Received: 12 January 2017 | Revised: 17 January 2018 | Accepted: 1 February 2018

DOI: 10.1111/gbb.12464

Genes, Brain and Behavior

ORIGINAL ARTICLE

No evidence of association of oxytocin polymorphisms with breastfeeding in 2 independent samples

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Funding information
QIMR Berghofer Medical Research Institute; Fundación Séneca, Grant/Award number: 08633/PHCS/08; Ministerio de Ciencia e Innovación, Grant/Award number: PSI11560-2009; National Institutes of Health, Grant/Award number: AA011998

Oxytocin has an important function in breastfeeding via its role in the milk ejection reflex and in attachment and bonding processes. Genetic factors account for a significant part of the individual differences in breastfeeding behavior. *OXT* and *OXTR* have been proposed as gene candidates for breastfeeding. Previous studies have focused on certain single-nucleotide polymorphisms (SNPs) within these genes, finding null or inconsistent results. The present study analyses the associations between a wide coverage of polymorphisms in *OXT* and *OXTR* and breastfeeding duration from 2 large and independent unselected samples comprising a total of 580 and 2112 female twin mothers from the Murcia Twin Registry (Spain) and QIMR Berghofer Medical Research Institute (Australia), respectively. A total of 19 SNPs in *OXT* and 137 in *OXTR* SNPs were covered in both samples. Effects of the *OXT* and *OXTR* polymorphisms on breastfeeding duration were calculated by means of linear regression controlling for age at survey time, educational level, interaction between age and educational level and principal components of genetic ancestry. The analyses were conducted independently in the 2 samples and also meta-analyzed. Although some SNPs were associated at an alpha level of .05 with breastfeeding, they did not survive multiple testing correction. We conclude that SNPs within or nearby *OXT* and *OXTR* are unlikely to have large effects on breastfeeding behavior.

KEYWORDS
association, breastfeeding, genetics, OXT, OXTR

1 | INTRODUCTION

There is substantial evidence to suggest that breastfeeding has significant and long-term biopsychosocial benefits for women, babies and society as a whole. Predictors of the initiation and duration of breastfeeding are diverse and act in complex interactions.[1] Among them, some of the most frequently reported are maternal age, ethnicity, socio-economic status, education, working conditions, obesity, interventions during labor, early postpartum contact and certain psychosocial factors, such as personality, self-efficacy or postnatal depression.[1–3]

Undoubtedly, biological factors (e.g. breast pain, soreness or milk production) also play a key role in the mother's disposition to breastfed and breastfeeding itself is a strongly hormone mediated process. Lactation depends on the action of prolactin, oxytocin, thyroid hormones, growth hormone, insulin and adrenal hormones. Specifically, oxytocin is responsible for the milk ejection reflex.[4] This neuropeptide is primarily produced in hypothalamic structures in the brain, and besides having effects in the brain as a neurotransmitter it is also released into the bloodstream where it functions as a hormone.[5] Receptors for oxytocin are found in peripheral tissues as well as in several areas of the brain.[6] During the lactation process, oxytocin is not only released in response to nipple stimulation, either by suction or manipulation, but it can also be released by visual, auditory or emotional stimuli, usually related to the baby.[7] All these stimuli activate the supraoptic and paraventricular nuclei of the hypothalamus, resulting in the release of oxytocin. From there, oxytocin reaches the breast through the blood, causing contractions of the myoepithelial cells

surrounding the alveolar cells and thus the release of milk.[7] Furthermore, the oxytocin system has been associated with mother-infant relationships and mothering.[8,9]

Genetics also plays a role in breastfeeding behavior. Three different twin and family studies from diverse cultural backgrounds (Spain, Australia and The Netherlands) have found significant heritability estimates (44%-70%) for initiation and duration of breastfeeding behavior.[10–12] This means that approximately half of the inter-individual variability observed in the behavior of breastfeeding is due to differences in genetic makeup, and the other half is due to differences in environmental-related experiences and measurement error.

Genes related to the production of oxytocin (*OXT*) or its receptor (*OXTR*) can be considered as strong biological candidates to study genetic influences on breastfeeding, by means of both the physiology of the lactation[4] and attachment and bonding processes.[8] The *OXT* gene encodes a precursor protein that is processed to produce oxytocin. The protein encoded by the *OXTR* gene belongs to the G-protein coupled receptor family and acts as a receptor for oxytocin. The functionality of *OXTR* polymorphisms is still unclear, but *OXTR* polymorphisms have been found to be related to plasma levels of oxytocin.[13] However, there is no clear evidence to date about a relationship between oxytocin-linked genes and breastfeeding behavior.

Jonas et al[14] reported a higher proportion of mothers with an A allele in the single-nucleotide polymorphism (SNP) rs2740210, in the *OXT* gene (downstream variant), exclusively breastfeeding at 3 and 6 months postpartum and partially breastfeeding at 12 in their primary sample. These effects were not found in their replication sample. In the replication sample, however, they found higher proportion of mothers with an A allele in rs4813625 (in *OXT*, upstream variant) breastfeeding (exclusively and partially) at 3 months. Additionally, they report an interaction of the rs2740210 with early life adversity to predict variation in breastfeeding duration. Conversely, Tharner et al[15] did not find significant associations between 2 SNPs in the *OXTR* gene, rs53576 and rs2254298 (both variants in intron 3), and breastfeeding duration. Both studies, however, report the results of only certain polymorphisms and their data come from mother's behavior with a specific child, regardless of the mother's conduct with her other children, if any. A preliminary genome-wide association study (GWAS) did not find any genetic variants that reached genome-wide significance for the average duration of breastfeeding to all the offspring.[11] However, the small sample size (N = 1521) lacked power to detect associations of small effect.

In summary, genes associated with oxytocin are biological candidates for breastfeeding behavior. To the best of our knowledge, there are only 2 previous empirical studies testing this hypothesis and they show no association between previously selected polymorphisms and breastfeeding. Nevertheless, these studies do not have a wide coverage of the candidate genes, which leaves open the possibility of an undetected effect.

Given the scarcity of data and the relevance of this question, the aim of the present study is to examine the association between the *OXT* and *OXTR* genes and breastfeeding duration from 2 large and independent samples from Spain and Australia, controlling for age and education effects.

2 | MATERIAL AND METHODS

2.1 | Participants and procedure

Two independent unselected samples of European ancestry with compatible phenotypic data were used for this study. The first of them was composed of 580 female twin mothers from the population-based Murcia Twin Registry (MTR), aged 52.4 (SD: 7.3; range = 43-69), born between 1940 and 1966, who had donated blood or saliva samples for DNA extraction. The MTR is a twin cohort in Spain whose reference population comprises all twin pairs who were born between 1940 and 1966 in the Murcia region. It is a research resource intended to stimulate research on the analysis of genetic factors related to health and health-related behaviors, including lifestyle, health promotion and quality of life. Participation in the MTR is voluntary, subjected to informed consent, and not remunerated. Information about the individuals comes from the databases available at the regional health system. They are included in the MTR if they meet the inclusion criteria: pairs with both members alive at the time of incorporation, residence in the region of Murcia, and no conditions or disability that may limit their voluntary participation. The MTR has gone through 4 waves of data collection using different interviewing techniques (personal or telephone) and, as of today, comprises more than 1200 twin pairs (same-sex and opposite-sex) and keeps an associated biobank with biological samples of part of the participants. Global cooperation rate across data collection waves and subsamples is 72.5%. Further information about the MTR characteristics and recruitment procedures can be found elsewhere.[16] For the present study, 580 females with DNA as well as phenotypic data available were selected from the MTR dataset. Biological and phenotypic data were not collected necessarily at the same moment. Phenotypic data were collected via personal (85.2%) or phone interview with retrospective questions on reproductive health, including those about breastfeeding for those women who had been mothers.

Participants in the second sample were 2112 parous female twins who had been mothers from QIMR Berghofer Medical Research Institute (QIMR) Health and Lifestyle studies (Cohorts I and II) who provided data on their breastfeeding completing a mailed questionnaire and for whom genotypic data is available. QIMR is a research institute in Australia. Since 1988, a series of studies of general health conditions have collected longitudinal data from more than 25 000 twins and family members. Women in Cohort I were born between 1892 and 1963 and women in Cohort II were born between 1964 and 1971. At the time of the survey (1988-1993), the mean age was 41.2 (SD = 11.7; range: 19-85). Women in Cohort I were approached for this survey as part of a follow-up of all complete twin pairs participating in the "1981 survey,"[17] being the response rate of 84.5%. Women in Cohort II were first ascertained as children in response to systematic appeals to parents through the Australian school systems and the mass media during the period 1980 to 1982, being the response rate for the present survey of 77.4% (global response rate for men and women). Further details of the sample, data collection and zygosity determination are described elsewhere for Cohort I[18] and Cohort II.[19]

This study was approved by the Murcia University Ethical Committee and the Queensland Institute of Medical Research Human Research Ethics Committee. All of the participants provided written informed consent.

2.2 | Genotyping and quality control

Participants from MTR provided blood (85.2%) or saliva samples. They were genotyped using the Illumina GSA Beadchip and imputed to 1000G Phase 3 Version 5. Participants from QIMR provided a saliva sample and were genotyped using the Illumina Hap370, Hap610K or Omni chips and imputed to 1000G Phase 1 Version 3.

For the present study, we selected all the SNPs available within a window of 10 000 kb upstream and downstream from *OXT* and *OXTR* that passed quality control filters (r^2 ≥ 0.6%, minor allele frequency ≥0.01), resulting in 73 SNPs in *OXT* and 222 SNPs in *OXTR* in the MTR sample and 29 SNPs for *OXT* and 169 for *OXTR* in the QIMR sample, with an overlap of 19 in *OXT* and 137 in *OXTR* variants with MTR. All SNPs were mapped in UCSC hg19/NCBI Build 37.

2.3 | Breastfeeding

Participants reported their number of children and the duration of breastfeeding in months (full or partial) for each of them via personal/phone interview (MTR cohort) or mailed questionnaire (QIMR cohort). The key question was equivalent between cohorts ("How long did you breastfeed each of your children for? Please include here either partial or full breastfeeding"). The average duration of breastfeeding (including zero for those who did not breastfeed) across all children was computed and transformed to Z-scores.

2.4 | Level of education

Level of education was categorized in 9 levels in the case of MTR (Illiterate, Reading & Writing, Primary Studies, General secondary education, Professional education level I, Superior secondary education, Professional education level II, University-Medium degree, and University-High degree) and in 6 levels in the QIMR sample (8-10 years' schooling, 11-12 years' schooling, Apprenticeship, diploma, etc., Technical/Teacher's College and University first degree and University post).

2.5 | Statistical analyses

Effects of the alleles of the *OXT* and *OXTR* polymorphisms (predictor) on breastfeeding duration (outcome) were calculated by means of linear regression controlling for age at survey time, level of education, the interaction between age and level of education and principal components of genetic ancestry (covariates). The analyses were conducted independently in the 2 samples with RAREMETALWORKER[20] to control for relatedness and zygosity. We used dosage genotype data (imputed genotypes to an expected allelic or genotypic count with a high confidence). Then, a sample size weighted meta-analysis of results of those SNPs present in both samples was carried out with METAL.[21] The appropriate marker filters were applied during meta-analysis. Finally, we conducted a gene-based test to test the aggregated effect of the SNPs in the meta-analysis results using GCTA-fastBAT.[22]

A threshold of *P* value = .0003 was established for the meta-analysis (Bonferroni correction). With the available sample size (*N* = 2692) at a α of .0003 for a common variant (MAF > 0.01), we can assume >90% to detect an effect explaining 1% of the variance in the meta-analysis. To detect an effect explaining 0.5% of the variance the power decreases to >50%.

3 | RESULTS

Women from MTR had 2.52 children on average (SD: 1.1; range: 1-9). The mean duration of breastfeeding was 3.5 months (SD = 3, range 0-13 months). Women from QIMR had an average of 2.54 children (SD: 1.2; range: 1-8) and breastfed for 5.1 months (SD: 4; range: 0-17). Table 1 shows the distribution of the level of education of the sample.

The SNPs within a window of 10 000 kb upstream and downstream from *OXT* and *OXTR* in *OXT* showing effects with *P* < .05 in each of the samples are shown in Table S1, Supporting Information. No polymorphisms in the *OXT* region showed associations with mean breastfeeding duration in MTR and 7 SNPs did in QIMR (best hit, rs6115779, β = .87; *P* value = .007). Regarding *OXTR*, 35 SNPs were significant at an alpha level of .05 in MTR (best hit, rs4493422, β = −0.17, *P* value = .001) and 19 were in QIMR (best hit rs237899, β = −0.96, *P* value = .008).

Table 2 shows the results for the top 20 SNPs of the meta-analysis of MTR and QIMR, which is restricted to variants present in the 2 samples (N SNPs = 156; 19 corresponding to *OXT* and 137 to *OXTR*), and the complete results are in Table S2. The total sample size included in the meta-analysis was N = 2692. The top hit was located in the *OXTR* region (rs237899, β = −2.76, *P* value = 0.006). Although 16 SNPs reached significance at an alpha level of .05, no significant

TABLE 1 Level of studies in the MTR and QIMR samples

Level of studies	*N* (%)
MTR (*N* = 580)	
Illiterate	1 (0.17%)
No studies (Reading & Writing)	63 (10.86%)
Primary studies	212 (36.55%)
General secondary education	164 (28.28%)
Professional education (level I)	58 (10%)
Superior secondary education	33 (5.69%)
Professional education (level II)	14 (2.41%)
University-medium degree	23 (3.97%)
University-high degree	12 (2.07%)
QIMR (*N* = 2112)	
11-12 years' schooling	14 (0.66%)
Apprenticeship, diploma, etc.	525 (24.86%)
Technical/Teacher's College	529 (25.05%)
University first degree	781 (36.98%)
University postgraduate training	164 (7.77%)
University post	99 (4.69%)

TABLE 2 Meta-analysis of QIMR and MTR, with N = 2692 (top 20 SNPs)

Index SNP	Chr:position (GRCh37/hg19)	A1/A2	*Z*-score	*P*-value	Direction (QIMR-MTR)
rs237899	3:8808515	A/G	−2.765	0.006	−
rs237900	3:8808696	A/G	−2.709	0.007	−
rs237889	3:8802483	T/C	2.683	0.007	++
rs53576	3:8804371	A/G	2.286	0.022	++
rs62243369	3:8802808	A/G	−2.265	0.024	−
rs62243370	3:8802813	A/G	−2.263	0.024	−
rs2254298	3:8802228	A/G	−2.172	0.030	−
rs2254295	3:8802292	T/C	2.134	0.033	++
rs237915	3:8810311	T/C	2.113	0.035	++
rs237902	3:8809184	A/G	−2.094	0.036	−
rs401015	3:8814012	T/C	2.092	0.036	++
rs180789	3:8813927	A/G	2.091	0.037	++
rs11706648	3:8796547	A/C	2.087	0.037	++
rs2268491	3:8800398	T/C	−2.063	0.039	−
rs237913	3:8810050	A/C	−2.018	0.044	−
rs2740197	20:3057258	A/G	−2.014	0.044	−+
rs2270464	3:8816940	A/G	−1.82	0.069	−
rs3806675	3:8811646	A/G	1.816	0.069	++
rs13319411	3:8803119	A/G	−1.776	0.076	−
rs4686302	3:8809222	T/C	1.738	0.082	++

Abbreviations: Chr, chromosome; SNP, single-nucleotide polymorphism. Column A1/A2 has the SNP alleles, with the first allele (A1) the reference allele for the frequency and *Z*-score columns. A threshold of *P*-value = .0003 was established for the meta-analysis (Bonferroni correction).

[a] Indicates heterogeneity in the observed effect sizes across samples (*p*-value <.5).

effects were observed in the results after correction for multiple testing (*P* value threshold = 0.0003). Some effect sizes were not homogeneous across samples, mainly due to opposite direction and they are indicated in both tables.

The gene-based test did not show significant associations with *OXT* or *OXTR*.

4 | DISCUSSION

The aim of the present study was to investigate associations between polymorphisms in the *OXT* and *OXTR* genes and average breastfeeding duration in 2 large population-based samples and to compare our results with previous report on the topic. Results show no significant effect of any *OXT* or *OXTR* SNPs over breastfeeding duration. Although some SNPs were associated with breastfeeding at an alpha level of .05, they did not survive correction for multiple testing, established at *P* value = .0003.

Those SNPs previously reported by Jonas et al[14] in one of their study samples could not be meta-analyzed since they did not pass the quality control filters at QIMR or MTR. The SNPs reported by Tharner et al,[15] showing no associations with breastfeeding are significant at an alpha level of .05 in our meta-analysis (rs53576, β = 2.29, *P* value = .022; rs2254298, β = −2.172, *P* value = .029). However, as pointed before, none of them survive correction for multiple testing and thus the results are consistent.

OXT and *OXTR* genes are obvious candidates for the analysis of the role of genetic factors on breastfeeding behavior. The physiological implication of oxytocin in lactation, and their plausible associations with social and pair-bonding behaviors make them biological candidates. Our results suggest that loci in these genes are unlikely to be associated with breastfeeding behavior, in line with previous null or inconsistent results.[14,15]

5 | LIMITATIONS

While our measure of breastfeeding is an estimate (and not an exact measure) of breastfeeding, our study has covered a wider region surrounding the *OXT* and *OXTR* genes and has examined its relationship with the average duration of breastfeeding to all children as opposed to only 1 child. However, candidate gene studies have known limitations regarding study power, population stratification and accuracy of assumptions about the gene function. Additionally, our study has drawbacks regarding phenotypic characterization that should be taken into account in future studies. The breastfeeding data used in this analysis are based on self-report, thus there is a possibility that these women gave socially desirable answers. Maternal recall has been found to be a valid and reliable estimate of breastfeeding initiation and duration, although its accuracy decreases as the period of recall increases further than 3 years.[23] Other authors, however, increase that period up to 6 years.[24] Moreover, previous analyses from the MTR and QIMR using the total sample of women providing data on breastfeeding (not limited to those women who were genotyped), in which part of the women had been resurveyed 2 years after their initial contact, have reported a good test-retest reliability:

r = 0.91 for n = 389 women in the MTR[10] and r = 0.96 for n = 341 in the QIMR cohort.[11] Hence, while recognizing the information was retrospective and this could entail a potential recall bias, we think that our data provide a good approach for a valid estimation of the associations. Another question is that our measure took breastfeeding as a global practice and differences between full and partial breastfeeding could not be accomplished. It could be argued, for instance, that the effect of oxytocin-related genes could be more salient for exclusive breastfeeding while formula feeding would depend more strongly on non-genetic factors (e.g. SES). However, there is a significant correlation between exclusive and partial breastfeeding[25] and previous estimates of significant heritability have been obtained from the same samples and outcomes.[10,11] A third possible limitation is that our samples could be selected and not representative of the general population with regard to breastfeeding. The fact that our sample comprised twins may mean an extra support system, in which sister twins reinforce each other to overcome problems and keep breastfeeding. The opposite is also possible and twins could discourage breastfeeding. Both possibilities may have an impact in the initiation or duration of breastfeeding in relation with the reference population. Unfortunately, there are no data available to test this hypothesis. Finally, this study has focused on the potential association of specific biological variables with the duration of breastfeeding and included age, level of education and their interaction as covariates. The environment has a relevant impact on how women chose to feed their babies and, in fact, may interact with other variables (e.g. SES) to promote or difficult breastfeeding[1] and a possible gene-environment effect cannot be completely discarded. Future studies should integrate the effects of those variables to have a complete picture of the individual differences found in breastfeeding behavior.

6 | CONCLUSIONS

In conclusion, our results do not support the existence of a significant effect from the *OXT* or *OXTR* genotypes upon breastfeeding duration. Given the reported consistent heritability of this phenotype, future studies tagging haplotypes to infer the candidate gene polymorphism genotypes or with larger sample sizes and hypothesis free approaches (GWAS) are necessary to detect the regions in these genes and the whole genome accounting for the effects produced by common genetic variants.

ACKNOWLEDGMENTS

We are grateful to the twins for their participation. This work was supported by the Seneca Foundation, Regional Agency for Science and Technology, Murcia, Spain (grant number 08633/PHCS/08), the Ministry of Science and Innovation, Spain (grant number PSI11560–2009) and the NIH (grant numbers AA013320, AA013321, AA013326, AA011998 and AA017688). LCC was supported by a fellowship provided by Seneca Foundation-Regional Agency for Science and Technology (19151/PD/13) and a QIMR Berghofer fellowship. SEM was supported by a fellowship from the NHMRC (APP1103623).

Conflict of interest

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Colodro-Conde L, Sánchez-Romera J, Lind P, et al. No evidence of association of oxytocin polymorphisms with breastfeeding in 2 independent samples. *Genes, Brain and Behavior*. 2018;e12464. https://doi.org/10.1111/gbb.12464