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The influence of RhD phenotype on toxoplasmosis- and age-associated changes in personality profile of blood donors

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Abstract: *Toxoplasma gondii*, a parasite infecting 20–60% of humans in various countries, influences the behaviour of infected animal and human hosts. Infected human subjects have changes in several of Cattell's and Cloninger's personality factors. Recently, three independent studies have shown that Rh-positive subjects are protected against the *T. gondii*-induced changes of reaction times and increased risk of traffic accidents. Here we searched for evidence of similar effects of RhD phenotype on toxoplasmosis- or aging-associated changes in the personality profile of about 302 blood donors. We found that Rh-positive and Rh-negative subjects responded differently to toxoplasmosis. In addition to the already known effects of toxoplasmosis on novelty seeking, self transcendence, superego strength and protension, we also found effects of RhD phenotype on ego strength, protension, and praxernia, as well as opposite effects of toxoplasmosis on ego strength, praxernia, ergic tension and cooperativeness in Rh-positive and Rh-negative subjects. Moreover, our results indicate that RhD phenotype might influence not only the effect of toxoplasmosis but also the effect of aging on specific personality traits.

Keywords: parasite, aging, manipulation, personality, blood group, Rh factor, Cattell 16PF, Cloninger TCI, Rhesus factor, aging, senescence

The RhD protein which is the *RHD* gene product and a major component in the Rh blood group system carries the strongest blood group immunogen, the D antigen (Carritt et al. 1997, Flegel 2006). This antigen is absent in a significant minority (about 16%) of the Caucasian population (Rh-negatives) due to *RHD* deletion or alternation. The structure homology data suggest that the RhD protein acts as an ion pump of uncertain specificity and unknown physiological role (Biver et al. 2006, Kustu and Inwood 2006). Except for the well known but artificial role of the RhD protein in haemolytic disease of newborns, no phenotypic expression of the absence or presence of RhD on the surface of erythrocytes of a subject was known for more than sixty years. Recently, two studies on two populations of blood donors, one of conscripts (Novotná et al. 2008) and the other of university students (Flegr et al. 2008), have shown that Rh-positive subjects, and RhD-positive heterozygotes in particular, are protected against latent toxoplasmosis-induced impairment of reaction times. A prospective cohort study performed on nearly four thousand military drivers has found that Rh-negative *Toxoplasma*-infected subjects have about three times higher probability of a traffic accident than Rh-negative *Toxoplasma*-free subjects or than Rh-positive (*Toxoplasma*-free or *Toxoplasma*-infected) subjects (Flegr et al. 2009). Another study performed on 980 pregnant women has found the RhD-positivity protects *Toxoplasma*-infected women against excessive weight gain in the first trimester of pregnancy (Kaňková et al. 2010).

The protozoan parasite *Toxoplasma gondii* infects 20–60% of the population in most countries, depending on climate, hygienic standards and cooking habits (Tenter et al. 2000). Postnatally acquired toxoplasmosis in immunocompetent subjects causes usually only mild disease, acute toxoplasmosis, which turns spontaneously into lifelong latent toxoplasmosis. Latent toxoplasmosis is characterized by the presence of the dormant cyst stage of the parasite mainly in the neural and muscular tissues and immunity against new *Toxoplasma* infections (Remington and Krahenbuhl 1982, Jones et al. 2001). Latent toxoplasmosis in humans is considered as clinically asymptomatic (Markell et al. 1999, Roberts and Janovy 2000). However, infected people seem to have impaired reaction times (Havlíček et al. 2001) and a higher risk of traffic accidents (Flegr et al. 2002, Yereli et al. 2006, Kocazeybek et al. 2009), possibly as a result of manipulation activity of *T. gondii* "aimed" to increase the chance of transmission from the intermediate to the definitive host, i.e. from any bird or mammal species to any feline species, by predation. Between 1994 and 2007, about twelve

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studies have reported that *Toxoplasma*-infected men and women differed in several personality factors measured with Cattell's 16PF questionnaire or Cloninger's TCI questionnaire from *Toxoplasma*-free subjects (see Flegr 2007 for review). The difference in personality traits increases with the duration of the infection, which suggests that the changes are induced by toxoplasmosis rather than that the risk of *Toxoplasma* infection is influenced by the personality profile of subjects (Flegr et al. 2000). Moreover, the results of about forty years of research performed on laboratory animals have shown that *T. gondii* is able to specifically influence the behaviour and temper of its intermediate hosts (Webster 2001), probably by regulating the concentration of neurotransmitters, dopamine and possibly also serotonin, in particular regions of the brain (Skallová et al. 2006, Hodková et al. 2007). It has even been recently reported that the genome of *T. gondii* contains two genes for aromatic amino acid hydroxylases, the enzymes not previously described in the protozoa, and these enzymes probably catalyze the rate-limiting step of the biosynthesis of dopamine in the brain of infected hosts (Gaskell et al. 2009).

The aims of the present study were 1) to find out whether Rh-positive subjects are protected not only against the impairment of reaction times and excessive weight gain in pregnancy but also against the toxoplasmosis-associated specific changes in personality traits and 2) to find whether RhD phenotype modifies not only the host response to *Toxoplasma* infection but also to other biological factors, namely the age of the host.

MATERIALS AND METHODS

Subjects. During the thrombocyte separation sessions, about eight hundred donors of thrombocytes at the blood transfusion centres of the Institute of Haematology and Blood Transfusion, Prague, and of the Zbraslav hospital, Prague, were asked to voluntarily participate in the research project and to sign the informed consent form. Five hundred and thirty-one (about 70%) of them consented to provide their psychological data and 5 ml of blood for serological examination. They were given the Cloninger's TCI and Cattell's 16PF questionnaire forms and a stamped envelope with the address of the Department of Parasitology, Faculty of Science, Charles University on it. Three hundred and two TCI questionnaire forms (57.2%) and 262 16PF questionnaire forms (49.4%) were filled in and returned. The recruitment of subjects and the data handling complied with the applicable regulations.

Personality tests. TCI (Temperament and Character Inventory) (Cloninger et al. 1994): The Czech translation of the TCI questionnaire (Kožený and Tisanská 1998) was used, with the translation of two questions being corrected and Cloninger's validation scale being substituted with Eysenck's Lie scale EP/R. The final questionnaire (Preiss and Klose 2003) contained all 238 TCI items and 12 Lie scale items. The subjects received written instructions to answer YES or NO for each item depending on whether or not the sentence in the questionnaire characterized their usual and most probable behaviour or feelings in the given situations.

Serological tests. Serological tests for toxoplasmosis were carried out in the National Reference Laboratory for Toxoplasmosis of the National Institute of Public Health, Prague, and serological tests for RhD and ABO blood group type were performed at the Institute of Haematology and Blood Transfusion, Prague. Specific anti-*Toxoplasma* IgG and IgM antibody concentrations were determined by ELISA (IgG: SEVAC, Prague; IgM: TestLine, Brno) optimized for early detection of acute toxoplasmosis (Pokorný et al. 1989) and by the complement fixation test (CFT) (SEVAC, Prague) which is more sensitive and therefore more suitable for the detection of old *T. gondii* infection (Warren and Sabin 1942). Titres of antibodies to *Toxoplasma* in sera were measured at dilutions between 1:8 and 1:1024. The subjects with negative results of IgM ELISA (positivity index < 0.9) and CFT titres higher than 1:8 were considered latent-toxoplasmosis positive.

Statistical analysis. The software Statistica® version 9.0 was used for General Linear Models (GLM) tests, log-linear analysis and for testing the assumptions, namely those of the normality of data distribution, normality of residuals and equality of error variances.

RESULTS

Three hundred and two blood donors (213 men and 89 women) were tested for specific immunity against *Toxoplasma*. The prevalence rates of *Toxoplasma* infection in men and women were 32.9% and 27.0%, respectively, and the rates of Rh-negative phenotype were 26.9% and 22.4%, respectively. The very high proportion of Rh-negative subjects reflected the higher demand for Rh-negative blood and therefore preferential selection of Rh-negative donors rather than the frequency of the *RHD* deletion in the general Czech population. No association between sex, RhD phenotype, ABO phenotype and *Toxoplasma* infection was found by the log-linear analysis.

Cloninger's TCI

The multivariate GLM with toxoplasmosis, RhD phenotype and age as three independent factors and with seven Cloninger's factors as dependent variables showed significant effects of age ($p < 0.0001$), RhD phenotype ($p = 0.011$), toxoplasmosis ($p = 0.011$) and toxoplasmosis-RhD interaction ($p = 0.001$). The univariate GLM analyses showed the effect of toxoplasmosis on novelty seeking (NS) ($p = 0.048$) and self transcendence (ST) ($p = 0.024$), the effect of RhD phenotype on harm avoidance (HA) ($p = 0.036$) and cooperativeness (CO) ($p = 0.009$) and the effect of toxoplasmosis-RhD interaction on reward dependence (RD) ($p = 0.046$) and cooperativeness (CO) ($p = 0.007$). For the strength and direction of the effects, see Table 1. The multivariate GLM analysis performed on a subset of men showed the effect of age ($p < 0.0001$), a non-significant effect of toxoplasmosis ($p = 0.061$) and the effect of RhD phenotype ($p = 0.013$) and of toxoplasmosis-RhD interaction ($p < 0.0001$). The multivariate GLM analysis performed on a subset of women found the effect of age ($p = 0.007$) and no significant effect of

Table 1. Effects of latent toxoplasmosis and RhD phenotype on Cloninger's personality factors. The table shows the significance (two-sided p), effect size (η^2) estimated by the univariate GLM and arithmetic means in particular subsets. The results significant in two-tailed tests are printed in bold. NS – novelty seeking, HA – harm avoidance, RD – reward dependence, SD – self-directedness, CO – cooperativeness, ST – self-transcendence, PE – persistence.

		All						Men						Women					
		p	η^2	toxo$^-$ RhD$^-$	toxo$^-$ RhD$^+$	toxo$^+$ RhD$^-$	toxo$^+$ RhD$^+$	p	η^2	toxo$^-$ RhD$^-$	toxo$^-$ RhD$^+$	toxo$^+$ RhD$^-$	toxo$^+$ RhD$^+$	p	η^2	toxo$^-$ RhD$^-$	toxo$^-$ RhD$^+$	toxo$^+$ RhD$^-$	toxo$^+$ RhD$^+$
NS	toxo	**0.048**	0.01	20.240	19.772	20.273	16.479	0.505	0.00	18.706	19.087	20.316	16.451	**0.050**	0.047	23.50	21.30	20.00	16.55
	RhD	**0.053**	0.01					0.256	0.01					0.153	0.025				
	toxo×RhD	0.195	0.01					0.109	0.01					0.875	0.00				
HA	toxo	0.136	0.01	14.860	14.430	17.545	14.521	0.098	0.01	14.059	14.427	18.211	13.706	0.729	0.00	16.563	14.435	13.333	16.600
	RhD	**0.036**	0.02					**0.030**	0.02					0.802	0.00				
	toxo×RhD	0.094	0.01					**0.013**	0.03					0.272	0.02				
RD	toxo	0.250	0.00	14.320	15.168	15.864	14.817	**0.020**	0.03	13.324	14.602	16.053	14.510	0.255	0.02	16.438	16.435	14.667	15.600
	RhD	0.715	0.00					0.636	0.00					0.701	0.00				
	toxo×RhD	**0.046**	0.01					**0.008**	0.03					0.748	0.00				
SD	toxo	0.502	0.00	27.420	26.510	25.955	29.338	0.828	0.00	28.471	26.155	25.789	29.216	0.418	0.01	25.188	27.304	27.000	29.650
	RhD	0.330	0.00					0.882	0.00					0.338	0.01				
	toxo×RhD	0.069	0.01					**0.032**	0.02					0.943	0.00				
CO	toxo	0.147	0.01	29.260	29.181	25.727	30.254	0.771	0.00	28.412	28.262	25.474	30.588	0.154	0.03	31.063	31.239	27.333	29.400
	RhD	**0.010**	0.02					**0.018**	0.03					0.545	0.00				
	toxo×RhD	**0.007**	0.02					**0.011**	0.03					0.565	0.00				
ST	toxo	**0.024**	0.02	15.740	14.329	13.318	12.746	0.115	0.01	14.765	14.282	12.526	13.333	0.530	0.00	17.813	14.435	18.333	11.250
	RhD	0.335	0.00					0.766	0.00					**0.008**	0.08				
	toxo×RhD	0.520	0.00					0.451	0.00					0.379	0.01				
PE	toxo	0.929	0.00	4.400	4.638	4.727	4.366	0.535	0.00	4.618	4.553	4.579	4.196	0.225	0.02	3.938	4.826	5.667	4.800
	RhD	0.936	0.00					0.596	0.00					0.985	0.00				
	toxo×RhD	0.389	0.00					0.726	0.00					0.237	0.02				
n				50	149	22	71			34	103	19	51			16	46	3	20

Table 2. Effects of latent toxoplasmosis on Cloninger's personality factors. The table shows the significance (two-sided p), effect size (η^2) estimated by the univariate GLM and arithmetic means in particular subsets. The results significant in two-tailed tests are printed in bold. For abbreviations see Table 1.

	All				Men				Women			
	p	η^2	toxo$^-$	toxo$^+$	p	η^2	toxo$^-$	toxo$^+$	p	η^2	toxo$^-$	toxo$^+$
NS	**0.002**	0.033	19.889	17.372	0.112	0.012	18.937	17.500	0.001	0.115	21.985	17.000
HA	0.411	0.002	14.548	15.319	0.557	0.002	14.336	14.929	0.510	0.005	15.015	16.458
RD	0.910	0.000	14.942	15.043	0.247	0.006	14.301	14.929	0.195	0.019	16.354	15.375
SD	0.084	0.010	26.793	28.457	0.167	0.009	26.769	28.286	0.251	0.015	26.846	28.958
CO	0.955	0.000	29.202	29.191	0.327	0.005	28.301	29.200	0.157	0.023	31.185	29.167
ST	**0.016**	0.019	14.750	12.872	0.134	0.011	14.483	13.114	**0.034**	0.051	15.338	12.167
PE	0.665	0.001	4.572	4.447	0.386	0.004	4.559	4.300	0.533	0.005	4.600	4.875
n			208	94			143	70			65	24

Table 3. Effects of RhD phenotype on Cloninger's personality factors. The table shows the significance (two-sided p), effect size (η^2) estimated by the univariate GLM and arithmetic means in particular subsets. The results significant in two-tailed tests are printed in bold. For abbreviations see Table 1.

	All				Men				Women			
	p	η^2	RhD$^-$	RhD$^+$	p	η^2	RhD$^-$	RhD$^+$	p	η^2	RhD$^-$	RhD$^+$
NS	0.113	0.009	20.289	18.733	0.491	0.002	19.404	18.252	**0.040**	0.051	22.947	19.864
HA	0.099	0.009	15.711	14.443	0.113	0.012	15.596	14.168	0.606	0.003	16.053	15.091
RD	0.794	0.000	14.895	15.068	0.956	0.000	14.474	14.594	0.957	0.000	16.158	16.182
SD	0.707	0.000	26.868	27.389	0.616	0.001	27.333	27.123	0.172	0.023	25.474	28.015
CO	0.092	0.010	28.184	29.507	0.117	0.012	27.421	29.006	0.912	0.000	30.474	30.682
ST	0.219	0.005	14.868	13.787	0.853	0.000	13.860	13.923	**0.003**	0.101	17.895	13.470
PE	0.792	0.000	4.500	4.543	0.673	0.001	4.596	4.426	0.273	0.015	4.211	4.818
n			76	221			57	155			19	66

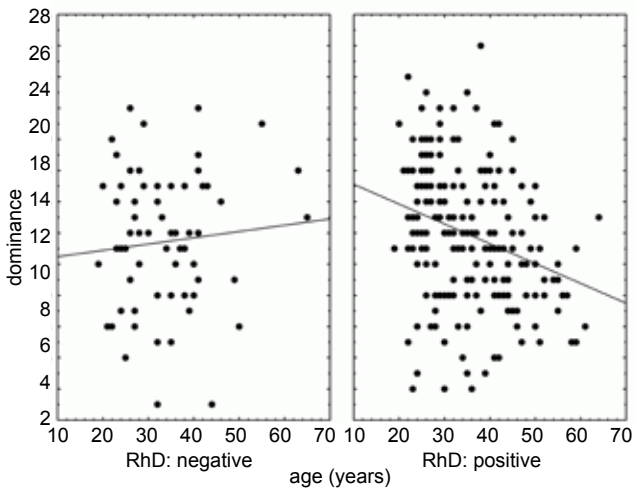

Fig. 1. Correlation between age and Cattell's dominance (E) in Rh-negative and Rh-positive subjects. The statistical significance of the RhD-age interaction estimated by the univariate GLM was 0.006.

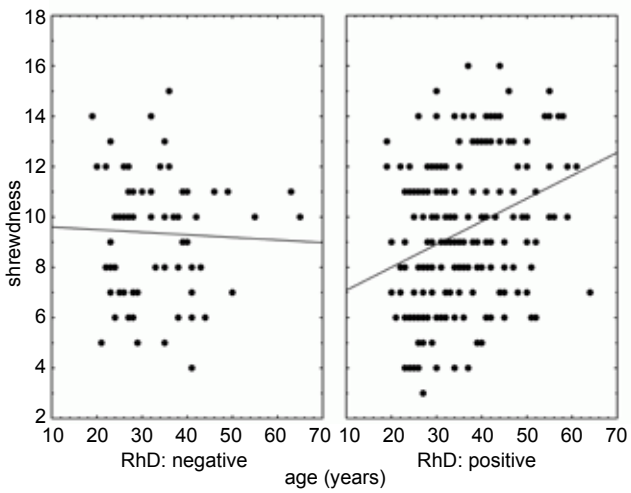

Fig. 2. Correlation between age and Cattell's shrewdness (N) in Rh-negative and Rh-positive subjects. The statistical significance of the RhD-age interaction estimated by the univariate GLM was 0.014.

toxoplasmosis ($p=0.137$), RhD phenotype ($p=0.075$) and toxoplasmosis-RhD interaction ($p=0.851$). The univariate GLM analyses performed separately for men and women showed that except for novelty seeking (NS) and self transcendence (ST), the particular effects of toxoplasmosis and RhD phenotype were stronger in men than women (Table 1).

The multivariate GLM with independent factors toxoplasmosis and age showed the effect of age ($p<0.0001$) and toxoplasmosis ($p=0.025$) for the whole data set, the effect of age ($p<0.0001$) and a non-significant effect of toxoplasmosis ($p=0.347$) for the subset of men, and the effect of age ($p=0.011$) and toxoplasmosis ($p=0.007$) for the subset of women. The multivariate GLM analyses with independent factors RhD phenotype and age showed the effect of age (all: $p<0.0001$, men: $p<0.0001$, women: $p<0.0001$) and the effect of RhD phenotype in women (all: $p=0.079$, men: $p<0.149$, women: $p<0.003$). Results of particular univariate GLM analyses are shown in Tables 2 and 3.

Cattell's 16PF

The multivariate GLM with toxoplasmosis, RhD phenotype and age as three independent factors and sixteen Cattells's factors as dependent variables showed the effects of age ($p<0.0001$), RhD phenotype ($p=0.020$), toxoplasmosis ($p=0.002$) and toxoplasmosis-RhD interaction ($p<0.001$). The univariate GLM analyses with independent factors toxoplasmosis, RhD phenotype and age showed the effect of toxoplasmosis on superego strength (G) ($p=0.001$), protension (L) ($p=0.011$) and shrewdness (N) ($p=0.026$), the effect of RhD phenotype on ego weakness (C) ($p=0.005$), protension (L) ($p=0.017$) and praxernia (M) ($p=0.033$) and the effect of toxoplasmosis-RhD interaction on ego weakness (C) ($p=0.003$), praxernia (M) ($p=0.005$) and ergic tension (Q_4) ($p=0.011$). For the strength and direction of the effects, see Table 4. Latent toxoplasmosis is known to have opposite effects on many Cattell's factors in man and women and therefore we repeated the same analysis separately for men and women. The multivariate GLM analysis performed on the subset of men showed the effects of age ($p<0.0001$), toxoplasmosis ($p=0.002$), RhD phenotype ($p=0.002$) and toxoplasmosis-RhD interaction ($p<0.001$). The multivariate GLM analysis performed on the subset of women did not find significant effects of age ($p=0.054$), toxoplasmosis ($p=0.326$), RhD phenotype ($p=0.618$) and toxoplasmosis-RhD interaction ($p=0.851$). For particular results of the univariate GLM analyses, see Table 4.

The multivariate GLM analyses with independent factors toxoplasmosis and age showed the effect of age (all: $p<0.0001$, men: $p<0.0001$, women: $p<0.0001$) and no effect of toxoplasmosis (all: $p=0.118$, men: $p=0.088$, women: $p=0.504$). The multivariate GLM analyses with independent factors RhD phenotype and age showed the effect of age (all: $p<0.0001$, men: $p<0.0001$, women: $p<0.0001$) and the effect of RhD phenotype in men (all: $p=0.082$, men: $p=0.036$, women: $p=0.262$). For particular results of the univariate GLM analyses, see Tables 5 and 6.

To reveal possible protective effects of RhD phenotype against biological factors other than toxoplasmosis, we included the RhD-age interaction into our models. The multivariate GLM analyses showed no significant effect of RhD-age interaction on Cattell's factors (all: $p=0.189$, men: $p=0.326$, women: $p=0.657$) or Cloninger's factors (all: $p=0.710$, men: $p=0.434$, women: $p=0.752$). However, the univariate GLM analyses showed the effect of RhD phenotype-age interaction on dominance (E) (all: $p=0.006$, men: $p=0.060$, women: $p=0.024$), see Fig. 1,

Table 4. Effects of latent toxoplasmosis and RhD phenotype on Cattell's personality factors. The table shows the significance (two-sided p), effect size (η^2) estimated by the univariate GLM and arithmetic means and number of cases in particular subsets. The results significant in two-tailed tests are printed in bold. A – afectothimia/schizothimia, B – low intelligence/high intelligence, C – ego weakness/high ego strength, E – submissiveness/dominance, F – desurgency/surgency, G – low superego strength/high superego strength, H – threctia/parmia, I – harria/premsia, L – alaxia/protension, M – praxernia/autia, N – naivete/shrewdness, O – untroubled adequacy/guilt proneness, Q_1 – conservatism/radicalism, Q_2 – group dependency/self sufficiency, Q_3 – low self-sentiment integration/ high strength of self-sentiment, Q_4 – low ergic tension/high ergic tension.

		All						Men						Women					
		p	η^2	toxo$^-$ RhD$^-$	toxo$^-$ RhD$^+$	toxo$^+$ RhD$^-$	toxo$^+$ RhD$^+$	p	η^2	toxo$^-$ RhD$^-$	toxo$^-$ RhD$^+$	toxo$^+$ RhD$^-$	toxo$^+$ RhD$^+$	p	η^2	toxo$^-$ RhD$^-$	toxo$^-$ RhD$^+$	toxo$^+$ RhD$^-$	toxo$^+$ RhD$^+$
A	toxo	0.618	0.00	10.707	11.060	11.857	10.403	0.245	0.01	10.000	10.217	11.556	9.958	0.927	0.00	12.071	12.951	13.667	11.526
	RhD	0.359	0.00					0.274	0.01					0.595	0.00				
	toxo×RhD	0.101	0.01					0.128	0.01					0.197	0.02				
B	toxo	0.173	0.01	8.390	8.068	7.524	7.836	0.248	0.01	8.407	8.163	7.833	7.667	0.203	0.02	8.357	7.854	5.667	8.263
	RhD	0.747	0.00					0.856	0.00					0.154	0.03	7.854			
	toxo×RhD	0.291	0.00					0.783	0.00					**0.045**	0.05				
C	toxo	0.145	0.01	14.439	14.323	11.810	15.194	0.100	0.01	15.074	14.337	11.667	15.500	0.890	0.00	13.214	14.293	12.667	14.421
	RhD	**0.005**	0.03					**0.017**	0.03					0.298	0.02				
	toxo×RhD	**0.003**	0.03					**0.001**	0.06					0.800	0.00				
E	toxo	0.976	0.00	13.171	14.053	13.762	13.358	0.848	0.00	12.444	14.370	13.444	13.708	0.916	0.00	14.571	13.341	15.667	12.474
	RhD	0.449	0.00					**0.048**	0.02					0.135	0.03				
	toxo×RhD	0.501	0.00					0.506	0.00					0.506	0.01				
F	toxo	0.274	0.00	11.951	12.617	11.095	11.836	0.795	0.00	10.407	12.283	10.333	12.000	0.877	0.00	14.929	13.366	15.667	11.421
	RhD	0.080	0.01					**0.005**	0.04					0.062	0.05				
	toxo×RhD	0.543	0.00					0.699	0.00					0.400	0.01				
G	toxo	**0.001**	0.04	11.171	11.331	13.619	12.448	**0.008**	0.04	11.148	11.152	13.500	12.250	0.064	0.05	11.214	11.732	14.333	12.947
	RhD	0.157	0.01					0.138	0.01					0.630	0.00				
	toxo×RhD	0.113	0.01					0.172	0.01					0.356	0.01				
H	toxo	0.310	0.00	11.829	12.586	10.857	11.582	0.798	0.00	10.741	12.174	10.667	11.750	0.337	0.01	13.929	13.512	12.000	11.158
	RhD	0.231	0.01					0.113	0.01					0.804	0.00				
	toxo×RhD	0.774	0.00					0.860	0.00					0.946	0.00				
I	toxo	0.708	0.00	9.439	8.977	8.143	9.806	0.792	0.00	8.037	7.848	7.333	8.917	0.533	0.01	12.143	11.512	13.000	12.053
	RhD	0.406	0.00					0.449	0.00					0.470	0.01				
	toxo×RhD	0.117	0.01					0.286	0.01					0.882	0.00				
L	toxo	**0.011**	0.03	12.098	10.511	10.524	9.597	0.135	0.01	11.222	10.565	10.611	9.521	**0.052**	0.05	13.786	10.390	10.000	9.789
	RhD	**0.017**	0.02					0.148	0.01					0.105	0.04				
	toxo×RhD	0.406	0.00					0.760	0.00					0.121	0.03				
M	toxo	0.580	0.00	11.854	11.511	10.143	12.687	0.976	0.00	11.704	11.772	10.167	13.333	0.384	0.01	12.143	10.927	10.000	11.053
	RhD	**0.033**	0.02					**0.007**	0.04					0.957	0.00				
	toxo×RhD	**0.005**	0.03					**0.008**	0.04					0.307	0.01				
N	toxo	**0.026**	0.02	8.902	9.286	10.286	9.821	0.083	0.02	9.111	9.196	10.333	9.604	0.287	0.02	8.500	9.488	10.000	10.368
	RhD	0.553	0.00					0.241	0.01					0.554	0.00				
	toxo×RhD	0.169	0.01					0.218	0.01					0.706	0.00				
O	toxo	0.579	0.00	10.195	10.323	11.333	9.896	0.459	0.00	9.593	9.913	11.222	9.313	0.854	0.00	11.357	11.244	12.000	11.368
	RhD	0.297	0.00					0.272	0.01					0.783	0.00				
	toxo×RhD	0.215	0.01					0.117	0.01					0.850	0.00				
Q_1	toxo	0.775	0.00	7.073	7.090	7.714	6.657	0.330	0.01	6.778	7.163	7.944	7.042	0.192	0.02	7.643	6.927	6.333	5.684
	RhD	0.441	0.00					0.925	0.00					0.497	0.01				
	toxo×RhD	0.359	0.00					0.358	0.00					0.924	0.00				
Q_2	toxo	0.341	0.00	12.146	11.436	10.810	11.731	0.071	0.02	12.630	12.065	11.000	11.458	0.749	0.00	11.214	10.024	9.667	12.421
	RhD	0.932	0.00					0.840	0.00					0.551	0.00				
	toxo×RhD	0.166	0.01					0.460	0.00					0.134	0.03				
Q_3	toxo	0.652	0.00	11.707	11.977	11.381	12.761	0.796	0.00	12.222	12.152	11.222	12.854	0.274	0.02	10.714	11.585	12.333	12.526
	RhD	0.136	0.01					0.211	0.01					0.668	0.00				
	toxo×RhD	0.320	0.00					0.158	0.01					0.720	0.00				
Q_4	toxo	0.145	0.01	12.024	12.910	14.810	12.104	**0.013**	0.03	10.815	12.543	15.444	11.729	0.208	0.02	14.357	13.732	11.000	13.053
	RhD	0.209	0.01					0.217	0.01					0.655	0.00				
	toxo×RhD	**0.011**	0.03					**0.001**	0.06					0.402	0.01				
n				41	133	21	67			27	92	18	48			14	41	3	19

Table 5. Effects of latent toxoplasmosis on Cattell's personality factors. The table shows the significance (two-sided p), effect size (η^2) estimated by the univariate GLM and arithmetic means in particular subsets. The results significant in two-tailed tests are printed in bold. For abbreviations see Table 4.

	All				Men				Women			
	p	η^2	toxo$^-$	toxo$^+$	p	η^2	toxo$^-$	toxo$^+$	p	η^2	toxo$^-$	toxo$^+$
A	0.707	0.001	10.942	10.778	0.577	0.002	10.139	10.406	0.287	0.015	12.740	11.843
B	0.304	0.004	8.141	7.802	0.238	0.007	8.201	7.728	0.948	0.000	8.002	8.041
C	0.930	0.000	14.351	14.396	0.922	0.000	14.521	14.461	0.828	0.001	13.978	14.193
E	0.679	0.001	13.771	13.537	0.833	0.000	13.828	13.687	0.490	0.006	13.694	12.961
F	0.261	0.005	12.468	11.805	0.662	0.001	11.913	11.603	0.212	0.021	13.698	12.405
G	**0.005**	0.030	11.335	12.655	**0.018**	0.029	11.189	12.554	0.084	0.039	11.640	13.008
H	0.279	0.004	12.372	11.511	0.719	0.001	11.845	11.503	0.149	0.028	13.571	11.456
I	0.581	0.001	9.080	9.382	0.397	0.004	7.932	8.461	0.489	0.006	11.613	12.167
L	**0.014**	0.023	10.863	9.847	0.061	0.019	10.722	9.826	0.137	0.029	11.168	9.937
M	0.250	0.005	11.579	12.066	0.129	0.012	11.708	12.458	0.688	0.002	11.272	10.944
N	**0.058**	0.013	9.200	9.876	0.151	0.011	9.182	9.779	0.192	0.023	9.237	10.170
O	0.987	0.000	10.249	10.240	0.889	0.000	9.755	9.839	1.000	0.000	11.372	11.370
Q_1	0.728	0.000	7.091	6.961	0.643	0.001	7.100	7.311	0.066	0.044	7.077	5.896
Q_2	0.904	0.000	11.548	11.492	0.136	0.012	12.117	11.325	0.069	0.044	10.274	12.030
Q_3	0.242	0.005	11.916	12.398	0.657	0.001	12.183	12.399	0.178	0.024	11.331	12.385
Q_4	0.935	0.000	12.723	12.771	0.404	0.004	12.188	12.753	0.316	0.013	13.929	12.773
n			180	88			124	66			56	22

and shrewdness (N) (all: p=0.014, men: p=0.007, women: p=0.558), see Fig. 2.

As a sort of negative control, we repeated all analyses with ABO phenotype used instead of RhD phenotype. The multivariate GLM analysis revealed no significant effect of ABO phenotype or ABO phenotype-toxoplasmosis interaction (results not shown). Of 69 univariate analyses (7 Cloninger's and 16 Cattell's factors, all subjects, men, women), only two, the tests of reward dependence (RD) in all subjects (p=0.048) and premsia (I) in all subjects (p=0.029), revealed significant effects of the studied factors (ABO phenotype-toxoplasmosis interaction). These effects were not significant after the Bonferroni correction for multiple tests.

DISCUSSION

"Asymptomatic" latent toxoplasmosis and RhD phenotype, i.e. the presence or absence of the D antigen on the membrane of erythrocytes, had a specific effect on the personality profile of blood donors estimated by Cattell's 16PF questionnaire and Cloninger's TCI questionnaire. Moreover, toxoplasmosis had both quantitatively and qualitatively different effects on the personality profile of Rh-positive and Rh-negative subjects.

The specific influence of latent toxoplasmosis on Cloninger's factors has already been described in a nearly identical set of blood donors for novelty seeking (NS) and self transcendence (ST) (Skallová et al. 2005) and in an independent set of military conscripts for novelty seeking (Novotná et al. 2005). The analysis of the statistical models containing both toxoplasmosis and Rh phenotype as independent factors identified not only possible effects of RhD phenotype on harm avoidance (HA), cooperativeness (CO) and self transcendence (ST) (in women) but also opposite effects of toxoplasmosis on reward dependence (RD) and cooperativeness (CO) in Rh-positive and Rh-negative subjects. Similarly, specific effects of latent toxoplasmosis on Cattell's personality factors strength of superego (G) and protension (L) have already been reported in university students (Flegr and Hrdý 1994), military conscripts (Novotná et al. 2005), blood donors (Skallová et al. 2005), childbearing age women (Flegr and Havlíček 1999) and males (Flegr et al. 1996) and females (Flegr et al. 2000) diagnosed with acute toxoplasmosis 2–12 years before personality testing, (see Flegr 2007 for review). Again, the current analysis of the statistical models containing both toxoplasmosis and Rh phenotype as independent factors identified possible effects of RhD phenotype on ego strength (C), protension (L), and praxernia (M) and opposite effects of toxoplasmosis on ego strength (C), praxernia (M), ergic tension (Q_4). and cooperativeness (CO) in Rh-positive and Rh-negative subjects. It should be stressed that not only toxoplasmosis-RhD interaction but also some of the main effects of toxoplasmosis and nearly all of the main effects of RhD phenotype can only be proved to exist in the models containing both analyzed factors, i.e. toxoplasmosis and RhD phenotype. Therefore, the previous unsuccessful attempts to prove the correlation between various personality traits and blood groups (Irvine and Miyashit 1965, Wiener 1965, Cattell 1972, Rogers and Glendon 2003, Wu et al. 2005) can be explained not only by the fact that the ABO rather than the Rh factor blood group system was usually studied, but also by the fact that the important confounding factor, toxoplasmosis, was not included in the analyzed models.

Four studies have already shown that the response of the human body to latent toxoplasmosis depends on RhD phenotype (Flegr et al. 2008, 2009, Novotná et al.

Table 6. Effects of RhD phenotype on Cattell's personality factors. The table shows the significance (two-sided p), effect size (η^2) estimated by the univariate GLM and arithmetic means in particular subsets. The results significant in two-tailed tests are printed in bold. For abbreviations see Table 4.

	All				Men				Women			
	p	η^2	RhD^-	RhD^+	p	η^2	RhD^-	RhD^+	p	η^2	RhD^-	RhD^+
A	0.727	0.000	11.005	10.839	0.420	0.003	10.552	10.127	0.856	0.000	12.337	12.505
B	0.896	0.000	7.983	8.030	0.989	0.000	8.047	8.040	0.780	0.001	7.823	8.000
C	0.081	0.011	13.637	14.612	0.163	0.010	13.809	14.733	0.253	0.018	13.109	14.336
E	0.298	0.004	13.261	13.899	**0.028**	0.026	12.682	14.274	0.145	0.028	14.742	13.073
F	0.101	0.010	11.415	12.471	**0.006**	0.039	10.222	12.327	0.064	0.045	14.866	12.805
G	0.413	0.003	12.061	11.642	0.244	0.007	12.169	11.438	0.762	0.001	11.833	12.097
H	0.275	0.005	11.353	12.297	0.143	0.011	10.583	12.095	0.665	0.003	13.491	12.794
I	0.742	0.000	9.027	9.225	0.737	0.001	7.934	8.165	0.473	0.007	12.302	11.681
L	**0.006**	0.028	11.470	10.234	0.186	0.009	10.935	10.235	**0.001**	0.134	13.058	10.217
M	0.279	0.004	11.383	11.884	0.064	0.018	11.269	12.268	0.378	0.011	11.750	10.971
N	0.823	0.000	9.489	9.402	0.301	0.006	9.724	9.252	0.241	0.019	8.836	9.746
O	0.743	0.000	10.385	10.192	0.716	0.001	9.978	9.738	0.843	0.001	11.515	11.271
Q_1	0.606	0.001	7.191	6.982	0.933	0.000	7.127	7.169	0.258	0.017	7.348	6.551
Q_2	0.691	0.001	11.738	11.534	0.795	0.000	12.007	11.856	0.868	0.000	10.956	10.779
Q_3	0.203	0.006	11.657	12.222	0.316	0.005	11.852	12.378	0.337	0.012	11.055	11.868
Q_4	0.659	0.001	12.931	12.649	0.627	0.001	12.640	12.281	0.847	0.001	13.761	13.518
n			66	201			49	141			17	60

2008, Kaňková et al. 2010). Psychomotor performance (reaction times) of Rh-negative subjects deteriorated after *Toxoplasma* infection while Rh-positive heterozygotes were permanently and Rh-positive homozygotes were temporarily protected against such decrease in psychomotor performance. Also, RhD-negative women with latent toxoplasmosis gained more weight during pregnancy than RhD-negative, *Toxoplasma*-free women or RhD-positive women. Results of the present study suggest that RhD phenotype also modulates toxoplasmosis-associated changes in the personality profile. In contrast to the primarily quantitative modulation of changes in reaction times, the modulation of some personality profile changes was also qualitative. For example, latent toxoplasmosis was associated with lower ego strength and lower cooperativeness in Rh-negative subjects, but with higher ego strength and higher cooperativeness in Rh-positive subjects. The toxoplasmosis-associated changes were always stronger in Rh-negative subjects, as was reported previously for psychomotor performance.

Our present results indicate that RhD phenotype modulates the responses of the body not only to *Toxoplasma* infection, but also to other factors. Here we observed different correlations between two Cattell's factors, namely dominance and shrewdness, and age in Rh-positive and Rh-negative subjects. In contrast to the response of the body to *Toxoplasma* infection, the correlation with the personality factors was stronger in Rh-positive subjects. It must be pointed out, however, that unlike the effect of RhD-toxoplasmosis interaction, the effect of RhD-age interaction on Cattell's personality profile estimated by the multivariate GLM was not significant and the significances in the univariate tests did not survive the Bonferroni correction. Therefore, the significant effects of RhD-age interaction observed in two of sixteen univariate GLM tests might be just artifacts of the multiple statistical tests and should be confirmed in other independent studies.

The function of the RhD molecule, the membrane pump that transports either ammonium or CO_2, has been revealed only recently, but its physiological role remains unknown (Biver et al. 2006, Kustu and Inwood 2006). Therefore, the speculation about the mechanisms involved in the modulation of toxoplasmosis- (or age-) associated changes in personality traits (or reaction times or weight gain) would be rather premature. Obviously, the difference between Rh-positive homozygotes and heterozygotes, namely permanent protection against the impairment of reaction times in heterozygotes and only temporary protection in homozygytes (Novotná et al. 2008), suggests that not (only) the RhD molecule but (also) a product of another gene in a strong genetic linkage with the *RHD* gene might be responsible for the observed biological and psychological effects of RhD phenotype (Flegel 2006). To test such hypotheses, genetic instead of common serological data needs to be collected from larger populations.

The origin and maintenance of the Rh polymorphism in the human population is an old evolutionary enigma, as the fitness of carriers of the rarer variant of the *RHD* gene (an Rh-negative woman in a predominantly Rh-positive population or an Rh-positive man in a predominatly Rh-negative population) is compromised by the risk of haemolytic disease of newborns (Fisher et al. 1944, Haldane 1944). The broad spectrum of behavioural effects of RhD phenotype described in this and previous studies, however, suggests that the Rh polymorphism can be stabilized by some form of frequency dependent selection.

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