 44.84$, p= 0.705; $X^2_{\text{ITA\_Avoidance}}(49)= 47.84$, p= 0.520; $X^2_{\text{SNG\_Anxiety}}(71)= 94.465$, p= 0.053; $X^2_{\text{SNG\_Avoidance}}(65)= 56.994$, p= 0.749).

Genotyping

With regards to the Italian sample, this study adopted the same DNA derivation and genotyping procedure used by Bonassi and colleagues[61], using ACGT, Inc. (Wheeling, IL). DNA was extracted from each kit using the Oragene DNA purifying reagent,

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Group	Subscale	Cronbach's α	Skewness	Kurtosis	Mean (SD)	Median
Total	Age	-	-	-	21.98 (2.71)	22
	PBI M_Care	0.92	-0.85	0.34	25.61 (7.51)	27
	PBI M_OverP	0.83	0.44	0.07	15.51 (6.88)	15
	PBI F_Care	0.93	-0.34	-0.52	22.04 (8.56)	23
	PBI F_OverP	0.83	0.71	0.45	12.38 (6.86)	12
	ECR Anxiety	0.92	0.17	-0.44	62.41 (20.79)	62
	ECR Avoidance	0.92	0.21	-0.50	38.76 (16.84)	39
Italy	Age	-	-	-	23.08 (3.87)	22
	PBI M_Care	0.93	-0.62	-0.36	27.72 (6.09)	28
	PBI M_OverP	0.89	0.69	0.12	16.51 (6.37)	16
	PBI F_Care	0.94	-0.54	-0.65	23.43 (9.38)	25
	PBI F_OverP	0.83	0.74	0.09	14.26 (7.05)	13
	ECR Anxiety	0.91	0.68	0.12	56.89 (20.27)	53
	ECR Avoidance	0.88	0.37	-0.48	35.15 (15.66)	33
Singapore	Age	-	-	-	21.51 (1.83)	21
	PBI M_Care	0.91	-0.66	-0.03	25.39 (6.99)	26
	PBI M_OverP	0.89	0.40	-0.01	15.09 (7.06)	15
	PBI F_Care	0.88	-0.28	-0.41	21.44 (8.14)	22
	PBI F_OverP	0.85	0.68	0.51	11.57 (6.63)	11
	ECR Anxiety	0.93	-0.04	-0.39	64.79 (20.61)	67
	ECR Avoidance	0.94	0.12	-0.51	40.31 (17.13)	40

Table 1. Summary of Cronbach's α, skewness and kurtosis for each subscale of the Parental Bonding Instrument and Experience in Close Relationships in both groups

subscale	M_Care	M_Overp	F_Care	F_Overp	Anxiety	Avoidance
M_Care		-0.295*	0.425*	-0.099	-0.062	-0.071
M_Overp	-0.307*		-0.165	0.394*	0.009	0.050
F_Care	0.549*	-0.247		-0.177	-0.159	-0.086
F_Overp	-0.130	0.390*	-0.366*		0.173	0.105
Anxiety	-0.374*	0.429*	-0.399*	0.198		0.375*
Avoidance	-0.341*	0.200	-0.205	0.082	0.352*	

Table 2. Correlation and significance values among questionnaire subscales; significance is adjusted for multiple tests. Values above the diagonal refer to the Singaporean sample, while values under the diagonal refer to the Italian sample. *p<.05

then concentrations were assessed through spectroscopy (NanoDrop Technologies, USA). As for the buccal mucosa samples collected from participants in Singapore, SNP rs53576 was genotyped as part of the Illumina Infinium Global Screening Array -24 v2.0. Concentrations for each sample were magnified through polymerase chain reaction (PCR) for the OXTr gene rs53576 region target. The forward and reverse primers that were used were 5-GCC CAC CAT GCT CTC CAC ATC-3 and 5-GCT GGA CTC AGG AGG AAT AGG GAC-3. For this DNA region, study participants having at least one G allele (G/G homozygotes or A/G) were classified into a single G-carriers group. The averaged distribution of the different genotypes in the Caucasian population is 8-16% for A/A homozygotes and 84-92% for G-carriers1000 Genomes project, ensembl.org, and the distribution in the Italian sample was 15% for A/A homozygous and 85% for G-carriers. Specifically, genotype frequencies were as follows: A/A = 14 (15.05%), A/G = 34 (36.56%), G/G = 45 (48,39%). Participant age ($X^2(15) = 9.6108$, $p = 0.8435$) and gender $X^2(1) = 0.4018$, $p = 0.5262$) did not significantly differ between the A/A and G groups. As for the Singaporean sample, the genotyping

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distribution in East-Asian population is 35-47% for A/A homozygotes and 53-65% for G-Carriers 1000 Genomes project, ensembl.org, whereas in our sample the distributions were 37% for A/A homozygotes and 63% for G-carriers. Participant age ($X^2(10)) = 4.6170$, $p = 0.9153$) and gender $X^2(1) = 0.1836$, $p = 0.6683$) did not significantly differ between the A/A and G groups.

Genotype	A/A	A/G	G/G	X^2	*p-value*
rs53576$_{ITA}$	14	34	45	2.297	0.087
rs53576$_{SNG}$	80	94	42	2.239	0.134

Table 3. Hardy-Weinberg Equilibrium results for OXTr rs53576 in the two groups

Statistical Analyses

The sensitivity of the statistical power was calculated using G*Power software (version 3.1), showing a medium effect size equal to 0.25 ($\alpha = 0.025$; Power = 0.80). Analysis of covariance was used to find interaction effects on the total sample. An univariate ANCOVA was performed for each of the ECR subscales, which were considered as dependent variables. The OXTr gene genotype rs53576 (G-carriers and A/A homozygous), and the two samples to which participants belonged (Italy and Singapore) were considered as a between-subjects variable and the four PBI subscales (maternal care, paternal care, maternal overprotection and paternal overprotection) were examined as continuous covariates. The sample was analyzed as a whole first (N=309), then, as interaction effects were shown in results, the Italian (N=93) and the Singaporean (N=216) groups were analyzed separately. Differential Bonferroni correction was applied (2 repeated measures, one for each dimension of adult attachment, $\alpha = 0.025$). In the Results and Discussion Sections, only interaction effects that survived the correction are discussed. Variables related to PBI subscales were divided into two groups ("Low" vs "High" PBI dimension) using the median split procedure. Finally, post-hoc analyses were computed adopting the Student's *t-test* comparing the low vs high PBI levels to examine hypothetical significant differences between the two genetic carriers on anxiety and avoidance in adult attachment.

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Author contribution

Conceptualization and Experimental Design: IC, GE, BL. Data Collection and Data Curation: IC Genetic Analysis: JNF. Data Analysis, Data Interpretation and Writing: IC. Graphic visualization: IC, GE. Revision: IC, AB, BL, JNF, SP, GE. Supervision: GE, BL.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The corresponding author is responsible for submitting a competing interests statement on behalf of all authors of the paper.

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