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Determination of Twin Zygosity in Brazil: A DNA Validation of Two Short Questionnaires

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Abstract

Twin research in Brazil is expanding and should be built upon solid foundations; this includes the use of zygosity questionnaires validated through DNA. Our goals were to adapt and validate two short zygosity questionnaires to Brazilian Portuguese using DNA testing of Brazilian twins. One hundred same-sex twin pairs (27 males and 73 females; 30.9±12.9 years) answered socio-demographic and two short zygosity questionnaires. Blood samples were collected for DNA extraction. A genetic analysis of 22 autosomic short tandem repeat plus amelogenin and DYS391 markers were conducted to obtain an individual genetic profile. According to DNA tests, 17% of twin pairs were classified as dizygotic and 83% as monozygotic. Both questionnaires correctly distinguished zygosity with 96.7% accuracy. These DNA validated zygosity questionnaires may foster twin research in Brazil, given the stronger methodology.
Keywords: monozygotic twin, dizygotic twins, zygosity, questionnaire validation

Resumo

Determinação da Zigosidade de Gêmeos no Brasil: Validação de dois questionários breves por meio de DNA. A expansão da pesquisa sobre gêmeos no Brasil deve ser construída sobre bases sólidas, incluindo questionários para avaliação de zigosidade validados por DNA. Nossos objetivos foram adaptar e validar dois questionários curtos de zigosidade para o português brasileiro usando testes de DNA. Cem pares de gêmeos brasileiros do mesmo sexo (27 homens e 73 mulheres; 30,9±12,9 anos) responderam a questionários sociodemográficos e dois questionários curtos de zigosidade. Amostras de sangue foram coletadas para extração de DNA. Uma análise genética de 22 marcadores autossômicos de repetições curtas em tandem mais amelogenina e marcadores DYS391 foi realizada para obter um perfil genético individual. Segundo o teste de DNA, 17% dos gêmeos foram classificados como dizigóticos e 83% como monozigóticos. Ambos os questionários distinguiram corretamente a zigosidade com 96,7% de acerto. Estes questionários para avaliar a zigosidade validados por DNA poderão ser usados em pesquisas com gêmeos no Brasil contribuindo para o aumento do rigor científico.
Palavras-chave: gêmeos monozigóticos, gêmeos dizigóticos, zigosidade, validação de questionário

Resumen

Determinación de la Cigosidad Gemelar en Brasil: una validación por ADN de dos cuestionarios breves. La expansión de la investigación de gemelos en Brasil debe construirse sobre una base sólida mediante cuestionarios validados por el ADN para evaluar la cigosidad. Nuestros objetivos fueron adaptar dos cuestionarios cortos al portugués brasileño y validar utilizando pruebas de ADN. Cien pares de gemelos brasileños del mismo sexo (27 hombres y 73 mujeres; 30,9+12,9 años) respondieron a cuestionarios sociodemográficos y a dos cuestionarios cortos de cigosidad. Se recogieron muestras de sangre para la extracción de ADN. Se realizó un análisis genetica de 22 repeticiones cortas en tándem autosómicas más los marcadores de amelogenina y DYS391 para obtener un perfil genético individual. Según el ADN, 17% de los gemelos eran dicigóticos y 83% eran monocigóticos. Ambos cuestionarios distinguieron correctamente la cigosidad con precisión del 96,7%. Estos cuestionarios de cigosidad validados por el ADN podrán utilizarse en la investigación de gemelos en Brasil, lo que contribuirá a aumentar el rigor científico.
Palabras clave: gemelos monocigóticos, gemelos dicigóticos, cigosidad, validación de cuestionario

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Among other behavioral science disciplines, psychological science has faced the prevailing problem of the replicability crisis undermining trust in the field (Anvari & Lakens, 2018; Open Science Collaboration, 2015). The solution to this crisis involves fostering world-wide collaboration (e.g., Moshontz et al., 2018), promoting open science/transparency, and also improving statistical and methodological practices (Lindsay, 2020). By promoting more accurate measures, we hope to help psychology move beyond the replicability crisis.

Behavioural genetics research using twins is one of the psychological fields less affected by the replication crisis, given that many findings have been extensively replicated (Plomin et al., 2016). The reasons for this status are: strong criticism against genetic influences on behaviour which created a reaction to perform high standard studies; long use of the improved statistics of effect size, power and confidence intervals; incentive for replication given the extensive twin registries with numerous data on these relatively difficult-to-find individuals; focus on the global net effect of genetic and environmental components of social variance; and genetic effect sizes being large (Plomin et al., 2016). The fact that the genetic and environmental components of individual variation on any physical or psychobehavioural traits can be studied using twins makes this a promising approach to advance a wide range of psychological topics.

Furthermore, geography is another important aspect of this crisis in psychology to be considered. Most studies have been conducted in Western, Educated, Industrialized, Rich and Democratic societies (WEIRD), thus not being representative of the human species (Henrich et al., 2010). Studies on African and Latin American populations are still rare, despite being large, diverse and intermixed (Rad et al., 2018). The development of an inclusive science of human mind across-cultures is urgent (Barrett, 2020). Funding restriction for science can critically limit the scope of possible research methods employed in developing countries. Thus, it is important to provide low-cost methodological alternatives to encourage high quality research on non-WEIRD populations.

The present research on the DNA validation of two short Brazilian Portuguese questionnaires for zygosity assessment was conceived with this general perspective. If the gold standard of DNA classification is not available for all participants, questionnaires may be an excellent alternative. Once validated by DNA results, the zygosity questionnaires can easily, and with very low cost, be used to accurately assess any aspects of psychosocial life in the underrepresented Portuguese speaking populations.

The Study

Brazil is a large and populous country, which is culturally, ethnically, and economically diverse. There are many opportunities for twin research (Segal, 2016a, 2016b, 2017a), including cities with high twinning rates (Santos et al., 2018, Tagliane-Ribeiro et al, 2011, 2012, Varella, Acquaviva et al., 2017, Varella et al., 2018). Interest in twinning in Brazil has "expanded enormously in recent years, engaging the interests and efforts of many investigators, students, and twins" (Segal, 2017a, p. 481). Many academic groups have created twin registries or panels, such as the *Painel USP de Gêmeos* [the USP Twin Panel] (Otta et al., 2019; Varella, Arantes et al., 2017), UFRN Twin Panel, UFPA Twin Panel, UnB Twin Panel, PUC-BA Twin Panel, The Brazilian Twin Registry (P. H. Ferreira et al., 2016), among possibly others. The future prospect for twin research in Brazil is definitely promising (Segal, 2016a, p. 292); hence, it is crucial to build upon these solid foundations.

Twin research in Brazil is mostly tracking of twinning rates and conducting of heritability studies. Knowledge about twinning rates in Brazil has grown from initial studies focused on a single hospital (Colletto et al., 2001; Colletto, 2003) to several hospitals (Colletto et al., 2003), to the biggest entire city in Brazil (Otta et al., 2016), and finally the entire country (Santos et al., 2018; Varella, Acquaviva et al., 2017, Varella et al., 2018). Twinning rates in Brazil increased from 8.80‰ in 2002 to 10.08‰ in 2013. Mothers' age and high socioeconomic status are among the predisposing factors for twinning (Santos et al., 2018; Varella, Acquaviva et al., 2017, Varella et al., 2018), which align with findings in other countries (Pison et al., 2015). Furthermore, we found differences among Brazilian regions. The Southeast (10.34‰) and the South (10.06‰) have the highest twinning rates and the North (7.32‰) has the lowest rates, with the Central-West (9.05‰) and the Northeast (8.68‰) situated between (Varella, Acquaviva et al., 2017, Varella et al., 2018). Among other possible causes, higher maternal age and greater use of assisted reproduction technologies in the most developed regions (Southeast and South), plus the socioeconomic and ethnic composition of each population, could

explain these regional discrepancies (Santos et al., 2018, Varella, Acquaviva et al., 2017, Varella et al., 2018).

In the most comprehensive meta-analysis of twin heritability studies conducted over the last 50 years (Polderman et al., 2015), from among 2,748 publications just 10 studies of Brazilian populations qualified for inclusion. Knowledge about twin heritabilities in Brazil encompasses many areas such as anthropometrics (e.g., Da Rocha et al., 1972; Reis et al., 2007), health and medicine (e.g., Alonso et al., 2014;, Bretz, Corby, Hart et al., 2005, Bretz et al., 2006; Bretz, Corby, Schork et al., 2005; Colletto et al., 1981; Custódio et al., 2007; Jacques, et al., 1977; Machado et al., 2010; Peterson et al., 2011; Rapaport et al., 1991; Su et al., 2008), and psychology (e.g., Salzano & Rao, 1976; Silva et al., 1975; Von Schantz et al., 2015). There is considerable room for methodological development, with special attention to sample size and validated measurements. To better understand the underrepresentation of Brazilian twin research in the international scientific scenario, we conducted a review of twin studies that were not included in the meta-analysis (Fernandes et al., 2021). We found that Brazilian studies on twins increased over time (<2000 = 7.2%, 2000-2009 = 31.7%, and 2010-2019 = 61.1%). Of 167 studies which reached eligibility, almost half were from Medicine (*N* = 73), followed by Psychology (*N* = 29), Odontology (*N* = 20), and Physical Education (*N* = 11). Surprisingly, in 80.2% the zygosity classification was based on twins'/parents' reports and not on scientifically-based zygosity assessment. The lack of rigorous criteria for zygosity classification justifies that the studies did not meet the basic requirement to be included in a meta-analysis (e.g., Polderman et al., 2015). Thus, here we focus on improving methodology for zygosity classification through DNA validated measurements. Placentation is a clue to zygosity, but misleading results can emerge from this practice (Segal, 2015). Around 30% of monozygotic twins were misclassified as dizygotic twins because the twins had two placentas (Ooki et al., 2004).

Trait heritability estimates based on twins require accurate knowledge of zygosity. The easiest, fastest, and least expensive ways of assessing zygosity use indirect methods, particularly self-report measures or parent report measures (Maia et al., 2007; Segal, 2017b). Questionnaire-based zygosity assessments are used worldwide, such as the 4-item questionnaire administered by the Danish Twin Registry (Christiansen et al., 2003), and the 2-item questionnaire used by the National Academy of Sciences – National Research Council (NAS-NRC) twin panel (Reed et al., 2005), both for more than half a century. Zygosity questionnaires typically focus on each twin's opinion about perceived similarity ("two peas in a pod" question), and confusion of identity by others. Evaluation of photographs of twin pairs taken under standardised conditions (facial close-up and full body view) by independent observers may complement the questionnaire-based zygosity assessment. The higher the similarity and misidentification by others, the higher the chances of a pair being monozygotic twins. The next step is to compare zygosity questionnaires with the direct and gold standard of nuclear DNA analysis.

Researchers face the challenge of accurately determining the zygosity of twins through self-report measures by validating the mostly used zygosity questionnaires with DNA genotyping. In general, most published DNA validations of zygosity questionnaires have shown high validity, comparing the classification of zygosity based on questionnaire to the classification obtained through blood polymorphism or DNA markers, or a combination of both (cf. Rietveld et al., 2000). The main difference among the questionnaires is the number of questions which can vary from approximately two to 20 (Rietveld et al., 2000). The main goal of the *Painel USP de Gêmeos* [the USP Twin Panel] (Otta et al., 2019; Varella, Arantes et al., 2017) is to encourage high-quality twin studies on psychological processes and behaviour in Brazil. Therefore, to foster higher zygosity accuracy in future twin research in Brazil, it is necessary to validate zygosity questionnaires using DNA. Short zygosity questionnaires are of special interest. To the best of our knowledge there are currently no DNA validated zygosity questionnaires in Brazil.

Aims

Our aim was to compare the results of two short zygosity questionnaires with DNA findings in a Brazilian twin sample. After careful consideration of the variety of zygosity questionnaires that have been already DNA validated, we decided to focus our DNA validation efforts on the shortest measures used for establishing zygosity with the highest accuracy: (a) the four questions used in the Danish Twin Registry (Christiansen et al., 2003) and (b) the two questions used in the National Academy of Sciences – National Research Council (NAS-NRC) twin panel (Reed et al., 2005). The original English versions of both instruments were translated into Brazilian Portuguese and adapted to the local culture

(see the 4-items questionnaire at Appendix on p. 474 in Otta et al., 2019, and on p. 17 in Otta & Fernandes, 2021).

Materials and Methods

Participants

The sample consisted of 100 Brazilian same-sex twin pairs (27 male and 73 female) with a mean age of 30.92 (*SD* = 12.87) years (range = 12 to 66 years), mostly from the state capital city of São Paulo. The majority of the sample self-declared as European descending (white) (78.2%); 8.9% mixed ethnicity (*pardo*), 5% Asian descent, 4.5% African descent (black), 1% indigenous descent, and 0.5% Arabic descent; 1.9% had missing data. Half the sample (51%) self-declared as belonging to the middle social class; 23.3% low middle class, 15.8% high middle class, 5% high class, and 2.5% low class; 2.5% had missing data. The educational level of 43.1% of participants was technical education/university graduate; 29.7% high school, 22.8% post-graduate, and 3% basic school; 1.4% had missing data. None of the twin pairs declared that they had already taken a DNA zygosity test. All participants signed an informed consent form, and the research protocol was approved by the Ethics Committee of the Clinical Hospital from Medical School, University of São Paulo (approval number: 2.470.639).

Instruments

The Zygosity Questionnaire of the Danish Twin Registry (Christiansen et al., 2003) is a 4-item questionnaire composed of two questions about physical similarities, and two questions about identity confusion (Appendix A). A pair is classified as monozygotic (MZ) if both twins: (1) describe themselves as strikingly similar and or, (2) state that teachers, classmates, family, and friends had difficulties in distinguishing them, and (3) answer that they had the same hair- and eye-colour in childhood. A pair is classified as dizygotic (DZ) if both twins: (1) describe themselves as no more alike than ordinary siblings, and (2) report that people never experience difficulties in telling them apart. Zygosity is classified as unknown (UZ) in cases of disagreement between twins' answers, and if they report themselves as strikingly similar but at the same time answer they had never been mistaken for each other.

The Zygosity Questionnaire of the National Academy of Sciences – National Research Council (NAS-NRC) twin panel (Reed et al., 2005) is a two-item questionnaire composed of one question about physical similarity, and another based on confusion of identity (Appendix B). A twin pair is classified as MZ if both answer affirmatively to the physical similarity and the confusion of identity questions. Negative answers to both questions led to classification as a DZ pair. Zygosity is classified as unknown (UZ) if the pair gives conflicting answers.

The Zygosity Questionnaire of the Danish Twin Registry and the Zygosity Questionnaire of the National Academy of Sciences – National Research Council (NAS-NRC) twin panel were translated into Brazilian Portuguese by Brazilian Portuguese-English bilingual researchers following the standard practice for scale adaptation (cf. Epstein et al., 2015; L. Ferreira et al., 2014). Independent translations were compared and extensively discussed by a team with experience in studying twins in order to come to a final agreement. A separate independent translator translated the questionnaire back into English. The back-translation was evaluated by a native English speaker. A preliminary pilot testing was conducted with a sample of twins (*n* = 30). The most delicate issue was the impossibility of literal translation of the expression "like two peas in a pod". The same had occurred in Denmark, where the expression "like two drops of water" was chosen as the Danish equivalent of "like two peas in a pod". Our team concluded for the appropriateness of the Brazilian saying "*Cara de um, focinho do outro*".

Participants were also separately asked to report whether they thought they were MZ, DZ, or did not know (i.e., their zygosity self-identification). This zygosity assessment by self-identification was based on answer concordance of a pair of twins. When a twin answered, 'I don't know' and his co-twin answered 'MZ' or 'DZ', the classification was based on the co-twin's response following the standard procedure of the Danish Twin Registry[1]. When they were discordant about zygosity (i.e., one answered 'MZ' and other 'DZ'), the classification attributed to the pair was 'UZ' or uncertain zygosity. Finally, if a twin pair answered, 'I don't know' or did not inform their opinion (missing values) they were also classified as 'UZ'.

Procedure

The twins were recruited in 2018 through mailing-lists and Facebook from the *Painel USP de Gêmeos* [the USP Twin Panel] (Otta et al., 2019; Varella, Arantes et al., 2017). They were then invited by telephone to come

together to our laboratory at the Institute of Psychology in University of São Paulo. After signing the informed consent term, co-twins separately and independently provided socio-demographic information, completed the two zygosity questionnaires all in the same order (i.e., first Christiansen et al., 2003, followed by Reed et al., 2005) and the zygosity self-identification question. Peripheral blood samples were collected from each individual's arm for DNA testing by qualified nurses. The blood samples were transported to the Medical School at University of São Paulo where the genotyping was performed.

DNA Genotyping

Genomic DNA was extracted from 5 ml of peripheral blood following standard salting-out procedure (Miller et al., 1988). DNA samples were quantified using Nanodrop 2000 (Thermofisher) and stored at -20°C. Each sample tube contained information on collection date, the twin pair number and the letter 'A' or 'B' corresponding to each individual within the pair.

The PowerPlex® Fusion System (Promega) kit was used to analyze 22 autosomal STR loci (D3S1358, D1S1656, D2S441, D10S1248, D13S317, D16S539, D18S51, D2S1338, CSF1PO, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, D22S1045, FGA, Penta E and Penta D) plus amelogenin and DYS391, according manufacturer's instructions, in order to genotype the individual profile. Genotyping was carried out using ABI 3130 Genetic Analyser (Applied Biosystems) and the analysis was performed using GeneMapper ID Software v4.0 (Applied Biosystems). The discrimination power of this DNA kit of forensic human identification is higher than 99.999%.

The zygosity diagnosis was done investigating the concordance between the genetic profile of each pair member: MZ twins have exactly the same genetic profile for these STR markers, while DZ twins show a varying number of shared alleles. The procedure of DNA genotyping was independent and blind to the twins' self-reports.

Data Analysis

Twins were classified as MZ, DZ or unknown zygosity (UZ) according to the rules of each questionnaire. Using the Kappa value, we performed: 1. the comparison of associations between the response of twins and co-twins of a pair; 2. the concordance between twins' responses to each item of the instruments; 3. the comparison of zygosity classification through questionnaires, self-identification, and DNA analysis; 4. the concordance between the zygosity classification by individual response and DNA analysis. Data were also analysed as a function of sex and zygosity using a two-sided Fisher's exact test.

Results

DNA Diagnosis

According to DNA analysis 17% of twin pairs were classified as DZ and 83% as MZ.

Self-Report Measures

We found significant agreement between the classification of the twin and his/her co-twin. The association was almost perfect for Questionnaire II (Reed et al., 2005) (Kappa value = 0.90, $p < 0.001$), and important for Questionnaire I (Christiansen et al., 2003) (Kappa value = 0.77, $p < 0.001$) and for self-identification (Kappa value = 0.64, $p < 0.001$).

For agreement between responses of the twin and his/her co-twin per item of questionnaire (Table 1), we found important (item 1: Kappa value = 0.70, item 2: Kappa value = 0.63, $p < 0.001$), moderate (item 3: Kappa value = 0.53, $p < 0.001$) and almost perfect (item 4: Kappa value = 0.83, $p < 0.001$) associations for Questionnaire I (Christiansen et al., 2003), and important (item 1: Kappa value = 0.75, $p < 0.001$) and regular (item 2: Kappa value = 0.34, $p < 0.001$) associations for Questionnaire II (Reed et al., 2005).

We also evaluated the concordance between DNA and the individual response per questionnaire item. Questionnaire I (Christiansen et al., 2003) showed significant regular (item 3: Kappa value = 0.30, $p < 0.001$), moderate (item 1: Kappa value = 0.47, $p < 0.001$) and substantial (item 2: Kappa value = 0.64, item 4: Kappa value = 0.77, $p < 0.01$) associations. Questionnaire II (Reed et al., 2005) presented important (item 1: Kappa value = 0.80, $p < 0.001$) and moderate (item 2: Kappa value = 0.43, $p < 0.001$) associations.

The concordance between DNA and the individual classification was significant and substantial (Kappa value = 0.78, $p < 0.001$) for Questionnaire I (Christiansen et al., 2003), and significant and almost perfect (Kappa value = 0.86, $p < 0.001$) for Questionnaire II (Reed et al., 2005). In contrast, the self-identification showed a poor and non-significant association (Kappa value = -0.04, $p > 0.001$).

In addition, when comparing the items of the questionnaire by content, different associations were observed: important for physical similarity; regular, moderate, and substantial associations for identity confusion, in addition to an important association for the similarity of eye and hair colour (Table 1).

Table 1. *Comparative Evaluation of Two Questionnaires for Zygosity Determination and Self-Identification*

Questionnaire		Christiansen et al. (2003)		Reed et al. (2005)		Self-identification	
	Question number	Twin * co-twin	DNA * twin	Twin * co-twin	DNA * twin	Twin * co-twin	DNA * twin
Kappa Coefficient of Concordance	1	0.70	0.47	0.75	0.80	0.64	-0.04
	2	0.63	0.64	0.34	0.43		
	3	0.53	0.30	N/A	N/A		
	4	0.83	0.77	N/A	N/A		
		Correct diagnosed	Misdiagnosed	Correct diagnosed	Misdiagnosed	Correct diagnosed	Misdiagnosed
Accuracy of Zygosity Determination	MZ	73 (96.0%)	3	77 (98.7%)	1	43 (68.3%)	20
	DZ	16 (100%)	0	12 (85.7%)	2	4 (26.7%)	11
	Total	89(96.7%)	3	89 (96.7%)	3	47 (60.3%)	31
	UZ	8		8		22	
		Men	Women	Men	Women	Men	Women
Accuracy of Zygosity Determination by Sex	MZ	95.4% (21/22)	96.2% (52/54)	100% (23/23)	98.1% (54/55)	66.7% (10/15)	68.7% (33/48)
	DZ	100% (4/4)	100% (12/12)	100% (3/3)	81.8% (9/11)	25% (1/4)	22.3% (3/11)
	Total	96.1% (25/26)	96,9% (64/66)	100% (26/26)	95,4% (63/66)	57.9% (11/19)	61% (36/59)

Accuracy of Zygosity Determination

According to self-identification, the 100 twin pairs were distributed as 54% MZ, 24% DZ and 22% UZ. Sixty percent of the twins (*N* = 47/78) had correct zygosity classification, according to the genetic analysis: 68.3% (*N* = 43/63) MZs and 26.7% (*N* = 4/15) DZ. MZ misclassification was significantly higher than DZ misclassification (*p* = 0.001, Fisher's exact test). Of 22 twin pairs who were unaware of their zygosity, 90.9% (*N* = 20) were MZ and 9.1% (*N* = 2) DZ.

The classifications obtained by both questionnaires showed a total accuracy of 96.7%. The concordance with the DNA classification was poor for self-identification (Kappa value = -0.04, $p > 0.001$) and almost perfect for Questionnaire II (Reed et al., 2005) (Kappa value = 0.87, $p < 0.001$) and for Questionnaire I (Christiansen et al., 2003) (Kappa value = 0.89, $p <$ 0,001). The accurately classified percentages were 96% (*N* = 73/76) MZs and 100% (*N* = 16/16) DZs using Questionnaire I (Christiansen et al., 2003); since there were only MZ misclassifications we could not perform the Fisher's exact test. The classification obtained by Questionnaire II (Reed et al., 2005), in turn, presented 98.7% (*N* = 77/78) MZs and 85.7% (*N* = 12/14) DZs, but the DZ misclassification was not significantly higher than MZ (*p* = 0.33, Fisher's exact test). In relation to the total sample of 100 twin pairs, 8% could not be classified (UZ), being 7% MZ and 1% DZ for the Questionnaire I (Christiansen et al., 2003) and 5% MZ and 3% DZ for Questionnaire II (Reed et al., 2005). Table 1 also presents the correct and incorrect classification rates for each questionnaire.

The overall accuracy of zygosity determination differed between males and females for self-identification (57.9% versus 61.0%, respectively). However, the difference in the distribution of misclassified MZ and DZ pairs in males and females was not significant (*p* = 1, Fisher's exact test). Accuracy did not differ between

men and women for Questionnaire I (Christiansen et al., 2003) (96.1% versus 96.9%). Both MZ males and females were misclassified. Accuracy differed for Questionnaire II (Reed et al., 2005) (100% versus 95.4%). Some females were misclassified. Fisher's exact test could not be performed for Questionnaire I and II. Table 1 presents the zygosity classification rates by sex.

Discussion

Our study illustrates a profitable way to advance the discussion surrounding the replication and the representability crises in Psychology. We addressed these issues performing a DNA validation of two short zygosity questionnaires, thus improving methodology, increasing the chances of replicability in a wide range of topics, and by focusing on a Brazilian twin sample, thereby increasing the geographical representability of psychological research.

We translated and adapted two short zygosity questionnaires for use in Brazilian Portuguese (i.e., Christiansen et al., 2003, with four questions; Reed et al., 2005, with two questions), and validated them using a DNA analysis in a Brazilian twin sample (same-sex twin pairs: 27 males and 73 females; *M* age = 30.92; 12 to 66 years). Both questionnaires correctly established zygosity with 96.7% accuracy, the same amount (8%) of Unknown Zygosity (UZ), and with a high Kappa index. This high accuracy rate is virtually the same as that found, respectively, for the Danish version of the 4-item questionnaire (96% for Christiansen et al., 2003), and the English version of the 2-item questionnaire (96.8% for Reed et al., 2005). Both instruments can be easily and confidently used to classify zygosity of same-sex twins in future Brazilian research.

Although minor, there were unusual aspects of each zygosity questionnaire that should be noted. Comparing zygosity misclassifications between MZs and DZs, the Questionnaire I (Christiansen et al., 2003) resulted in minor misclassifications (4%) and only for MZs, whereas the Questionnaire II (Reed et al., 2005) resulted in slightly more misclassification for DZ twins (14.3%) than for MZ twins (1.3%), although the difference was not statistically significant. The Questionnaire II (Reed et al., 2005) presented higher concordance between twin and co-twin classification (Kappa value = 0.90, $p < 0.001$) than the Questionnaire I (Christiansen et al., 2003) (Kappa value = 0.77, $p < 0.001$). The concordance between DNA and individual classification is also higher for Questionnaire II (Reed et al., 2005) (Kappa value = 0.86, $p < 0.001$) than for Questionnaire I (Christiansen et al., 2003) (Kappa value = 0.78, $p < 0.001$). This indicates that Questionnaire I (Christiansen et al., 2003) is slightly better for diagnosing the zygosity of DZ twins, but offers lower confidence in classification based only on one co-twin. In contrast, Questionnaire II (Reed et al., 2005) presents more evenly distributed misdiagnoses, although this form is slightly less effective for diagnosing the zygosity of DZ twins than MZ; however, it offers higher confidence in classification based on just one twin of the pair. Given that twin researchers sometimes are unable to recruit both twins of the pair, this is important information to consider.

Comparing the accuracy of the zygosity assessment in males and females, no difference was found between the groups, either for self-identification or questionnaire I (Christiansen et al., 2003). However, questionnaire II (Reed et al., 2005) was less accurate for females. Our sample included more than twice as many females than males, but the efficacy of the zygosity assessment was quite similar in both sexes. This result is in accordance with the literature for other countries; for instance, no difference was found for sex in Christiansen et al. (2003). This is an encouraging result which demonstrates the wide applicability of questionnaire I for zygosity classification.

Analysing the concordance with DNA per item of each questionnaire, twin and co-twin presented high agreement for all items, except for item 2 of Questionnaire II (Reed et al., 2005), which is about confusion by family and school. The Questionnaire I (Christiansen et al., 2003) has different items to ask for confusion by family members and in school. Our results indicate that the differentiation is relevant. Excessively reducing the number of items may impair zygosity determination. These analyses by questionnaire items are also interesting because they show the contribution of each item content for the zygosity classification. In Questionnaire I (Christiansen et al., 2003), items 4 and 2, about eye and hair colour similarity in childhood and confusion in school, respectively, presented the best agreement with DNA. In Questionnaire II (Reed et al., 2005), item 1 about physical similarity in childhood presented the best agreement with DNA.

In contrast to the zygosity questionnaires, the twin's opinion or self-identification about their zygosity presented unsatisfactory and unreliable results. First, the accuracy of self-identification was 60.3%, with a

substantial concordance between twins. This moderate level of accuracy may give the false impression of a good index, which is not really the case because it was more inaccurate in MZ than in DZ pairs. The concordance with the DNA was considered 'low' or 'weak', far from the 'almost perfect' agreement of the results from both questionnaires. Moreover, this index misdiagnosed over three times more twin pairs than each zygosity questionnaire. Finally, more than a fifth of the sample did not have an opinion about their zygosity, which left uncategorized a substantial proportion of the twins who are relatively rare to find in the population (Otta et al., 2016; Santos et al., 2018; Varella, Acquaviva et al., 2017, Varella et al., 2018). Thus, it is definitely unwise to only rely upon a twin's opinion or self-identification about their zygosity for research or medical purposes. Twins and their parents may have been misinformed as to whether they were MZ or DZ because doctors themselves were uncertain, thinking approximately 30 percent of MZ twins have separate placentas and amniotic sacs (Ooki et al., 2004).

The main limitation of this research is the size, structure, and origin of the sample. Contrary to the Danish Twin Registry and to the National Academy of Sciences – National Research Council, both with half a century of history, the *Painel USP de Gêmeos* [the USP Twin Panel] is recent and with a limited number of twin pairs registered (Otta et al., 2019; Varella, Arantes et al., 2017). This explains the relatively modest size of the present sample. As in many twin studies, MZ twins are much more willing to participate than DZ, and female twins are more willing than male twins, which explains the asymmetry of our sample (Lykken et al., 1987). Despite having participants from 12 to 66 years of age, the majority of the sample consisted of young to middle adults; thus, we did not have enough participants of different age ranges to perform age-related analysis. Lastly, the majority of the participants were white, educated, middle social class from São Paulo State, which does not fully represent the plurality of the Brazilian population. However, our results were very similar to the original research from Denmark and the US, both studies with much larger samples, and arguably of a population much more different from São Paulo State than are the other Brazilian regions.

We have translated and adapted two short zygosity questionnaires to Brazilian Portuguese and validated their results against the DNA genotyping in a sample of Brazilian same sex twins presenting a very high overall accuracy and reliability. This finding is consistent with the literature and supports the accurate use of questionnaire-based zygosity assessment to distinguish same-sex twins for heritability research. We hope to foster more high-quality twin research in Brazil by offering these accurate and reliable zygosity assessments and promoting psychological science chances of replicability and of a global representability.

Declaration of Interest Statement

The authors declare no conflict of interests.

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[1] According to the Data Manager of the Danish Twin Registry (L. A. Larsen, personal communication, January 13, 2020), regarding self-perception of zygosity the following practice is adopted: When one co-twin's answer is misleading/missing and the other member answers MZ or DZ, they considered the zygosity of the pair the more informative answer.

Appendix

Appendix A

English version of the Zygosity Questionnaire of the Danish Twin Registry (Christiansen et al., 2003) and corresponding Brazilian Portuguese version

Question	Original English version	Brazilian Portuguese Version
1	Do you and your twin look — like two peas in a pod?* — like two ordinary siblings? — not very much alike?	Sobre o quanto são parecidos(as), marque apenas uma alternativa. Você e seu(sua) irmão(ã) gêmeo(a): — são tão parecidos fisicamente como diz o ditado "cara de um, focinho do outro". — são parecidos fisicamente quanto dois irmãos biológicos não gêmeos — não são parecidos fisicamente, como vizinhos.
2	In school, is/was it difficult for your teachers and friends to tell you apart?	Na escola, é/era difícil para seus professores e colegas distinguirem um(a) do(a) outro(a)? ( ) SIM ( ) NÃO
3	Is/was it difficult for your family and friends to tell you apart?	É/era difícil para sua família ou amigos diferenciarem um(a) do(a) outro(a)? ( ) SIM ( ) NÃO
4	In childhood, did you and your twin have both the same eye color and the same hair color?	Na infância, você e seu(sua) irmão(ã) tinham ambos(as) a mesma cor de olhos e a mesma cor de cabelo? ( ) SIM ( ) NÃO

Appendix B

English version of the Zygosity Questionnaire of the National Academy of Sciences – National Research Council (NAS-NRC) twin panel (Reed et al., 2005) and correspondent Brazilian Portuguese version

Question	Original English version	Brazilian Portuguese Version
1	As children were you and your twin alike as two peas in a pod or of only ordinary family resemblance?	Quando crianças você e seu(sua) irmão(ã) gêmeo(a) eram: — muito parecidos fisicamente como no ditado "a cara de um, focinho do outro". — ou tinham apenas a semelhança familiar comum?
2	In childhood, did your parents, brothers and sisters, or teachers have trouble in telling you apart?	Na escola, é/era difícil para seus professores e colegas distinguirem um(a) do(a) outro(a)? ( ) SIM ( ) NÃO

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