RESEARCH ARTICLE

Worse Health Status and Higher Incidence of Health Disorders in Rhesus Negative Subjects

Jaroslav Flegr¹*, Rudolf Hoffmann², Mike Dammann¹

¹ Division of Biology, Faculty of Science, Charles University in Prague, Prague, Czech Republic, ² Department of Hematology, Na Homolce Hospital, Prague, Czech Republic

* flegr@cesnet.cz

Abstract

Rhesus-positive and Rhesus-negative persons differ in the presence-absence of highly immunogenic RhD protein on the erythrocyte membrane. The biological function of the RhD molecule is unknown. Its structure suggests that the molecular complex with RhD protein transports NH₃ or CO₂ molecules across the erythrocyte cell membrane. Some data indicate that RhD positive and RhD negative subjects differ in their tolerance to certain biological factors, including, Toxoplasma infection, aging and fatigue. Present cross sectional study performed on 3,130 subjects showed that Rhesus negative subjects differed in many indices of their health status, including incidences of many disorders. Rhesus negative subjects reported to have more frequent allergic, digestive, heart, hematological, immunity, mental health, and neurological problems. On the population level, a Rhesus-negativity-associated burden could be compensated for, for example, by the heterozygote advantage, but for Rhesus negative subjects this burden represents a serious problem.

Introduction

Polymorphism in the Rhesus factor, namely the existence of a large deletion in the RHD gene [1] in a substantial fraction of the human population, has been an evolutionary enigma since the discovery of this factor in the 1930’s [2–5]. Theoretically, neither the RhD-negative allele can successfully spread in the RhD positive population nor the RhD-positive allele can spread in the RhD negative population [6,7]. Before the introduction of prophylactic treatment in 1968, a positive frequency dependent selection systematically penalized the less abundant allele because lots of children of RhD-negative women in the mostly RhD-positive population as well as children of RhD- positive men in the mostly RhD-positive population were dying of hemolytic anemia. It has been suggested that this polymorphism can be stabilized when the disadvantage of carriers of the locally rarer allele is counterbalanced by higher viability of their heterozygote children or by another form of frequency-dependent selection [6]. In the past seven years, several studies have demonstrated that Rhesus positive and Rhesus negative subjects differ in resistance to the adverse effects of parasitic infections, aging, fatigue and smoking [7–13]. A study performed on 250 blood donors has further shown that the resistance to effects of toxoplasmosis is higher in Rhesus positive heterozygotes than in Rhesus positive...
homozygotes and substantially higher than in Rhesus negative homozygotes [7]. This is the first direct evidence for the role of selection in favour of heterozygotes in stabilization of the RHD gene polymorphism in human populations. Such a mechanism is reminiscent of widely known situations with polymorphism in genes associated with sickle cell anaemia in geographic regions with endemic malaria [14].

The results of previous studies suggest that RhD negative homozygotes could have a worse health status than RhD positive population consisting of RhD positive homozygotes and heterozygotes. These results, however, were obtained on either rather small or rather specific populations, e.g. military personnel [13] or pregnant women [12]. To obtain more reliable data about situation in more typical populations, we run a large questionnaire study in a population of healthy Czech and Slovak volunteers. Using an electronic questionnaire distributed with a Facebook-based snowball method [15], we have screened a population of 3,130 subjects for indices of various health problems as well as for incidences of 225 diseases and disorders.

Methods

Ethics Statement

Only subjects older 18 years were invited and allowed to start the internet test. We erased data of 7 subjects who claimed to be younger as well as the data of all subjects who did not respond how old they were. The study, including the method of obtaining an electronic consent with a participation in the study (by pressing a particular button), was approved by the IRB of the Faculty of Science, Charles University (Komise pro práci s lidmi a lidským materiálem Přírodovedecké Fakulty Univerzity Karlovy)—No. 2014/21.

Subjects

The subjects were invited to participate in the study using a Facebook-based snowball method [15] by posting an invitation to participate in “an experiment searching for associations between the blood group of a subject and his/her personality, performance, morphology and health” on the wall of the Facebook page “Guinea pigs” for Czech and Slovak nationals willing to take part in diverse evolutionary psychological experiments (www.facebook.com/pokusnikralici). The participants were informed about the aims of the study on the first page of the electronic questionnaire: “The subject of the present study is searching for associations between the blood group of a subject and his/her personality, performance, morphology and health. If you can check up on what your blood group is, please do it now. “They were also provided with the following information: “The questionnaire is anonymous and obtained data will be used exclusively for scientific purposes. Your cooperation in the project is voluntary and you can terminate it at any time by closing this web page. If you can check up on what your blood group is, please do it now. We need the data from subjects with all blood groups, not only from Rh negative subjects. Therefore, please share the link to this questionnaire with your friends, for example on Facebook. Press the “continue” button if you agree to your anonymous participation in the study”. The share button was pressed by 480 participants, which resulted in obtaining data from 4,286 responders in total between 28.4. 2014–9.3. 2015. Data file is available as the S1 File.

Questionnaire

The anamnestic questionnaire was prepared by two medical doctors and was distributed as a Czech/English Qualtrics survey (http://1url.cz/q05K). It contained two categories of questions. The first of them monitored presence and intensity of general and specific health problems of
responders. The responders were asked to subjectively rate of their allergic, cancer, digestive, fertility, genitourinary, heart, hematological, immunity, mental health, metabolic including endocrine, musculoskeletal, neurological, respiratory organs, sense organs, and sexual life problems using 6-points Likert scales. The second group of questions tried to collect objective information reflecting the health status of responders. We asked the responders, for example, how many drugs prescribed by doctors they currently take per day, how many of “different herbs, food supplements, multivitamins, superfoods etc.” they currently take per day, how many times they used antibiotics during the past 365 days. We also provided the responders lists of about 250 disorders (separated to 15 categories) and asked them to tick which of them they were diagnosed with. The questionnaire contained, among others, also the following questions: “What is your Rh blood group?” with three options: a) I do not know / I am not sure, b) negative (this is the less frequent variant) c) positive (the more frequent variant). Implicitly, the answer a) (I do not know/I am not sure) was checked.

Statistical methods
Before statistical analysis, suspicious data (too high or too short body height, too low or too high body mass or age, too short duration of the test etc.) were filtered out (26 cases). In the test, we also measured simple reaction times, operational, short-term and long-term memory, psychomotor performance, intelligence and personality profiles. However, here we have analyzed only data concerning health status.

SPSS v. 21. was used for all statistical tests. Ordinal and binary data were analyzed by partial Kendall’s correlation test [16,17]. This test measures strength and significance of association between binary, ordinal and continuous data regardless of their distributions. This technique enabled us to control for one confounding variable, for example the age of a responder. The Excel sheet for computing partial Kendall’s Tau and the significance between variables A and B after the variable C is controlled based on Kendall Tau’s AB, AC and BC. It is available here: http://web.natur.cuni.cz/flegr/programy.php (item no. 12) and in S2 File. Certain diseases have very different incidence in men and women. Also, some biological factors, including RhD phenotype, could have different impacts on men and women. Therefore, we performed all analyses for all responders and also separately for the male and female responders.

Results
Descriptive statistics of data
Among 4,286 Czech and Slovak participants of a subsequent case-control study, 3,130 subjects (840 RhD positive men, 317 RhD negative men, 1,337 RhD positive women and 636 RhD negative women) provided information about their gender and RhD phenotype. RhD negative subjects, especially women, have higher motivation to care about, and to remember, their RhD phenotype. Therefore, the frequency of RhD negative subjects (30.4%) differed from the 16% general frequencies within the Czech and Slovak populations and also between men (27.4%) and women (32.2%). The mean age of RhD positive men (37.6, S.D. 13.5) was approximately the same as that of RhD negative men (37.7, S.D. 12.7), t(1153) = -0.10, P = 0.923. RhD positive women were younger (33.6, S.D. 11.9) than RhD negative women (35.2, S.D. 12.7), t(1923) = 2.74, P = 0.006. The numbers of men and women in the particular age strata were comparable, with the exception of the 21–30 age stratum, which consisted of 363 men and 842 women (Fig 1).
Correlation of RhD phenotype with self-reported health problems (ordinal variables)

Twenty-two dependent variables (mostly ratings of particular health problems on a scale from 1–6, 1: “no problems at all”, 6: “frequent or serious”) were ordinal and had a highly skewed distribution. Therefore, the nonparametric partial Kendall’s correlation test (which enables to control one confounding variable, here the age) was used to search for an association between the RhD phenotype and the intensity of fifteen categories of health problems (allergic, cancer, digestive, fertility, genitourinary, heart, hematological, immunity, mental health, metabolic including endocrine, musculoskeletal, neurological, respiratory organs, sense organs, and sexual life problems) and also another six health-related variables, namely the numbers of drugs prescribed by doctors that the subject currently takes per day, numbers of “different herbs, food supplements, multivitamins, superfoods etc.” the subject currently takes per day, how many times the subject has used antibiotics during the past 365 days, how many times the subject was required to seek acute medical care for a serious illness (not injury) that lasted more than 3 days during the past 5 years, how many specialized medical doctors the subject had to regularly attend (not for prevention) at least once in the past two years, how often the subject felt tired (not after exertion, e.g. sports) and how often the subject has experienced a headache. The results showed that the RhD negative subjects had more serious health problems in 6 of 22 analyzed variables than the RhD positive subjects (Table 1).

Association between RhD phenotype and incidence of particular diseases (binary variables)

After rating each particular category of health problems, the subjects were asked to identify which specific disorders they suffered from on the lists of 225 disorders. They were also asked to identify which specialized medical doctors they had to visit regularly (not for prevention) at
least once in the past two years from a list of 10 types of specialists. The associations were analyzed with the partial Kendall’s Tau correlation test with age being a covariate. One hundred fifty four (154) of 225 diseases/disorders were reported by at least 10 subjects. Within this subset, 31 significant associations with RhD negativity (21 positive and 10 negative) were expressed in all subjects. In male subjects, the number of significant association was 35 (19 positive and 16 negative) while in female subjects the number of significant associations was 30 (18 positive and 12 negative). The expected number of false significant results for 462 statistical tests was not 96 but 23. For example, RhD negative men more often reported certain mental health disorders including panic disorders, antisocial personality disorders and attention deficits, ticks, fasciculation, thyroiditis, immunity disorders, allergies, especially skin allergies, excessive bleedings, anemia, osteoporosis, liver disease, infectious diseases and acute diarrhea diseases, while they less often reported gall bladder attacks, coeliac disease, maligestion, malabsobtion, warts, some types of cancers and prostate hypertrophy. RhD negative women reported more frequently psoriasis, constipation and diarrheas, ischemic diseases, type 2 diabetes, some types of cancers, lymphatic nodes swelling, vitamin B deficiency, thrombosis, tonsil stones, too high sex desire, precocious puberty, urinary tract infections, scoliosis and they less often reported hearing loss, weight loss, hypoglycemia, glaucoma, fasciculation and warts. RhD

Table 1. Difference in various health status related variables between RhD negative and RhD positive subjects.

| problems          | all       | men       | women      |
|-------------------|-----------|-----------|------------|
|                   | Tau       | p         | Tau        | p          | Tau        | p          |
| allergic          | 0.018     | 0.153     | 0.016      | 0.444      | 0.012      | 0.450      |
| cancer            | 0.008     | 0.514     | -0.053     | **0.012*  | 0.031      | **0.052*   |
| digestive         | 0.034     | **0.008*  | -0.016     | 0.443      | 0.050      | **0.002*   |
| fertility         | -0.009    | 0.475     | 0.045      | **0.035*  | -0.042     | **0.009*   |
| genitourinary     | 0.006     | 0.628     | -0.026     | 0.223      | -0.003     | 0.844      |
| heart & vascular  | 0.031     | **0.015*  | 0.001      | 0.971      | 0.047      | **0.003*   |
| hematological     | 0.028     | **0.028*  | -0.028     | 0.185      | 0.031      | **0.053    |
| immunity          | 0.034     | **0.007*  | 0.023      | 0.268      | 0.024      | 0.126      |
| metabolic         | -0.006    | 0.672     | -0.020     | 0.342      | -0.017     | 0.296      |
| musculoskeletal   | 0.015     | 0.264     | -0.039     | **0.069    | 0.035      | **0.034*   |
| mental health     | 0.013     | 0.322     | 0.049      | **0.024*  | -0.012     | 0.460      |
| neurological      | 0.016     | 0.225     | 0.060      | **0.005*  | -0.013     | 0.417      |
| respiratory org.  | -0.003    | 0.798     | -0.002     | 0.918      | -0.008     | 0.613      |
| sense organs      | -0.014    | 0.279     | -0.033     | 0.120      | -0.012     | 0.454      |
| sexual life       | -0.012    | 0.369     | -0.011     | 0.615      | -0.007     | 0.649      |
| medicine/day      | 0.049     | **0.000*  | 0.047      | **0.025*  | 0.045      | **0.004*   |
| herbs/day         | -0.001    | 0.942     | 0.045      | **0.030*  | -0.039     | **0.014*   |
| antibiotics/year  | 0.014     | 0.269     | -0.022     | 0.290      | 0.023      | 0.136      |
| acute care/5 years| 0.002     | 0.883     | 0.016      | 0.429      | -0.013     | 0.418      |
| doctors/2 years   | 0.018     | 0.162     | -0.017     | 0.425      | 0.025      | 0.122      |
| tired (frequency) | 0.026     | **0.046*  | 0.010      | 0.637      | 0.019      | 0.238      |
| headache (frequency) | 0.013   | 0.321     | 0.024      | 0.264      | -0.020     | 0.214      |

Number of responders varied between particular questions and was about 1,000 for men and 1,800 for women. Mostly significant effects of age on health status were controlled in present partial Kendall Tau test. Positivity of Tau indicates that RhD negative subjects have higher values of particular health related variables, i.e., a worse health status. Significant results (P < 0.05) and trends (P < 0.10) are printed in bold. Asterisks indicate results significant in two-sided tests. Values < 0.0005 are coded as 0.000.

doi:10.1371/journal.pone.0141362.t001
negative subjects had to make more frequent visits to medical professionals specializing in oto-laryngology ($P = 0.021$), psychiatry ($P = 0.008$), gynecology ($P < 0.001$), and dermatology ($P = 0.014$) (the theoretical number of false positive results was 0.5). Table 2 shows the associations between RhD negativity and disease incidences while Table 3 shows the associations between RhD negativity and visiting specialized doctors.

**Discussion**

The RhD negative subjects expressed many indices of a worse health status. Men, women or both sexes reported more frequent allergic, digestive, heart, hematological, immunity, mental health and neurological problems. They also reported the usage of more drugs prescribed by doctors per day, attended more specialized doctors, namely, dermatologists, gynecologists, internal medicine doctors, neurologists, and psychiatrists (men) in the past two years, a higher frequency of headaches and being tired more often than RhD positive subjects. Incidence of various diseases and disorders also differed between RhD negative and RhD positive subjects, mostly being higher in the former.

RhD negative subjects have increased the risk of developing of certain heart diseases, respiratory diseases and some immunity and autoimmunity related diseases, for example rheumatoid arthritis. The general pattern suggests that RhD negative subjects could have problems with autoimmunity, could be more resistant to infections of viral origin and could be less resistant to infections of bacterial origin.

The mechanism of the effect of the RhD phenotype on human health status is not clear. RhD protein together with strongly homologous RhCE protein and with also homologous RhAG glycoprotein are all components of a membrane complex of which the function is not quite clear. It is most probably involved in NH$_3$ transport and possibly also in CO$_2$ transport [18,19]. This complex is associated with spectrin-based cytoskeleton and therefore plays an important role in maintaining the typical shape (biconcave discoid) of human erythrocytes [20]. The biological functions of complexes containing the RhD protein are unknown. However, they might be involved in NH$_3$/NH$_4^+$ detoxification of organs. Ammonia, the product of protein catabolism is extremely toxic, especially for brain cells and must be quickly removed from the sensitive organs. It was observed that the concentration of ammonium is three times higher in red cells than in plasma [20] and it was further suggested that the RhD containing complex plays a key role in its capturing and its transport to the kidneys and the liver [20]. It was also suggested that the complex might participate in intracellular pH regulation [20] and consequently also in the regulation of local oxygen tension. It was suggested that RhD-negativity-associated anoxia in certain parts of the nervous system could be responsible for physiological (and also behavioral) effects of the RhD phenotype [21]. The variation of the oxygen tension in various organs and tissues could, of course, influence also other biological functions, including the functions of the immune system. This could explain why RhD negativity seems to be associated with neurological, mental health and immunological disorders. The probable roles of the RhD-containing complex in keeping the normal morphology and adhesiveness of red cells (for review see [20]) could be responsible for the observed associations of RhD negativity with some haematological and inflammation-related diseases, including arthritis.

Limitations and strength of present study: Using very effective Facebook-based snow-ball method we obtained data from a large number of subjects. However, most of them were relatively young people (mean age was 35.4). Most of the diseases and disorders with the largest public health impact (but possibly not the largest economic impact) start at a higher age in developed European countries such as the Czech and Slovak Republics. This can largely distort the whole picture of the RhD negativity impacts on public health. Future studies (which could
| Disorder | Rh+ Dis- | Rh+ Dis+ | Rh- Dis+ | Rh- Dis+ | Tau | P  |
|----------|----------|----------|----------|----------|-----|----|
| Pharyngitis | 294 591 600 1379 | -0.030 | 0.017* | 100 185 255 498 | -0.012 | 0.570 |
| Bronchitis, pneumonia | 686 199 1519 460 | -0.009 | 0.476 |
| Rhinitis, tonsillitis | 367 518 847 1132 | 0.015 | 0.240 |
| Ectoparasites, e.g. lice | 738 147 1649 330 | 0.002 | 0.868 |
| Scabies | 868 17 1930 49 | -0.018 | 0.147 |
| Helminthiasis | 844 41 1867 103 | -0.012 | 0.325 |
| Acute diarrhea dis. | 724 161 1679 00 | 0.040 | 0.001* |
| Acquired immunodef. | 866 19 1935 44 | -0.003 | 0.796 |
| Flu and flu-like virosis | 297 588 632 1347 | -0.013 | 0.312 |
| Boreliosis | 801 84 1807 172 | 0.011 | 0.391 |
| Other thick born dis | 878 7 1962 17 | -0.003 | 0.797 |
| Sexually transmit. dis. | 870 15 1934 45 | -0.018 | 0.142 |
| Hepatitis A, E | 878 7 1955 24 | -0.022 | 0.083 |
| Hepatitis B | 879 6 1970 9 | 0.013 | 0.303 |
| Herpes zoster | 832 53 1855 124 | -0.007 | 0.553 |
| Herpes, oral or genital | 623 262 1410 569 | 0.008 | 0.531 |
| Meningoencephalitis | 872 13 1960 19 | 0.021 | 0.087 |
| Inflamm. of middle ear | 618 267 1400 579 | 0.011 | 0.379 |
| Eye infections | 784 101 1739 240 | -0.009 | 0.467 |
| Other infectious dis. | 823 62 1874 105 | 0.034 | 0.000* |
| Skin allergy | 686 199 1558 421 | 0.013 | 0.310 |
| Bacterial skin infect. | 822 63 1861 01 | 0.013 | 0.285 |
| Warts | 600 285 1244 735 | -0.045 | 0.000* |
| Lymphatic modes swelling | 840 45 1883 96 | 0.000 | 0.588 |
| Mononucleosis | 778 107 1728 251 | -0.007 | 0.587 |
| Tonsil stones | 657 183 1534 330 | 0.047 | 0.000* |
| Skin allergy | 646 224 1482 462 | 0.022 | 0.075 |
| Food allergy | 765 105 1698 246 | -0.007 | 0.577 |
| Respiratory allergy | 535 335 1193 751 | 0.001 | 0.935 |
| Other allergies | 806 64 1814 130 | 0.011 | 0.361 |
| Autoimmunity | 803 50 1815 94 | 0.019 | 0.127 |
| Rheumatoid arthritis | 831 22 1880 29 | 0.033 | 0.008* |
| Haematological autoimmunity dis. | 848 5 1896 11 | 0.000 | 0.985 |
| Thyroiditis | 781 72 1789 120 | 0.038 | 0.002* |
| Immunodeficiency | 813 40 1824 85 | 0.006 | 0.651 |
| Bechterew’s dis. | 847 6 1902 7 | 0.022 | 0.088 |
| Other immunolg. dis. | 796 57 1830 79 | 0.054 | 0.000* |
| Psoriasis | 829 24 1847 42 | 0.017 | 0.177 |
| Stomach or duodenal ulcer | 810 34 1840 62 | 0.017 | 0.178 |
| Chronic gastritis | 827 17 1845 57 | -0.030 | 0.018* |
| Liver disease | 806 38 1841 61 | 0.031 | 0.014 |
| Diarrheas | 647 197 1496 406 | 0.027 | 0.034* |
| Constipation | 701 143 1638 264 | 0.042 | 0.001* |
| Malignancy, food intolerance | 733 111 1646 256 | -0.002 | 0.845 |
| Malabsorption | 822 22 1864 38 | 0.019 | 0.137 |
| Bulimia, anorexia | 824 20 1846 56 | -0.015 | 0.247 |

Worse Health of Rh Negative Subjects

(Continued)
### Table 2. (Continued)

| Condition                        | All (Men | Women | Tau | P   | All (Men | Women | Tau | P   | All (Men | Women | Tau | P   |
|----------------------------------|---------|-------|-----|-----|---------|-------|-----|-----|---------|-------|-----|-----|
| Flatulence                       |         |       |     |     |         |       |     |     |         |       |     |     |
| Weight loss                      |         |       |     |     |         |       |     |     |         |       |     |     |
| Other digestive.                 |         |       |     |     |         |       |     |     |         |       |     |     |
| Pyrosis reflex                   |         |       |     |     |         |       |     |     |         |       |     |     |
| Gall bladder attack              |         |       |     |     |         |       |     |     |         |       |     |     |
| Coeliac disease                  |         |       |     |     |         |       |     |     |         |       |     |     |
| Hypertensive disease             |         |       |     |     |         |       |     |     |         |       |     |     |
| Ischaemic disease                |         |       |     |     |         |       |     |     |         |       |     |     |
| Other heart dis.                 |         |       |     |     |         |       |     |     |         |       |     |     |
| Excessive bleeding               |         |       |     |     |         |       |     |     |         |       |     |     |
| Thrombosis                       |         |       |     |     |         |       |     |     |         |       |     |     |
| Atrial fibrillation              |         |       |     |     |         |       |     |     |         |       |     |     |
| Anemia                           |         |       |     |     |         |       |     |     |         |       |     |     |
| High leukocytes level            |         |       |     |     |         |       |     |     |         |       |     |     |
| Other problems with leukocytes   |         |       |     |     |         |       |     |     |         |       |     |     |
| Other blood diseases             |         |       |     |     |         |       |     |     |         |       |     |     |
| High platelets level             |         |       |     |     |         |       |     |     |         |       |     |     |
| Low platelets level              |         |       |     |     |         |       |     |     |         |       |     |     |
| Excessive bleeding               |         |       |     |     |         |       |     |     |         |       |     |     |
| Accented blood clotting          |         |       |     |     |         |       |     |     |         |       |     |     |
| Iron deficiency                  |         |       |     |     |         |       |     |     |         |       |     |     |
| Lymphatic nodes swelling         |         |       |     |     |         |       |     |     |         |       |     |     |
| Vitamin B12 deficiency           |         |       |     |     |         |       |     |     |         |       |     |     |
| Type 1 diabetes                  |         |       |     |     |         |       |     |     |         |       |     |     |
| Crohn's disease                  |         |       |     |     |         |       |     |     |         |       |     |     |
| Immunodeficiency                 |         |       |     |     |         |       |     |     |         |       |     |     |
| Type 2 diabetes                  |         |       |     |     |         |       |     |     |         |       |     |     |
| Hypothyroidism                   |         |       |     |     |         |       |     |     |         |       |     |     |
| Hyperthyroidism                  |         |       |     |     |         |       |     |     |         |       |     |     |
| Inborn metabolic dis.            |         |       |     |     |         |       |     |     |         |       |     |     |
| Obesity                          |         |       |     |     |         |       |     |     |         |       |     |     |
| Hypoglycemia                     |         |       |     |     |         |       |     |     |         |       |     |     |
| Osteoporosis                     |         |       |     |     |         |       |     |     |         |       |     |     |
| Delayed puberty                  |         |       |     |     |         |       |     |     |         |       |     |     |
| Precocious puberty              |         |       |     |     |         |       |     |     |         |       |     |     |
| Amenorrhea                       |         |       |     |     |         |       |     |     |         |       |     |     |
| Other metabolic dis.             |         |       |     |     |         |       |     |     |         |       |     |     |
| Melanoma and other skin cancer   |         |       |     |     |         |       |     |     |         |       |     |     |
| Breast cancer                    |         |       |     |     |         |       |     |     |         |       |     |     |
| Cervix uter cancer               |         |       |     |     |         |       |     |     |         |       |     |     |
| Other cancer diseases            |         |       |     |     |         |       |     |     |         |       |     |     |
| Urinary tract infections         |         |       |     |     |         |       |     |     |         |       |     |     |

(Continued)
| Table 2. (Continued) |
|----------------------|
| **All**              |
| Rh-| Rh-Dis*| Rh-| Rh-Dis*| Rh-| Rh-Dis*| Tau | P               |
|----------------------|
| **Men**              |
| Rh-| Rh-Dis*| Rh-| Rh-Dis*| Rh-| Rh-Dis*| Tau | P               |
|----------------------|
| **Women**            |
| Rh-| Rh-Dis*| Rh-| Rh-Dis*| Rh-| Rh-Dis*| Tau | P               |

**Nephrosis, glomerulonephritis**

| 779 | 11 | 1778 | 21 | 0.009 | 0.501 |

**Bladder infection, cystitis**

| 725 | 65 | 1666 | 133 | 0.014 | 0.269 |

**Prostate hypertrophy**

| 787 | 3 | 1773 | 26 | -0.051 | 0.000* |

**Gynaecological infections**

| 644 | 146 | 1518 | 281 | 0.038 | 0.004* |

**Cervical precancer or cancer**

| 778 | 12 | 1774 | 25 | 0.005 | 0.725 |

**Obstetric complications**

| 760 | 30 | 1743 | 56 | 0.015 | 0.262 |

**Recurrent abortions**

| 777 | 12 | 1773 | 26 | 0.001 | 0.943 |

**Kidney stones**

| 778 | 12 | 1756 | 43 | -0.030 | 0.024* |

**Other genitourinary dis.**

| 771 | 19 | 1759 | 40 | 0.006 | 0.669 |

**Glioma**

| 798 | 4 | 1793 | 23 | -0.006 | 0.006* |

**Cataracts, clouding of the lens**

| 793 | 9 | 1796 | 20 | -0.002 | 0.880 |

**Refractive errors**

| 438 | 364 | 982 | 834 | -0.006 | 0.646 |

**Hearing loss**

| 773 | 29 | 1717 | 99 | -0.041 | 0.002* |

**Macular degeneration**

| 791 | 11 | 1797 | 19 | 0.011 | 0.319 |

**Strabismus**

| 786 | 16 | 1777 | 39 | -0.006 | 0.669 |

**Sense of smell problems**

| 789 | 13 | 1765 | 51 | -0.037 | 0.005* |

**Sense of taste prob.**

| 800 | 2 | 1807 | 9 | -0.018 | 0.167 |

**Ringing in the ears**

| 741 | 61 | 1671 | 145 | -0.010 | 0.465 |

**Other sense organs dis.**

| 767 | 35 | 1745 | 71 | 0.011 | 0.415 |

**Sense of motion problems**

| 772 | 30 | 1757 | 59 | 0.011 | 0.403 |

**Amblyopia, lazy eye**

| 768 | 34 | 1728 | 88 | -0.014 | 0.269 |

**Extremity neuroopathy**

| 802 | 8 | 1822 | 24 | -0.015 | 0.242 |

**Multiple sclerosis**

| 805 | 5 | 1840 | 6 | 0.021 | 0.108 |

**Epilepsy**

| 805 | 5 | 1830 | 16 | -0.013 | 0.319 |

**Migraine**

| 627 | 183 | 1450 | 396 | 0.014 | 0.287 |

**Other neurologic dis.**

| 793 | 17 | 1812 | 34 | 0.008 | 0.555 |

**Stuttering**

| 799 | 11 | 1819 | 27 | -0.003 | 0.812 |

**Tics**

| 780 | 30 | 1771 | 75 | -0.007 | 0.601 |

**Muscle twitch, fasciculation**

| 753 | 57 | 1705 | 141 | -0.010 | 0.443 |

**Cramps**

| 746 | 64 | 1700 | 146 | 0.000 | 0.971 |

**Unipolar depressive disorders**

| 765 | 31 | 1742 | 78 | -0.009 | 0.469 |

**Bipolar disorder**

| 786 | 10 | 1800 | 20 | 0.007 | 0.587 |

**Anxiety disorders**

| 745 | 51 | 1720 | 100 | 0.019 | 0.136 |

**Alcohol use disorders**

| 787 | 9 | 1803 | 17 | 0.009 | 0.509 |

**Drug use disorders**

| 788 | 8 | 1797 | 23 | -0.011 | 0.396 |

**Post traumatic disorder**

| 785 | 11 | 1787 | 33 | -0.016 | 0.221 |

**Obsessive compulsive dis.**

| 783 | 13 | 1783 | 37 | -0.012 | 0.338 |

**Panic disorder**

| 767 | 29 | 1772 | 48 | -0.027 | 0.035* |

**Insomnia primary**

| 727 | 69 | 1655 | 165 | -0.005 | 0.685 |

**Learning disability**

| 767 | 29 | 1761 | 59 | 0.011 | 0.389 |

---

Worse Health of Rh Negative Subjects (Continued)
Worse Health of Rh Negative Subjects

Table 2. (Continued)

|                      | All | Men | Women |
|----------------------|-----|-----|-------|
| Borderline personality disorder | 787 | 9 1804 | 16 0.013 0.325 |
| Antisocial personality disorder | 787 | 9 1802 | 18 0.008 0.554 |
| Attention deficit, hyperactivity | 772 | 24 1779 | 41 0.024 0.068 |
| Other mental health dis. | 769 | 27 1777 | 43 0.030 0.021* |
| Erectile dysfunction | 783 | 31 1746 | 83 -0.021 0.105 |
| Too low sex appenticity | 654 | 160 1473 | 356 0.001 0.930 |
| Too high sex appenticity | 749 | 65 1695 | 134 0.012 0.368 |
| Too low sex potency | 809 | 5 1811 | 18 -0.020 0.123 |
| Quality of sex | 724 | 90 1628 | 201 0.000 0.999 |
| Other sexological dis. | 791 | 23 1762 | 67 -0.021 0.101 |
| Paraphilias (mild) | 942 | 11 2157 | 20 0.011 0.349 |
| Spondylosis, spondylitis | 774 | 9 1772 | 18 0.004 0.733 |
| Backbone pain | 517 | 266 1168 | 622 -0.010 0.430 |
| Osteoporosis | 765 | 18 1764 | 26 0.027 0.037* |
| Rheumatoid arthritis | 758 | 25 1741 | 49 0.010 0.467 |
| Scoliosis | 646 | 137 1538 | 252 0.046 0.000* |
| Scheuermann’s disease | 774 | 9 1762 | 28 -0.017 0.189 |
| Other musculoskeletal dis. | 758 | 25 1745 | 45 0.020 0.127 |
| Osteoarthrosis | 742 | 41 1706 | 84 0.008 0.555 |
| Bronchitis | 721 | 62 1634 | 156 -0.016 0.228 |
| Asthma | 701 | 82 1677 | 213 -0.019 0.139 |
| Recurrent infections | 699 | 84 1585 | 205 -0.012 0.345 |
| Other respiratory dis. | 756 | 27 1734 | 56 0.007 0.569 |

Numbers of RhD negative subjects without particular disorders, RhD negative subjects with particular disorders, RhD positive subjects without particular disorders, RhD positive subjects with particular disorders, partial Kendall’s Tau and statistical significance, respectively, are shown in six columns of each section. The effect of age on health status was controlled in partial Kendall’s correlation (non-parametric) test. Positive Tau corresponds to a positive association and negative B to a negative association of RhD negativity with incidence of particular disorder. Significant results (p < 0.05) and trends (p < 0.10) are printed in bold. Asterisks indicate results significant in two-sided tests. p values < 0.0005 are coded as 0.000. The effect size is shown as Tau.

doi:10.1371/journal.pone.0141362.t002

be easily done in countries with available national databases of medical records of all citizens) should aim to recruit middle age and senior subjects. Our study compared the health status of RhD negative subjects (16% in general population of Czech and Slovak Republics) with RhD positive subjects, i.e., with the health status of mixed population of RhD positive homozygotes (36% of the general populations within the Czech and Slovak Republics) and heterozygotes (48% the general populations in the Czech and Slovak Republics). The results of the published case-control studies on the effects of the RhD genotype on psychomotor performance [22,23], as well as the heterozygote advantage hypothesis, however, suggest that the health status of RhD positive homozygotes and heterozygotes differs. In further studies concentrated on particular disorders, smaller populations of subjects should be RhD genotyped using molecular biology techniques and then the health status of all three RhD genotypes have to be compared. In
In the present study, the health status data were collected using a questionnaire. This enabled study of the effects of the RhD phenotypes on rarer disorders using a large population sample. Of course, more precise and more detailed data could be obtained from medical records. Primarily, we have run the study to confirm or disprove the alarming results of a previous small scale studies performed on non-typical populations. However, we had no a priori hypotheses which health-related variables should correlate with RhD phenotype or which disorders should

### Table 3. Differences between RhD positive and RhD negative participants in specialised medical doctors the subject had to regularly visit at least once in the past two years.

|                      | RhD-V- | RhD-V+ | RhD+V- | RhD+V+ | tau | p     |
|----------------------|--------|--------|--------|--------|-----|-------|
| Internal medicine    | 710    | 101    | 1651   | 213    | 0.011 | 0.400 |
| Otolaryngology       | 720    | 91     | 1690   | 174    | 0.030 | 0.021*|
| Neurology            | 747    | 64     | 1729   | 135    | 0.010 | 0.419 |
| Psychiatry           | 746    | 65     | 1751   | 113    | 0.036 | 0.006*|
| Gynecology           | 641    | 170    | 1539   | 325    | 0.045 | 0.000*|
| Surgery              | 766    | 45     | 1740   | 124    | -0.020 | 0.114 |
| Infectology          | 797    | 14     | 1838   | 26     | 0.013 | 0.318 |
| Orthopedics          | 705    | 106    | 1643   | 221    | 0.017 | 0.188 |
| Dermatology          | 680    | 131    | 1602   | 262    | 0.029 | 0.023*|
| Other Doctors        | 610    | 201    | 1357   | 507    | -0.025 | 0.051 |

|                      | All    | Men    | Women  |
|----------------------|--------|--------|--------|
| Internal medicine    | 241    | 617    | 92     |
| Otolaryngology       | 242    | 646    | 63     |
| Neurology            | 252    | 669    | 40     |
| Psychiatry           | 254    | 686    | 23     |
| Gynecology           | 270    | 707    | 2      |
| Surgery              | 249    | 649    | 60     |
| Infectology          | 266    | 691    | 18     |
| Orthopedics          | 244    | 633    | 76     |
| Dermatology          | 240    | 626    | 83     |
| Other Doctors        | 223    | 556    | 153    |

Columns 2–5 show numbers of RhD- or RhD+ subjects that had to (V+) and had not to (V-) visit a doctor of particular specialisation within the past 2 years. Columns 6 and 7 show Tau and P computed with partial Kendall’s correlation between two binary variables, i.e. the RhD phenotype and the Visiting doctor, controlled for the confounding variable age of a subject. Positivity of Tau indicates that RhD negative subjects have had to more frequently visit a doctor of particular specialization. Significant results (P < 0.05) and trends (P < 0.10) are printed in bold. Asterisks indicate results significant in two-sided tests. Values < 0.0005 are coded as 0.000.

doi:10.1371/journal.pone.0141362.t003
occur more frequently in RhD negative subjects. Therefore, the present study had a more or less explorative character. Hence, we have reported the results of statistical tests without formal correction to multiple tests. It should be noted, however, that, for example, we have obtained 41% positive results for the ordinal health status variables and 20% positive results for binary health status variables. Theoretically, only 5% of false positive results should be expected in multiple tests. The main strength of the present study is the absence of any sieve effect, which could result in publication bias in other types of studies. Positive results of particularly observational or experimental studies and partly also meta-analytic studies, could be an artefact of intentional or unintentional “cherry-picking”; i.e. preferential or even exclusive publication of positive results. In our study we have searched for the effects of the RhD phenotype on all diseases and all disorders having high enough incidences in the Czech population (n = 154) and we have reported all, both positive and negative results.

Conclusions
Some of the associations observed the present study were relatively strong and some of them concerned rather frequent disorders. Therefore, the total impact of frequency of RhD negative homozygotes in the general population on public health could be large.

The aim of the present study was to search for indices of validity of the heterozygote advantage hypothesis, namely for the indices of impaired health status of RhD negative subjects. It must be reminded, however, that the observed specific disease burden of the RhD negative subpopulation is in an agreement with predictions of this hypothesis but does not prove its validity. The higher disease burden in RhD negative homozygotes could be compensated either by increased fitness of heterozygotes (heterozygote advantage hypothesis) or by still unknown selection pressure in favor of RhD negative subjects. In this context, the shorter reaction times of RhD negative, Toxoplasma-free blood donors [7] and university students [8] and higher intelligence in RhD negative, Toxoplasma-infected soldiers [11] should be remembered. It could be speculated to what extent the highly uneven distributions of RHD minus alleles in world populations might be the result of a founder event and a gene flow [24] and to what extent it is also modulated by specific selection pressures caused by differences in the geographical distribution of a disease or diseases.

Supporting Information
S1 File. Excel file containing the data set.
(XLSX)

S2 File. Excel sheet for computing the partial Kendall correlation test.
(XLS)

Acknowledgments
We would like to thank Zdeněk Hodný Ins. Mol. Genetics, Czech Academy of Science, for his help with preparing the medical questionnaire and Charlie Nichols for his help with English version of the paper.

Author Contributions
Conceived and designed the experiments: JF. Performed the experiments: JF RH MD. Analyzed the data: JF. Contributed reagents/materials/analysis tools: JF. Wrote the paper: JF RH MD.
References

1. Wagner FF, Flegel WA (2000) RHD gene deletion occurred in the Rhesus box. Blood 95: 3662–3668. PMID: 10845894
2. Haldane JBS (1942) Selection against heterozygosis in Man. Eugenics 11: 333–340.
3. Hogben L (1943) Mutation and the Rhesus reaction. Nature 152: 721–722.
4. Fisher RA, Race RR, Taylor GL (1944) Mutation and the rhesus reaction. Nature 153: 106–106.
5. Li CC (1953) Is the Rh facing a crossroad? A critique of the compensation effect. American Naturalist 87: 257–261.
6. Feldman MW, Nabholz M, Bodmer WF (1969) Evolution of the Rh polymorphism: A model for the interaction of incompatibility, reproductive compensation and heterozygote advantage American Journal of Human Genetics 21: 171–193. PMID: 4976959
7. Novotná M, Haviček J, Smith AP, Kolbeková P, Skallová A, et al. (2008) Toxoplasma and reaction time: Role of toxoplasmosis in the origin, preservation and geographical distribution of Rh blood group polymorphism. Parasitology 135: 1253–1261. doi: 10.1017/S003118200800485X PMID: 18752708
8. Fleg J, Novotná M, Lindová J, Haviček J (2008) Neurophysiological effect of the Rh factor. Protective role of the RhD molecule against Toxoplasma-induced impairment of reaction times in women. Neuroendocrinology Letters 29: 475–481. PMID: 18766148
9. Fleg J, Klose J, Novotná M, Berenreitterová M, Haviček J (2009) Increased incidence of traffic accidents in Toxoplasma-infected military drivers and protective effect RhD molecule revealed by a large-scale prospective cohort study. BMC Infectious Diseases 9: art. 72.
10. Fleg J, Preiss M, Klose J (2013) Toxoplasmosis-associated difference in intelligence and personality in men depends on their Rhesus blood group but not ABO blood group. PLoS ONE 8.
11. Ka ková Š, Šulc J, Fleg J (2010) Increased pregnancy weight gain in women with latent toxoplasmosis and RhD-positivity protection against this effect. Parasitology 137: 1773–1779. doi: 10.1017/S0031182010000661 PMID: 20602855
12. Kočová Š, Smolík J, Fleg J (2010) Increased pregnancy weight gain in women with latent toxoplasmosis and RhD-positivity protection against this effect. Parasitology 137: 1773–1779. doi: 10.1017/S0031182010000661 PMID: 20602855
13. Flegr J, Geryk J, Volny J, Klose J, Cernochova D (2012) Rhesus factor modulation of effects of smoking and age on psychomotor performance, intelligence, personality profile, and health in Czech soldiers. PLoS ONE 7: e49478. doi: 10.1371/journal.pone.0049478 PMID: 23209579
14. Allison AC (1954) The distribution of the sickle-cell trait in East Africa and elsewhere, and its apparent relationship to the incidence of subtertian malaria- Protection afforded by sickle-cell trait against subtertian malarial infection. Transactions of the Royal Society of Tropical Medicine and Hygiene 48: 312–318. PMID: 13187561
15. Kankova S, Flegr J, Calda P (2015) The influence of latent toxoplasmosis on women's reproductive function: four cross-sectional studies. Folia Parasitologica 62.
16. Siegel S, Castellan NJ (1988) Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill. xxiii, 399 p.
17. Kočová Š, Kodym P, Flegr J (2011) Direct evidence of Toxoplasma-induced changes in serum testosterone in mice. Experimental Parasitology 128: 181–183. doi: 10.1016/j.exppara.2011.03.014 PMID: 21458453
18. Kustu S, Inwood W (2006) Biological gas channels for NH3 and CO2: evidence that Rh (rhesus) proteins are CO2 channels. Transfusion Clinique et Biologique 13: 103–110. PMID: 16563833
19. Flegel WA (2011) Molecular genetics and clinical applications for RH. Transfusion and Apheresis Science 44: 81–91. doi: 10.1016/j.transci.2010.12.013 PMID: 21277262
20. Le Van Kim C, Colin Y, Cartron JP (2006) Rh proteins: Key structural and functional components of the red cell membrane. Blood Reviews 20: 93–110. PMID: 15961204
21. Prandota J (2012) Rhesus-associated glycoprotein (RhAG) phenotype of the red blood cells modulates T. gondii infection-associated psychomotor performance reaction times and changes in the human personality profile. Impaired function of the CO2, AQP1, and AQP4 gas channels may cause hypoxia and thus enhance neuroinflammation in autistic individuals. In: Gemma C, editor. Neuroinflammation: Pathogenesis, Mechanisms and Management. New York: Nova Publishers. pp. 423–439.
22. Wakefield J, Salway R (2001) A statistical framework for ecological and aggregate studies. Journal of the Royal Statistical Society: Series A (Statistics in Society) 164: 119–137.
23. Guthrie KA, Sheppard L (2001) Overcoming biases and misconceptions in ecological studies. Journal of the Royal Statistical Society: Series A (Statistics in Society) 164: 141–154.

24. Anstee DJ (2010) The relationship between blood groups and disease. Blood 115: 4635–4643. doi: 10.1182/blood-2010-01-261859 PMID: 20308598