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VALIDATION OF ZYGOSITY ASSESSMENT BY A SELF-REPORT QUESTIONNAIRE IN A SAMPLE OF ADULT SERBIAN TWINS[2]

Validation of a twin zygosity-estimating questionnaire, The Questionnaire of Twins' Physical Resemblance, created by Oniszczenko et al. and used in European and Serbian twin studies, was carried out on a sample of 222 pairs (176 monozygotic, 46 dizygotic) of adult twins (average age 24.6). Four discriminant functions, use of different sets of indicators (zygosity questionnaire items), were applied in order to obtain the most correct and accurate estimates of zygosity. The first function was a predefined function used in European twin studies, the following two functions contained sets of 18 and 24 freely estimated indicators respectively, while the last one utilized the items with most consistent contributions to zygosity prediction. The analytic procedure included cross-validation, whereby the sample was randomly split into two subsamples, comprising 107 and 115 twin pairs. The results pointed to successful (over 90% correct) identification of monozygotic twins, and sizeably lower correctness in identifying dizygotic twins. Overall correctness of estimation exceeded 90%, with the small set of best-performing indicators. The results encourage questionnaire estimation of zygosity, and raise the issue of improving the classification procedure in dizygotic twins.

Key words: behavioral genetics, questionnaire assessment of zygosity, twin studies

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Introduction

Correct estimation of twins' zygosity is a crucial prerequisite for the validity of results of the twin studies (Lenau et al., 2017). Currently, several procedures are available for the assessment of zygosity. Undoubtedly most efficient are the DNA analytic procedures, which reduce estimation error to less than 1% (Becker et al., 1997; Lenau et al., 2017). However, the cost of such analyses (Lenau et al., 2017) may still be a challenge for large studies or studies in underprivileged regions. Therefore, besides DNA analyses, or instead of them, the questionnaire assessment of zygosity is often used as an alternative (Joseph, 2004). Technical aspects of the questionnaire-based zygosity estimation in twin studies imply identification of indicators with best discriminant power (by means of discriminant analysis or similar analytic procedures), and the use of the extracted discriminant function in subsequent estimations.

The efforts to improve the accuracy of zygosity questionnaires were evident in recent decades. In adult twin samples, overall classification rates in most cases exceeded 90%, and occasionally amounted to approximately 98% (e.g., Jarrar et al., 2018; Joseph, 2004; Lenau et al., 2017; Ooki, Yamada, Asaka, & Hayakawa, 1990; Peeters, Van Gestel, Vlietinck, Derom, & Derom, 1998), whereby one or more zygosity questionnaires were applied.

In order to reliably determine twins' zygosity, the questionnaires employ a number of indicators, which are assumed to discriminate well between monozygotic and dizygotic twins (Oniczenko, Angleitner, Strelau, & Angert., 1993). Such indicators are sometimes labelled as biological and physical characteristics (Lenau et al., 2017). The aforementioned set of indicators includes "objective" estimates such as height, eye colour, natural hair colour, blood type, earlobe shape, etc. Indicators based on "subjective" assessment are also considered, and they mostly refer to mistaking twins for one another: in childhood, by family, friends, acquaintances, teachers, colleagues, strangers, in photographs taken recently, etc. Relevant are also the data about chronic and acute medical conditions. These features have been shown to be reliable indicators of zygosity in self- and peer- ratings. Reportedly, the questions referring to twins being as similar as "peas in the pod" have been particularly informative (Joseph, 2004).

However, classification of dizygotic twins may still be a challenge. While there are findings that suggest similar or equal precision of MZ and DZ twin's classification, or even better classification rates for DZ twins (Jarrar et al., 2018; Lenau et al., 2017), there are results that point to the contrary (Lenau et al., 2017; Ooki et al., 1990).

A zygosity questionnaire most frequently used in European twin studies is the The Questionnaire of Twins' Physical Resemblance by Oniszczenko et al. (1993). The questionnaire has been successfully applied in BilSat (Kandler et al., 2012), JetSSA (Stößel, Kämpfe, & Riemann, 2006), GOSAT (Spinath, Angleitner, Borkenau, Riemann, & Wolf, 2002), and TwinLife (Lenau et al., 2017) studies.

Within and beyond these studies, the questionnaire has demonstrated excellent classification rates (Lenau et al., 2017).

The Oniczenko et al. (1993) questionnaire has also been applied in the Serbian twin study, conducted within the research project "Psychological foundations of mental health: hereditary and environmental factors" (e.g., Nikolašević, Bugarski - Ignjatović, Milovanović, & Raković, 2014). The zygosity classification function used in the BilSat study (Kandler et al., 2012), similarly, but not identically to the function applied in cohorts 3 and 4 of the TwinLife study (Lenau & Hahn, 2017), has been used in Serbia so far. However, the predictive validity of the measure has not been validated yet in a sample of Serbian twins. Despite robustness of the phenomenon, it is not warranted that the Bilsat/TwinLife classification procedure will be as efficient in Serbian culture as it is in its original form. A number of issues should be addressed: does the original classification procedure (as shown in Lenau & Hahn, 2017) discriminate well between adult MZ and DZ twins in Serbia? Would an extended set of indicators perform better? Would it be possible to select the most discriminative items and develop a brief, but efficient classification tool? All these questions are subordinate to the principal aim of the current study: to identify the set of indicators, which most efficiently discriminate between monozygotic and dizygotic twins in the adult Serbian sample. The results are expected to help in future self-report zygosity assessment in behavioral genetic studies.

Method

Sample and Procedure

A sample of 222 twins (111 twin pairs; 70% female participants in total), whose average age was 24.6 (*SD* = 7.64), took part in the study. The DNA test results suggested that 176 twin pairs were monozygotic, while 46 twin pairs were dizygotic. Prior to the analyses, 37 undoubtedly dizygotic (different-sex) twin pairs were excluded from the study. For the purposes of cross-validation, following, but not mirroring the procedure used in the reference study of Lenau et al. (2017), the sample was randomly split into two sub-samples. The first sub-sample included 87 monozygotic and 20 dizygotic twin pairs (average age 24, *SD* = 7.92), while the second one included 89 monozygotic and 26 dizygotic twin pairs (average age 25.16, *SD* = 7.36). The data were collected from 2011 to 2018, by administering the questionnaire to participants (twins) in a form of a standardized interview, with the standard clause of confidentiality. A smaller number of twins who were not able to attend the interview completed the questionnaires at their homes and returned them by mail. The zygosity questionnaire was not administered to the twin pairs of different sexes. Twins were recruited as a part of the wide Serbian national project "Psychological Foundations of Mental Health: Hereditary and Environmental Factors".

Instrument

The Questionnaire of Twins' Physical Resemblance (Oniszczenko et al., 1993). This questionnaire is a self-report measure containing 31 sets of items (plus 19 demographic questions) referring to the above mentioned biological and physical indicators. The questionnaire can be applied as the standard self-report, paper-pencil format, or in the form of a standardized interview. The measure, scoring procedures, and the discriminant functions used for classification, are described in detail in Lenau & Hahn (2017). Certain indicators, carrying the extensions 1 and 2, have been calculated in two different variants, and entered as such in the functions (for details see Lenau et al., 2017; Lenau & Hahn, 2017).

Data analysis

The criterion used for the validation of the zygosity questionnaire was the result of DNA zygosity estimation, carried out by method of micro-satellites (Becker et al., 1997).

According to the standard procedure, all indicators used in the analyses were calculated from "raw" responses to questionnaire items. The procedure was primarily based on the calculation of differences in responses by the twins from each pair. The final indicators values ranged from 0 to 1, whereby the scoring was such that the value 1 points to monozygosity, value 0 to dizygosity, while the value 0.5 was assigned to the cases where zygosity could not be estimated with sufficient reliability. Thus, although most labels of indicators in Tables 3, 5 and 8 contained the word "differences" (for the sake of comparability with other studies), the reader should interpret them according to the scoring procedure described above.

Classification procedures were carried out by discriminant function analyses, using the "MASS" (Venables & Ripley, 2002) package in R 3.5.1 (R Core Team, 2018). The "lda" function from the "MASS" package performed linear discriminant analysis, with the possibility of cross-validation. Thus, the functions developed in the first subsample were applied in the second, and vice versa. In the entire sample, the function was estimated independently from the ones derived and cross-validated in the subsamples. A linear discriminant analysis was used according to methodology presented in Lenau et al. (2017), assuming that such decision would facilitate the comparability of results. Prior probabilities were set to 80:20 for MZ and DZ twins respectively.

The following discriminant / classification functions were tested:

a) The function developed in the BilSat study (Kandler et al., 2012), similar to the function applied in the cohorts 3 and 4 of the TwinLife study (Lenau et al., 2017). The function was weighted according to the BilSat original function (Kandler et al., 2012; weights for the TwinLife cohorts 3 /4 function is presented in Lenau & Hahn, 2017);

b) the function comprising the indicators used in the BilSat study (Kandler et al., 2012), whereby the discriminant coefficients (weights) were freely estimated;
c) the function freely estimated in the sample of adult Serbian twins, based on the extended set of indicators described in Lenau et al. (2017) and Lenau and Hahn (2017), including twins' own belief about their zygosity, as well as the "peas in the pod" statement;
d) the discriminant function based on the best-discriminating items selected from the previous functions. The criterion for the selection was the following: items which standardized discriminant coefficients were stable across samples were chosen to be included in the analysis.

Results

Weighted BilSat Function: Classification Rates

In the first step of the study, classification rates of the discriminant function obtained from the BilSat study were estimated (Table 1).

Table 1
Function 1: BilSat - classification rates

	Subsample 1	Subsample 2	Total
MZ (%)	98.63	96.20	97.37
DZ (%)	55.88	63.89	60.00
Total (%)	85.05	86.09	85.59

Note. MZ – monozygotic twins, DZ – dizygotic twins.

The results show excellent classification rates for monozygotic twins, and unsatisfactory rates for dizygotic twins. Correct classification rates are approximately 85% to 86%. These results suggest that the adjustment of the "original" classification procedure to Serbian sample would be recommended. Nevertheless, having in mind that the study has identified Serbian monozygotic twins with almost perfect correctness (Table 1), we cannot dispute validity of the indicators, and tend to see this result as corroborating the robustness of the phenomenon.

BilSat Function: Freely Estimated Coefficients

With the discriminant coefficients estimated freely, correct classification rates improve substantially, with correct classification rates approximating 90% (Table 2).

Table 2

Function 2: Classification- freely estimated coefficients and group centroids

				Group centroids		
	Subsample 1	Subsample 2	Total	Subsample 1	Subsample 2	Total
MZ (%)	95.29	92.31	94.32	-0.45	-0.75	-0.55
DZ (%)	72.73	79.17	67.39	2.27	2.13	2.22
Total (%)	90.65	89.57	88.74	-	-	-

Note. MZ – monozygotic twins, DZ – dizygotic twins.

Still, the correctness in identifying dizygotic twins is still unsatisfactory. At the same time, instability of indicators' contributions over subsamples is evident, with some of the standardized discriminant coefficients varying not only regarding size, but also regarding the sign (Table 3).

Table 3

Function 2: Standardized discriminant coefficients

	Subsample 1	Subsample 2	Total
difference in height	-0.17	-0.04	-0.05
difference in hairiness	-0.10	-0.33	-0.23
difference in skin colour	0.07	0.08	0.07
difference in sweating	-0.09	-0.13	-0.11
difference in eye colour	-0.05	0.05	-0.01
difference in blood type	-0.15	0.15	0.02
difference in hair type	-0.36	-0.34	-0.32
difference in eye colour 2	-0.69	-0.04	-0.37
difference in ear lobes	0.28	0.28	0.24
parent's effort to keep apart	-0.09	-0.14	-0.06
difference in sickness	-0.05	-0.24	-0.09
mistaken in childhood	-0.29	-0.40	-0.34
mistaken by siblings 1	-0.08	-0.09	-0.08
mistaken by teachers 1	-0.20	-0.17	-0.13
mistaken by people meeting first time 1	-0.49	-0.58	-0.53
mistaken by parents 2	0.30	-0.21	0.01
mistaken by teachers 2	0.07	-0.09	-0.06
mistaken in a photograph	-0.29	0.07	-0.05

While this result could be partly attributed to the sample size and somewhat unfavourable conditions for cross-validation, it also suggests that modification of the function (either its extension or reduction) may improve its correctness in classification.

Extended Set of Predictors – Freely Estimated Coefficients

The results suggest that the extension of predictor set have not significantly improved classification rates (Table 4). They also show that the problem encountered in Function 2, namely ‘the instability of indicators’ contributions across functions, remains.

Table 4
Function 3: Classification based on extended set of predictors and group centroids

				Group centroids		
	Subsample 1	Subsample 2	Total	Subsample 1	Subsample 2	Total
MZ (%)	93.18	92.22	94.89	-0.47	-0.74	-0.58
DZ (%)	73.68	76.00	69.57	2.37	2.29	2.34
Total (%)	89.72	88.70	89.64	-	-	-

Note. MZ – monozygotic twins, DZ – dizygotic twins.

Standardized, freely estimated discriminant coefficients on the extended set of predictors are presented in Table 5.

Table 5
Function 3: Standardized discriminant coefficients – extended set of items

	Subsample 1	Subsample 2	Total
difference in height	-0.14	-0.05	-0.07
difference in hairiness	-0.14	-0.46	-0.29
difference in skin colour	0.03	0.06	0.09
difference in sweating	-0.14	-0.19	-0.11
difference in eye colour	-0.03	0.22	0.00
difference in blood type	-0.21	-0.26	-0.27
difference in rhesus factor	0.11	0.48	0.39
difference in hair type	-0.36	-0.37	-0.33
difference in eye colour 2	-0.67	-0.15	-0.38
difference in ear lobes	0.27	0.28	0.27
parent's effort to keep apart	-0.06	-0.01	0.04
difference sickness	0.00	-0.27	-0.08
mistaken in childhood	-0.23	-0.82	-0.40
mistaken by parents 1	-0.21	-0.31	-0.23
mistaken by siblings 1	0.06	-0.09	-0.02
mistaken by friends 1	-0.18	0.30	0.03
mistaken by teachers 1	-0.20	-0.14	-0.14
mistaken by people meeting first time 1	-0.65	-1.07	-0.62
mistaken by parents 2	0.43	-0.14	0.08
mistaken by siblings 2	-0.23	0.00	-0.05
mistaken by friends 2	0.34	-0.17	0.06
mistaken by teachers 2	0.02	-0.09	-0.06
mistaken by people meeting first time 2	-0.08	0.82	0.03
mistaken in photograph	-0.24	0.05	-0.07
peas in a pod	0.16	0.05	0.10
own belief	-0.21	0.12	-0.03

Among the salient indicators that remain invariant or "partially invariant" across samples, there are physical features such as differences in eye colour, blood group, and hairiness, but also indicators of mistaking twins by parents, teachers, people met for the first time. Curiously, some of the physical indicators appear to be indicative of dizygosity, such as the difference (or similarity) in rhesus factor and ear lobes. This also applies to “peas in the pod” similarity statement, which contribution is modest (even negligible), but with consistently positive sign.

Selected Items

In the final phase of the study, ten “stable” indicators were selected according to their coefficients in Function 3, and entered into the analysis. Selection of the best-discriminating items apparently contributed not only to coefficient stability (with some exceptions, such as difference in hairiness and rhesus factor), but also to classification correctness, with correctness rate in subsamples around 91%, and the overall correctness in the entire sample also being 91%. Although these results were favourable, the problem of correctly identifying dizygotic twins remained, with correctness approximating 80%, but not exceeding it (Table 6).

Table 6
Function 4: Ten best - discriminating items and group centroids

				Group centroids		
	Subsample 1	Subsample 2	Total	Subsample 1	Subsample 2	Total
MZ (%)	94.32	94.32	93.82	-0.44	-0.63	-0.54
DZ (%)	78.95	77.78	79.55	2.10	2.07	2.17
Total (%)	91.59	90.43	90.99	-	-	-

Note. MZ – monozygotic twins, DZ – dizygotic twins.

Standardized, freely estimated discriminant coefficients on the selected set of items are presented in Table 7.

Table 7
Function 4: Standardized discriminant coefficients based on 10 selected items

	Subsample 1	Subsample 2	Total
difference in height	-0.22	-0.07	-0.08
difference in hairiness	0.02	-0.31	-0.19
difference in blood type	-0.09	0.11	0.02
difference in hair type	-0.36	-0.25	-0.31
difference in eye colour 2	-0.63	-0.01	-0.30
mistaken in childhood	-0.18	-0.52	-0.39
mistaken by parents 1	-0.04	-0.29	-0.19
mistaken by people meeting first time 1	-0.55	-0.50	-0.51
difference sweating	-0.05	-0.12	-0.10
mistaken by teachers 1	-0.10	-0.19	-0.12

Discussion

This study was conducted in order to validate "The Questionnaire of Twins' physical resemblances" (Onisczenko et al., 1993), which might be regarding as something of a standard in European behavioral genetic studies. Generally, the questionnaire and classification procedures based on it performed well in our sample, but certain adjustments were nevertheless necessary. The results of this research were nearly in line with the results of the previous research that spoke in favour of high classification rates (>90%) of zygosity questionnaires (e.g., Jarrar et al., 2018; Joseph, 2004; Lenau et al., 2017; Ooki et al., 1990; Peeters et al., 1998).

One of the most important, though expected, findings concerns better performance of the "freely estimated" functions compared to the predefined function derived in the BilSat / TwinLife studies. Although this result may be regarded as self-explanatory, still it is important to mention that the "predefined" classification procedure has performed satisfactorily in identification of monozygotic twins. Whether this result is due to cultural factors, specific self-assessment of Serbian dizygotic twins, or relatively small number of dizygotic twin pairs in this study, is yet to be resolved.

What seems to be a persistent issue is the assessment of dizygotic twins. In this study, correctness of their classification has not been up to our expectations, despite prior probabilities set to fairly liberal 80:20 in favour of monozygotic twins, reflecting the sample structure. A possible hypothesis based on this result could be that the Serbian dizygotic twins tend to accentuate their similarities, instead of differences. Therefore, qualitative or quantitative examination of their responses on the zygosity questionnaire may help clarify this issue. Nevertheless, the classification of dizygotic twins has been improved by modification of the discriminant function, suggesting that further work in this area may yield more favourable results. What could be recommended for the future studies would be the application of less traditional classification procedures, such as learning-based algorithms or discriminant analysis based on different estimation methods. However, in case of Serbian twin samples, it would be highly recommendable to apply these procedures with larger samples of dizygotic twins.

One of the crucial limitations of the study is the sample size. This is the issue that cannot be resolved quickly, however further validity checks of the questionnaire are expected as the number of participants increases. In this study, we relied on the traditional classification procedure based on discriminant function analysis, deliberately choosing not to apply more recent or (arguably) more sophisticated procedures. This decision was in accordance with the aims of this study: as the first validation study of this sort in Serbia, its goal was to test the existing methodology before making any recommendations for future studies.

The study has shown that (at least in Serbian twins) reasonably correct estimation is possible with a relatively small number of reliable indicators which are contained in The Questionnaire of Twins' Physical Resemblance (Onisczenko et al., 1993). This does not mean that more elaborate sets of indicators are unnecessary or unwelcome (it is quite the opposite, for the sake of reliability and validity of estimation). Rather, this result suggests that an experienced researcher, whenever DNA analyses are unavailable, could rely on a small set of features to estimate zygosity with an acceptable error rate. Although this study does not bring a definitive solution to the problem of the questionnaire estimation of zygosity in Serbian twins, it at least highlights the risks that the researchers should be aware of and take into account.

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VALIDACIJA PROCENE ZIGOTNOSTI UPITNIČKIM PUTEM NA UZORKU ODRASLIH BLIZANACA IZ SRBIJE

Najprecizniji metodi kojima se u bihejvioralno - genetičkim studijama procenjuje zigotnost blizanaca jesu procedure DNK analize. Medutim, budući da su za sprovođenje ovakvih analiza potrebna nezanemarljiva finansijska sredstva, istraživači se cesto odlučuju na nesto manje preciznu, ali finansijski znatno manje zahtevnu alternativu - primenu upitnika za procenu zigotnosti. Tačnost klasifikacije, odnosno tačnog prepoznavanja monozigotnih i dizigotnih blizanaca u velikom broju studija prevazilazi 90%, što je dovoljan razlog za njihovu široku primenu. U srpskoj bihejvioralno - genetičkoj studiji za procenu zigotnosti primenjuje se upitnik Oniščenka i saradnika, nazvan ,,Upitnik fizičkih sličnosti medu blizancima”. Pored fizičkih karakteristika, ovaj instrument obuhvata biološke markere zigotnosti i markere koji se odnose na ,,mešanje” blizanaca (pogrešno prepoznavanje jednog kao drugog od strane bliskih i nepoznatih osoba). Osnovni cilj istraživanja prikazanog u ovom radu jeste validacija ovog instrumenta. Osnovna istrazivačka pitanja koja se tom prilikom postavljaju odnose se na primenljivost ,,predefinisanih” diskriminativnih funkcija (s unapred određenim ponderima za indikatore), korišćenih u inostranim studijama, i na mogućnost identifikacije optimalnog seta prediktora zigotnosti na srpskom uzorku. Pri tome, kriterijum za procenu predstavljaju rezultati procene zigotnosti DNK analizom, koji se smatraju maksimalno pouzdanim. U istraživanju su učestvovala 222 para blizanaca istog pola, starosti približno 24 godine, ispitana u okviru blizanačke studije u periodu 2011 - 2018. Kao osnov za procenu zigotnosti, korišćen je upitnik Oniščenka i saradnika, a analitička procedura obuhvatala je evaluaciju kvaliteta predikcije zigotnosti na osnovu četiri diskriminativne funkcije: predefinisane funkcije razvijene u okviru BilSat studije u Nemačkoj, dve slobodno procenjene funkcije s 18, odnosno 24 prediktora, kao i funkcije koja obuhvata indikatore koji su u ovim analizama pokazali najveću diskriminativnu moć. Svaka analiza podrazumevala je unakrsnu validaciju na dva nasumično formirana poduzorka, (N1 = 107, N2 = 115), pri čemu su oba obuhvatila približno 80% monozigotnih i približno 20% dizigotnih blizanaca. Rezultati upućuju na visoku uspešnost predefinisane funkcije u identifikaciji monozigotnih, ali ne i dizigotnih blizanaca. Tačnost klasifikacije povećava se primenom ,,slobodno procenjenih” funkcija, mada procenat tačno identifikovanih dizigotnih blizanaca i dalje nije zadovoljavajući. Skup najboljih indikatora daje najuspešniju predikciju generalno, pri čemu tačnost prelazi 90%, ali prepoznavanje dizigotnih blizanaca pokazuje se kao problem koji tek očekuje zadovoljavajuće rešenje.

Ključne reči: bihejvioralna genetika, blizanačka studija, upitnička procena zigotnosti