ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LXI, N1, 102 SECTIO D 2006

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Searching for endophenotype in schizophrenia – working memory dysfunction in patients with schizophrenia and their first-degree relatives

Although genetic epidemiology studies have provided strong evidence that genetic factors play an important role in the etiology of schizophrenia, however, in view of etiological-genetic and clinical-phenotypical complexity of this disease finding reliable genes liable for the genesis of schizophrenia still remain open questions. The construct that might help in overcoming those difficulties is the term of endo-phenotype, i.e. trait marker of genetic liability, for the first time suggested by Gottesman and Shields in 1967 (2). The current criteria for endophenotype were elaborated on the grounds of Gershon and Golin's suggestions (6): I. Endophenotype must be connected with the disease in general population, i.e. to be characterized by diagnostic specificity. II. Endophenotype should be stable, condition-independent. III. Endophenotype should be inherited. IV. Endophenotype should differentiate the ill from their healthy siblings. V. Among the relatives, when a probationer has got an endophenotype, the very endo-phenotype should be observed more extensively among not diseased members of family than in general population.

At present the 'candidate' to the names of endophenotype markers in schizophrenia among others are thought to be as follows: neurophysiology – antisaccade, smooth – persuit eye movement, P50 auditory ERPs, P300 oddball ERPs; neuroimaging – reduced level of hippocampal NAA/CRE, disturbed activation of dorso-lateral prefrontal cortex (DLPFC), neurocognition – working memory, thought disorder, executive functioning (3). Promising endophenotype candidate that was often researched in schizophrenia was working memory, a capacity-limited system that enables the holding of information while the individuals works on a problem (1). The most frequently used tests applied for measuring this function are Wisconsin Card Sorting Test WCST, Trail Making Test, Stroop Test, Verbal Fluency Test (4, 9, 11). As it appears from the meta-analysis carried out by Szoke et al. (15) concerning existing differences of executive functions in healthy siblings of patients ill with schizophrenia and control group (criterion V for endophenotype) vast heterogeneity of the results mostly gets out of the methodological differences. When considering the researches with the use of WCST there is a possibility of employing at least several possible methods in carrying it out, e.g. classical, Nelson's, Milner's and computerized. It is noteworthy that the Nelson's method and the computerized version of WCST seem to be more sensitive than the classical method in detecting impairments in relatives, but it requires carrying out a vast number of researches using those methods to confirm the above-mentioned thesis (15). That is why carrying out the researches seemed to be interesting, whose methodological bases will be comparing with previously conducted researches, thereby a reliable comparison of final results will be possible.

This study aimed to verify the thesis on endophenotypical character working memory, i.e., its II, III and IV criterion relating to the existence of differences between the group of patients ill with paranoid schizophrenia and the control group (criterion III)

as well as their healthy siblings and the control group (criterion IV), and to explore possible relationships between disturbances in working memory and the data connected with the course of illness (criterion II).

MATERIAL AND METHODS

In our research we used: 1. Socio-demographic Questionnaire, constructed by the authors, containing personal data and the data connected with the disease. 2. The Wisconsin Card Sorting Test_WCST®Computer Version 4 Research Edition (WCST:CV4™) (with instructions in Polish) as a standard test used to assess working memory and executive functions (7). The Raw scores for Percentage of Errors (% E), Perseverative Responses (PR), Percentage of Perseverative Responses (% PR), Perseverative Errors (PE), Percent Perseverative Errors (% PE), Non-Perseverative Errors (NPE), Percentage of Non-Perseverative Errors (% NPE), Number of Categories Completed (CC), Trials to Complete First Category (WCST 1-cat), Percent Conceptual Level Responses (% CONC), Categories Achieved (CA), Total Errors (TE), Percent Failure to Maintain Set (% FTMS) were analyzed. 3. Mini-International Neuropsychiatric Interview (M.I.N.I. PLUS) (14).

Statistical methods. The WCST Raw Scores variables are not normally distributed (according to the Kolmogorov-Smirnov test, p-value < 0.05). Because of that fact nonparametric tests were applied in the research. To test the hypothesis that WCST Raw Scores are equal in the three groups the Mann Whitney U test No assumptions about the shape of populations and equality of standard deviations are required by that test. The U test is a nonparametric alternative to the t-test. As the size of the two samples increases the sampling distribution of the U statistic tends to become normally distributed and the Z statistic may be applied.

To test the influence of the DUP on the WCST scores Spearman r correlation coefficient (r_s) was applied. Spearman r_s can be thought of as the regular Pearson product moment correlation coefficient. Median and Coefficient of Quartile Deviation (V_q) was applied instead of mean and standard deviation.

The group investigated were subjects above eighteen years old with the diagnosis of the paranoid schizophrenia in the phase of the remission (n = 44) according to ICD-10, and their healthy siblings (n = 47) and the control group (N = 22) (mentally healthy and without any history of mental disease in families, without addictions or serious somatic diseases that might influence the results of neuropsychological tests), chosen according to the level of education and age. The members of the control group had been recruited mainly among our Department personnel. The state of the mental status of siblings and controls was rated with the MINI scale.

RESULTS

Table 1. Socio-demographic data and those connected with the disease of the researched groups

	Patients N=44		Siblings N=47		Control N=22	
Sex	N	%	N	%	N	%
Female	15	34.1	24	51.1	9	40.9
Male	29	65.9	23	48.9	13	59.1
	X	SD	X	SD	X	SD
Age (years)	26.73	5.67	26.87	6.83	26.80	6.26
Education (years)	14.71	2.72	14.94	2.92	14.83	2.82
Disease outset (years)	21.03	4.21				
Disease duration (years)	6.52	5.08				
Duration of untreated psychosis (months)	9.60	12.46				

The data obtained for the investigated group were introduced in Table 1. As it has been presented in Table, in the group of patients men outnumbered women like in the control group, whereas in the sibling group the rate of en to men was similar. The researched groups did not significantly differ in age or education.

DIFFERENCES IN PERFORMANCE OF WCST BETWEEN THE RESEARCHED GROUPS

Table 2. Medians and V_Q for the WCST Raw Scores variables in the analysis

		TE	% E	PR	% PE	PE	% PE	NE	% NE	CONC	%CONC	CC	WCST 1-cat
Patients	Me	27.00	24.00	13.00	13.00	12.00	13.00	12.00	11.00	66.00	70.00	6.00	18.00
	V_Q	70%	49%	71%	46%	65%	40%	56%	41%	11%	24%	25%	64%
Siblings	Me	12.00	16.00	6.00	8.00	6.00	8.00	5.50	7.00	64.50	81.50	6.00	11.00
	V_Q	39%	24%	33%	23%	25%	17%	55%	48%	5%	8%	0%	13%
Control	Me	11.00	14.00	5.00	7.00	5.00	7.00	5.00	6.00	64.00	85.00	6.00	12.00
	V_Q	23%	21%	20%	7%	10%	7%	50%	33%	4%	5%	0%	29%

In the first instance criterion IV for endophenotype was verified by comparing obtained results in WCST in the group of patients and their siblings (Table 3). As it results from Table 3, patients with schizophrenia committed more total errors ($p<0.001$), perseverative ($p<0.0001$) and nonperseverative errors ($p<0.004$), succeeded on fewer categories ($p<0.01$), made more perseverative responses ($p<0.001$), needed more trials to succeed at the first category ($p<0.004$) and gave significantly lower conceptual level responses ($p<0.002$) than the siblings did. The only parameters that were not different in both groups were: Conceptual Level Responses, and Failure to Maintain Set.

Table 3. Differences in performance of WCST between the groups of patients and their siblings

	TE	% E	PR	% PR	PE	% P E	NPE	% NPE	CONC	% CONC	CC	WCST 1st	FTMS
U	428.50	435.00	395.00	408.00	404.00	426.00	489.50	527.50	761.00	462.50	577.00	491.50	656.00
Z	-3.438	-3.376	-3.768	-3.648	-3.683	-3.476	-2.852	-2.488	-.223	-3.108	-2.554	-2.862	-1.350
p	.001	.001	.000	.000	.000	.001	.004	.013	.824	.002	.011	.004	.177

The group of patients obtained lower results than the control group in all WCST categories, although in Conceptual Level Responses, RS Failure to Maintain Set, they did not reach statistical significance (Table 4). As it appears from Table 5, the obtained differences on the WCST performance level did not reach statistical significance.

Table 4. Differences in performance of WCST between the group of patients and control group

	TE	% E	RP	% RP	PE	% PE	NPE	% NPE	CONC	% CONC	CC	WCST 1st Cat.	FTMS
U	99.00	93.000	100.50	106.50	104.50	109.50	102.00	106.00	266.00	99.50	170.50	181.50	185.00
Z	-3.259	-3.375	-3.237	-3.124	-3.162	-3.069	-3.205	-3.133	-.067	-3.250	-2.335	-1.689	-1.779
p	.001	.001	.001	.002	.002	.002	.001	.002	.947	.001	.020	.091	.075

Table 5. Differences in performing of WCST between the group of healthy siblings and the control group

	TE	% E	P R	% PR	P E	% PE	NPE	%NPE	CONC	% CONC	CC	WCST 1st Cat	FTMS
U	136,00	135,00	143,50	153,00	141,50	140,50	132,50	128,00	176,00	127,00	154.00	165,50	142,00
Z	-1,117	-1,145	-,916	-,655	-,975	-1,013	-1,222	-1,346	,000	-1,367	-1,215	-,304	-1,104
p	,264	,252	,360	,512	,329	,311	,222	,178	1,000	,172	,224	,761	,269

RELATIONS BETWEEN THE DATA CONNECTED WITH THE COURSE OF THE DISEASE AND THE WCST

In the next stage of work we searched for relations between the data connected with the course of the disease and the WCST performance level. The obtained data show that the age at which first psychotic symptoms appeared had no influence on conducting WCST. However, the duration of untreated psychosis worsened the obtained results. The DUP was recoded into four groups on the basis of the quartiles' rank (Table 6).

Table 6. Relations between the duration of untreated psychosis and performance of WCST

		DUP (quartiles)
Total Errors	Correlation Coefficient	0.252
	Sig. (1-tailed)	0.041
% Errors	Correlation Coefficient	0.261
	Sig. (1-tailed)	0.035
Perseverative Responses	Correlation Coefficient	0.236
	Sig. (1-tailed)	0.051
% Perseverative Responses	Correlation Coefficient	0.235
	Sig. (1-tailed)	0.052
Nonperseverative Errors	Correlation Coefficient	0.248
	Sig. (1-tailed)	0.043
% Nonperseverative Errors	Correlation Coefficient	0.240
	Sig. (1-tailed)	0.048

As it appears from Table 6, increasing duration of untreated psychosis was connected with an enlargened number of errors made, both perseverative and nonperseverative ones, as well as total errors. As it appears from Table 7, the disease duration significantly influenced Conceptual Level Responses and Failure to Maintain Set.

Table 7. Correlations among duration of psychosis and WCST performance factors

	Conceptual Level Responses	Failure to Maintain Set
Chi-Square	9.915	7.491
df	2	2
Asymp. Sig.	0.007	0.024

THE FRACTION OF INDIVIDUALS WITH SCHIZOPHRENIA AND THEIR SIBLINGS, WHO ARE DESIGNATED AS HAVING ABNORMAL WORKING MEMORY

We use in our analysis the definition of abnormal working memory as a value higher than "mean + 2 SD" for controls. For measurement of % Perseverative Errors the mean for control group X = 7.64, SD = 1.96 and Mean +2*SD = 11.56. On the basis of above results 57.1% of patients, 15.6% of siblings and 9% of controls were characterized as having abnormal working memory.

DISCUSSION

The preponderance of data suggests that cognitive deficits in schizophrenia are present more often among affected persons compared with controls (15). The researches conducted in the recent years show that the disturbances of cognitive processes in different intensification appear in case of 62–95% of the ill with schizophrenia, whereas among the healthy people their frequency does not exceed 10% (4, 8) although the fraction of individuals with schizophrenia who are designated as having abnormal working memory varies with the test employed, the clinical population studied, and the definition of abnormal (e.g. 1.5 or 2 standard deviation units below the mean for controls). If consideration is given only to studies of large numbers of cases (about 100) and controls, most reports describe 25% to 50% of persons with schizophrenia as falling in the variably defined deficit range for working memory (2). The data from our study with 51% of patients, 15.6% of siblings and 9% of persons from the control group having abnormal working memory (% PE) confirm other authors' results (2).

Similarly to the majority of researches concerning working memory we obtained the results confirming the criterion IV for the Endo-phenotype for schizophrenia with reference to its parameter, i.e. differences statistically significant between the group of patients and their healthy siblings. In Rybakowski and Borkowska's research comparing the performance of WCST among schizophrenics and first degree relatives (parents) and the control group there were obtained the results indicating the difference between the group of patients and their healthy parents in the following categories: N-P, P, CC and % CONC (12). Similarly, in our investigations we found some differences in the same range. Other authors' research results, however, confirm the lack of differences between both groups (13). The group of healthy siblings in our research did not vary in statistically significant way in comparison with the control group, in contrast to the above-mentioned authors' results, in the research of those, the healthy parents differed from the control group in WCST CC, % CONC. In Saud et al's work those differences reached statistical significance in relation to WCST % P (13).

In our research we found the existence of differences on the level of WCST (TE, %T, PR, % PR, NE, % NE) performance, but in reference to no category did the result appear to be statistically significant. Consequently, in our research the criterion V for endophenotypical marker

was not fulfilled with reference to the differences between the group of siblings and the control group. Maybe the increase of the number of researched groups, by increasing statistical discrimination power of the evaluations would enable us to find statistically significant differences. However as it appears in Szoke et al.'s meta-analysis, relatives of schizophrenic patients have wide, although not severe, impairment of executive functions and the sensitivity to different tests for impairments is not the same (15). Their research results suggest that fluency tests are more sensitive to impairments in relatives of schizophrenic patients than the other three tests: WCST, Stroop and TMT. In our research the duration of untreated psychosis had the influence on the level of WCST performance. The similar results were obtained by Galińska et al. (5). In contrast to the other authors results (13) deficits exhibited by the group with schizophrenia in our investigation were related to the duration of illness. As a matter of fact, the relations concerning psychosis durations are not numerous and include two measurements of WCST: Conceptual Level Responses and Failure to Maintain Set, but they doubt about fulfilling criterion II for endophenotype – the feature independent of state. The authors of the research fully acknowledged the limitations of the research (small groups), which could lead to false-negative results because of limited statistical power. In conclusion we should emphasize that though working memory is one of the most important 'candidates' for the name of endophenotypical marker for schizophrenia, yet the choice of tools of measurement seems to play a crucial role.

CONCLUSIONS

1. Patients with schizophrenia perform comparing to their siblings and control subjects significant worst on the Wisconsin Card Sorting Test (WCST), not only on the "classical" WCST measurements (perseverative errors and number of categories), as well as on more rarely reported scores. 2.We found differences between siblings and control subjects, but the differences were not statistically significant. 3. The duration of untreated psychosis has the influence on the level of WCST execution. 4. 57.1% of patients, 15.6% of siblings and 9.0% of controls were characterized as having abnormal working memory (in % Perseverative Errors). 5.The criterion IV was fulfilled in full for endophenotype with reference to working memory in schizophrenia, whereas II and V were not confirmed in our researches.

Acknowledgement. This work was supported by Polish Committee of Scientific Research (KBN) Grant No. 3 P05B 030 24.

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SUMMARY

This study aimed to verify the thesis on endophenotypical character working memory, i.e., its II, III and IV criterion relating to the existence of differences between the group of paranoid schizophrenia patients and the control group (criterion III) as well as between their healthy siblings and the control group (criterion IV), to explore possible relationships between disturbances in working memory and the data connected with the course of illness (criterion II). Subjects were 44 patients with the diagnosis of the paranoid schizophrenia in the phase of the remission, their 47 healthy siblings and 22 controls chosen according to the level of education and age. We used: WCST® Computer Version 4 Research Edition and Mini-International Neuropsychiatric Interview. Patients with schizophrenia comparing to their siblings and control subjects, achieve significantly worse results on the Wisconsin Card Sorting Test (WCST), not only on the "classical" WCST measurements (perseverative errors and number of categories completed), but also on more rarely reported scores. 2) We found differences between siblings and control subjects, but the differences were not statistically significant. 3) The duration of untreated psychosis and duration of illness have the influence on the level of WCST performance. 4) 57.1% of patients, 15.6% of siblings and 9.0% of controls were characterized as having abnormal working memory (in % of Perseverative Errors). 5) The criterion IV was totally fulfilled for endophenotypicality with reference to working memory in schizophrenia, whereas II and V were not confirmed in our researches.

W poszukiwaniu endofenotypu dla schizofrenii – dysfunkcja pamięci operacyjnej u osób chorujących na schizofrenię i ich krewnych pierwszego stopnia

Celem naszego badania była weryfikacja tezy o endofenotypowym charakterze pamięci roboczej, tj. jej II, III i IV kryterium dotyczącego istnienia różnic pomiędzy grupą pacjentów chorych na schizofrenię paranoidalną a grupą kontrolną (kryterium III), ich zdrowym rodzeństwem i grupą kontrolną (kryterium IV) oraz poszukiwanie związków pomiędzy zaburzeniami w funkcjonowaniu pamięci roboczej i danymi związanymi z przebiegiem choroby (kryterium II). Badaniami przy pomocy WCST objęto grupę 44 pacjentów ze schizofrenią paranoidalną wg ICD-10 w fazie remisji, ich zdrowe psychicznie rodzeństwo (N= 47) oraz grupę kontrolną (N=22). Istotnie gorsze wyniki wykonania WCST przez pacjentów ze schizofrenią w porównaniu ze zdrowym rodzeństwem dotyczą nie tylko „ klasycznych'' pomiarów WCST (tj.

procentu popełnionych błędów perseweracyjnych i liczby poprawnie ułożonych kategorii), ale także rzadziej opisywanych pomiarów tego testu. Stwierdzono istnienie różnic pomiędzy grupą rodzeństwa i kontrolną w poziomie wykonania WCST, ale te różnice nie osiągnęły istotności statystycznej. Długość nieleczonej psychozy i trwania choroby miały wpływ na wyniki WCST. U 51 % pacjentów, 15,6% rodzeństwa i 9% osób z grupy kontrolnej stwierdzono nieprawidłowe wyniki wykonania WCST (% błędów perseweracyjnych). W pełni zostało spełnione kryterium IV endofenotypowości w odniesieniu do *working memory* w schizofrenii, II zaś i V nie znalazło potwierdzenia w naszych badaniach.