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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 August 2013; accepted: 07 October 2013; published online: 21 October 2013.
Citation: Kato TA, Watabe M and Kanba S (2013) Neuron-glia interaction as a possible glue to translate the mind-brain gap: a novel multi-dimensional approach toward psychology and psychiatry. Front. Psychiatry 4:139. doi: 10.3389/fpsyt.2013.00139
This article was submitted to Neuropsychiatric Imaging and Stimulation, a section of the journal Frontiers in Psychiatry.

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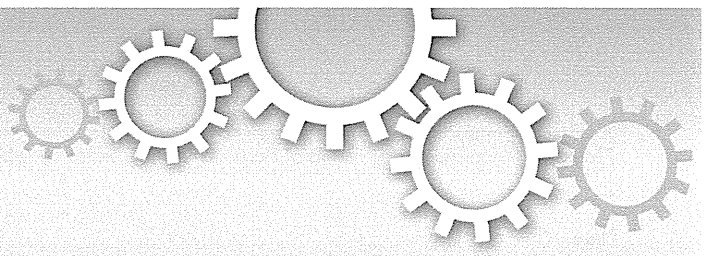

OPEN

SUBJECT AREAS:
MICROGLIA
COOPERATION
PSYCHOLOGY
DECISION

Received
17 December 2012

Accepted
27 March 2013

Published
18 April 2013

Correspondence and requests for materials should be addressed to M.W. (motokiw@gmail.com & motokiw@aoni.waseda.jp) or T.A.K. (takahiro@npsych.med.kyushu-u.ac.jp)

* These authors contributed equally to this work.

Minocycline, a microglial inhibitor, reduces 'honey trap' risk in human economic exchange

Motoki Watabe[1]*, Takahiro A. Kato[2,3]*, Sho Tsuboi[4], Katsuhiko Ishikawa[5], Kazuhide Hashiya[5], Akira Monji[2], Hideo Utsumi[3] & Shigenobu Kanba[2]

[1]Organization for Japan-US Studies, Waseda University, 513 Waseda Tsurumaki-cho, Shinjuku-ku, Tokyo 162-0041, Japan, [2]Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi Higashi-ku, Fukuoka 812-8582, Japan, [3]Innovation Center for Medical Redox Navigation, Kyushu University, 3-1-1 Maidashi Higashi-ku, Fukuoka 812-8582, Japan, [4]Department of Psychology, Graduate School of Letters, Kyoto University, Yoshida Honmachi, Sakyo-ku, Kyoto 606-8051, Japan, [5]Faculty of Human Environment Studies, Kyushu University, 6-19-1 Hakozaki, Higashi-ku, Fukuoka 812-0053, Japan.

Recently, minocycline, a tetracycline antibiotic, has been reported to improve symptoms of psychiatric disorders and to facilitate sober decision-making in healthy human subjects. Here we show that minocycline also reduces the risk of the 'honey trap' during an economic exchange. Males tend to cooperate with physically attractive females without careful evaluation of their trustworthiness, resulting in betrayal by the female. In this experiment, healthy male participants made risky choices (whether or not to trust female partners, identified only by photograph, who had decided in advance to exploit the male participants). The results show that trusting behaviour in male participants significantly increased in relation to the perceived attractiveness of the female partner, but that attractiveness did not impact trusting behaviour in the minocycline group. Animal studies have shown that minocycline inhibits microglial activities. Therefore, this minocycline effect may shed new light on the unknown roles microglia play in human mental activities.

In movies, a female spy often wins the trust of her male target using her physical attractiveness. The male target usually suspects that she is a spy, but because of her attractiveness, he becomes amorously entangled with the female spy despite concerns regarding her trustworthiness. For males, allocating valuable resources to physically attractive females may be evolutionarily adaptive, in that it may increase the probability of producing attractive offspring under natural selection. However, this tendency toward resource allocation to attractive females creates 'noise' that complicates decisions in short-term economic exchanges, leading to the tendency to 'honey trap' males with this behaviour.

In an economic exchange, attractiveness in a female increases sexual arousal in a male that automatically (without careful evaluation of her trustworthiness) facilitates trusting behaviour. While these traits should be adaptive in terms of mate-choice[1], experimental studies have shown that they also affect decisions in social and economic exchange[2,3]. These traits lead to the question of how males can avoid the honey trap.

Recent studies with human subjects show that minocycline, a commonly used tetracycline antibiotic, may facilitate focus on appropriate environmental cues for social decision-making, possibly by reducing noise and other factors (e.g. personality and arousal) that can obstruct decisions. In an economic exchange, one study showed that subjects treated with minocycline make more sober decisions compared to participants treated with placebo[4]. In another study, participants were given dextroamphetamine and those treated with minocycline report less of a 'high' feeling compared to those who did not receive minocycline[4]. Minocycline is also known to improve symptoms associated with psychiatric disorders such as schizophrenia and depression[5–7]. There are past studies examining the effects of physical attractiveness on cooperation in social/economic exchange in different sex pairs, but no study has examined the effects of minocycline on such behaviour in different sex pairs. The hypothesis of this study was that minocycline reduces the risk of the honey trap effect and leads to more appropriate decisions in a short-term economic exchange, through a reduction in the noise triggered by physical attractiveness.

In this experiment, 98 healthy males played a trust game with 8 photographed young females after a 4-day oral treatment course of either minocycline or placebo. Looking at a picture showing a female's face, male players decided how much out of 1300 yen (approximately 13 USD) they would give to each female. Males then evaluated

Table 1 | Mean scores and results of *t*-tests comparing major variables

Item		Conditions		*t*-test	
		Placebo (n = 48)	Minocycline (n = 50)	*t*-value	*p*
Age (years)	Mean	**21.30**	**21.63**	−1.50	0.138
	SD	1.364	1.875		
Offering Rate (0 to 1)	Mean	**0.61**	**0.49**	1.90	0.062
	SD	0.329	0.277		
Mean Attractiveness of All Pictures	Mean	**3.08**	**2.78**	1.37	0.175
(0: Not at all – 10: Perfectly)	SD	1.119	1.076		
Mean Trustworthiness of All Pictures	Mean	**5.52**	**5.37**	0.63	0.528
(0: Not at all – 10: Perfectly)	SD	0.968	1.349		
Mean State Anxiety Score	Mean	**2.11**	**2.28**	−1.50	0.138
(1: Not at all – 10: Very much so)	SD	0.514	0.573		
Mean Trait Anxiety Score	Mean	**2.26**	**2.27**	−0.03	0.979
(1: Not at all – 10: Very much so)	SD	0.514	0.578		

how trustworthy each female was and how physically attractive she was using a 11-point Likert Scale (0: Not at all – 10: Perfectly so). Of note, all of the photographed females had actually decided, in advance, to choose 'betray' against the male players. Therefore, male participants played with untrustworthy female partners, but were unaware of the deception. The impact of attractiveness and trustworthiness on the amount of money given to female partners was analysed. The independent variables were the evaluations/scores of physical attractiveness and trustworthiness given by the male participants.

Results

Table 1 summarizes the mean scores for the major variables and results of a *t*-test used to compare the placebo and minocycline conditions. Consistent with previous reports in which trust games were conducted between healthy male participants[8,9], the offering rate differed marginally between conditions. The State and Trait Anxiety Inventory (STAI)[10] was measured and no significant differences were found for either State or Trait Anxiety scores between conditions.

The primary hypothesis of this study was that the minocycline group would be less affected by the attractiveness of pictured females than the placebo group. To test this hypothesis, an ANOVA was performed with condition (minocycline vs. placebo) and attractiveness (high vs. low) as independent variables and the offering rate of money by participants as the dependent variable. The attractiveness score was not normally distributed ($P = 0.0004$), therefore the score was sub-divided into 2 categories (high vs. low). **Figure 1** shows the mean offer rate by condition and the level of attractiveness. There is a significant interaction effect between condition and attractiveness (F (1,776) = 7.78, $P = 0.005$). Consistent with the primary hypothesis, participants in the placebo group gave larger amounts of money when the partner was more attractive, while participants in the minocycline group did not. According to a simple main effect test, a main effect of attractiveness was detected in the placebo group ($P = 0.0004$), but not in the minocycline group ($P = 0.223$). In addition, Figure 1 shows that, for partners with high attractiveness, the offering rate in the placebo group was significantly higher than in the minocycline group ($P = 0.0004$), but not for less attractive partners ($P = 0.590$).

Discussion

This study demonstrated that minocycline is the first drug shown to reduce the honey trap effect on young males. A previous report using a trust game with an anonymous male partner showed that

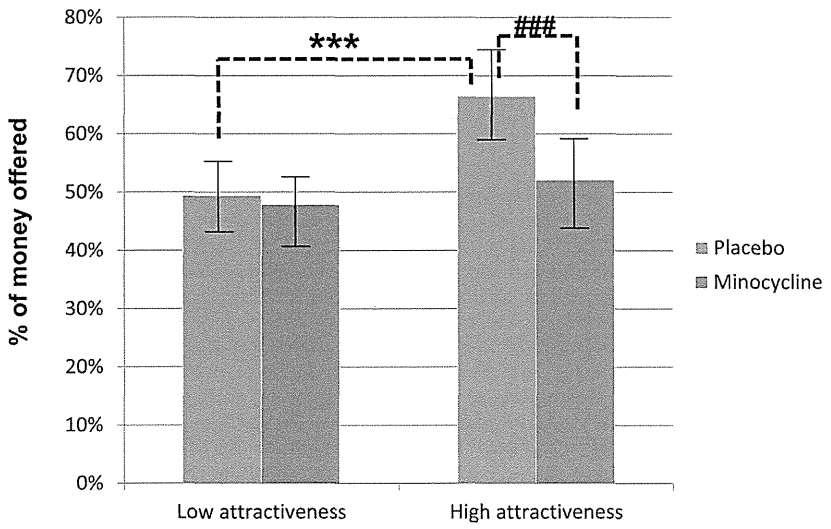

Figure 1 | Mean Offering Rate (percentage of money offered) by the Male Participants to Less- and More-Attractive Female Partners. Error bars represent the standard deviation for each condition. *** For the placebo group, the offering rate to highly attractive female partners is higher than that to partners with low attractiveness ($P = 0.0004$). ### The offering rate to highly attractive partners in the placebo group is higher than that in the minocycline group ($P = 0.0004$).

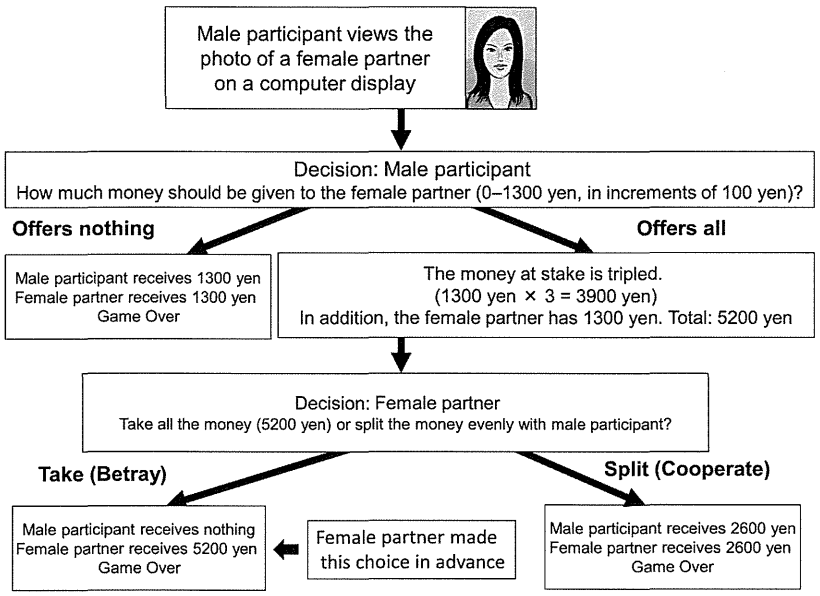

Figure 2 | Trust Game Structure with the Most Extreme Cases.

minocycline reduces decision-making based on personality and trait[9]. Rather, minocycline facilitated decision-making based on situational factors such as game structure and evaluation of others' trustworthiness[8,9]. Consistent with evidence that minocycline attenuates the subjective high feeling associated with dextroamphetamine treatment[4], the current results indicate that minocycline may reduce the effect of arousal and lead to sober decision-making. Recent clinical trials suggest that minocycline improves symptoms in patients with schizophrenia and depression[5–7]. In the current experiment, anxiety was measured and no difference was identified between the minocycline and placebo conditions. Future studies should clarify the effects of treatment with minocycline on psychological processes including mood, impulsivity, and cognitive performance in both healthy volunteers and patients with psychiatric disorders.

In rodent models, minocycline is the most commonly used drug for suppressing microglial activity in the brain[11–13]. In addition, a clinical trial with human subjects has shown that a long-term minocycline treatment (200 mg/d) suppresses microglial activation in various areas of the brain including the putamen, thalamus, and frontal cortex[14]. Microglia are glial cells with immunologic/inflammatory functions that contribute to various brain pathologies, including neurodegenerative diseases[15–17] and psychiatric disorders (e.g. schizophrenia[18–20] and autism[21,22]). Recent animal-model studies have shown that stress increases microglial activation[23–25] and causes anxiety-like behaviours[26]. This behavioural change can be modulated with minocycline treatment[26]. In addition, recent evidence from rodent studies showed that in normal brains, microglia make direct contact with synapses[27–31], suggesting that in this study minocycline may change synaptic reactions by suppressing microglial activity. The amygdala, one of the brain regions most affected by minocycline[32], is activated during judgments of trustworthiness in human faces[33,34]. However, no studies have investigated how microglial activation directly contributes to human social decision-making and how these effects are modulated by minocycline. Taken together, these results suggest that microglial activity in the amygdala may modulate cognitive and emotional processes involving physical attractiveness and evaluation of trustworthiness.

Other possible effects of minocycline should be taken into account. Apart from inhibiting microglial activation, minocycline has also been reported to interact with brain glutamate and dopamine neurotransmission[35] and to have direct effects on neuronal cells[36]. Some reports suggest positive links between microglia and glutamate and dopamine interaction[37,38]. Further research should be performed to clarify the effects of this potential interaction. The dose of minocycline (200 mg/d) used in this experiment was based on previous reports with human subjects[4,14] and different doses may have different effects. Therefore, further trials should be conducted to investigate the effect of minocycline dosing.

To date, the biological mechanisms that underlie the honey trap effect remain poorly understood and no drug has been conclusively proven to attenuate honey trap effects during human social decision-making. The results of the present study suggest that minocycline is the first drug to have a novel pharmacologic function in humans—inhibition of honey trap effects. The current findings may shed new light on the mechanism underlying microglial effects on human mental activities and represent a novel psychopharmacologic approach for modulation of microglia.

Methods

This double-blind randomised trial, one of a series of trust game studies with human male subjects[9], was approved by the Kyushu University Ethical Committee under the administration of the UMIN Clinical Trials Center (**UMIN000004803**). After a complete description of the study, all participants provided written informed consent. Either minocycline or placebo was administered to participants for 4 days, after which they participated in a trust game[39].

Subjects. Participants were recruited using on-campus advertisements. Therefore, all participants were undergraduate or graduate students at Kyushu University. Healthy adult males (age range, 20–30 years) who were capable of providing informed consent were included. Participants were excluded if they met any of the following 4 criteria: (1) any history of experiencing side effects associated with antibiotics, including minocycline; (2) any history of severe heart, liver, or kidney disease; (3) a history of allergic syndromes; and (4) any history of psychiatric disorders. Their mental and physical health was confirmed via interview with a psychiatrist (TAK). After this screening process, 101 healthy adult males were enrolled in the study.

Drug administration. Participants received a hand-out describing their detailed dosing schedule. They were asked to record the exact time each dose was taken, and to keep and submit all capsule packaging, as evidence of medication administration. Participants began the medication (either minocycline or placebo) on the evening of Day 1 and continued taking the medication twice daily (morning and evening) for 3 additional days. The game experiment was conducted on Day 5. Participants were instructed to take the last capsule 3 h prior to their scheduled appointment time, ensuring that all participants had similar drug levels during the actual experiment.

Each capsule contained either 100 mg minocycline (in the treatment group) or 100 mg lactose (in the placebo group). This minocycline dose (200 mg/d) is within the typical range for daily dosing used to treat infections[40] and has also been used in recent clinical trials[4,6,14]. Using a double-blind procedure in advance, participants were randomly assigned to either the treatment group or the placebo group.

Procedure. After 4 days of drug administration, participants were interviewed by a physician regarding drug side effects, other medications, and adherence to the drug administration protocol. Participants then took part in the following trust game.

Trust Game with photographed female partners. In this 2-player game[39], each player was initially given 1300 JPY. The first player (the male participant) then decided how much of the 1300 JPY to give to the second player (the female partner). The amount of money given to the female partner was tripled and the female partner then decided whether to split her money equally with the male participant (namely, cooperate) or to take the entire amount of money (namely, betray). The trust game structure illustrating the most extreme cases is shown in **Figure 2**. All of the female partners were photographed and had decided in advance to take the entire amount of money. However, the male participants were not aware of this decision.

The male participant's decision regarding how much money to give to the female partner is thought to reflect the level of trust the male participant places in his partner. The amount of money given was expected to function as a behavioural measure of the trust the male participant has in the female partner. In this experiment, male participants had no information about the female partner except for a photograph. Therefore, it is likely that male participants based their decisions regarding how much to trust each female partner, on impressions formed on the basis of the photos. After the experiment, each participant was paid an amount of money corresponding to the result of a randomly selected game from all 8 games.

Photo materials. Prior to the experiment, 61 young females were recruited using on-campus advertisements (mean age, 20.08 years; SD, 1.31 years). Each female participant was asked how they would behave in the role of the female partner in the trust game described above, especially in the case of an anonymous male participant that had chosen to give them the entire amount of money. Eleven participants answered 'take the entire amount' rather than 'split equally'. Eight female participants gave permission to use their photos in the experiment (mean age, 19.88 years; SD, 0.93 years). The photographs included the head and shoulders, with a neutral facial expression. During the experiment, each participant was asked if they knew each of the female partners shown in the photographs, in order to avoid confounding effects associated with previous acquaintance. However, there were no acquaintances identified among the participant pairs.

Statistical analyses. Ninety-eight Japanese males, out of 101 initially enrolled, completed all experiments (mean age, 21.49 years; SD, 1.65 years). Of the participants, 3 (1 in the minocycline condition and 2 in the placebo condition) failed to complete the experimental procedure, so the analyses were performed with data from the 98 participants. All data analyses were performed with SPSS (Version 19, IBM Corp., Armonk, NY USA).

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Acknowledgments

This work was financially supported by a Grant-in-Aid from the Japan Society for the Promotion of Science (JSPS) to Dr. Kato, Dr. Watabe, and Dr. Kanba. We thank graduate and undergraduate students from Kyushu University and Waseda University for their assistance and are indebted to all our participants.

Author contributions

Conceived and designed the experiments: T.A.K., M.W. Performed the experiments: T.A.K., M.W., S.T., K.I. Analysed the data: M.W. Contributed reagents/materials/analysis tools: T.A.K., M.W., K.H., A.M., H.U., S.K. Wrote the paper: M.W., T.A.K.

Additional information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Watabe, M. *et al.* Minocycline, a microglial inhibitor, reduces 'honey trap' risk in human economic exchange. *Sci. Rep.* 3, 1685; DOI:10.1038/srep01685 (2013).

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Minocycline Modulates Human Social Decision-Making: Possible Impact of Microglia on Personality-Oriented Social Behaviors

Takahiro A. Kato[1,2*◔], Motoki Watabe[3*◔], Sho Tsuboi[4], Katsuhiko Ishikawa[5], Kazuhide Hashiya[5], Akira Monji[1], Hideo Utsumi[2], Shigenobu Kanba[1]

1 Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, **2** Innovation Center for Medical Redox Navigation, Kyushu University, Fukuoka, Japan, **3** Graduate School of Economics, Waseda University, Waseda, Japan, **4** Department of Psychology, Graduate School of Letters, Kyoto University, Kyoto, Japan, **5** Graduate School of Human-Environment Studies, Kyushu University, Fukuoka, Japan

Abstract

Background: Microglia, one of the glial cells, play important roles in various brain pathologies including psychiatric disorders. In addition, microglia have recently been proved to monitor synaptic reactions via direct-touching even in normal brain. Human microglia may modulate various social/mental functions, while microglial social/mental roles remain unresolved especially in healthy humans. There is no known drug with the specific effect of modulating microglia. Therefore, using minocycline, a tetracycline antibiotic and the most famous microglial inhibitor, is one of the best alternative approaches to clarify microglial functions on human social/mental activities.

Methodology/Principal Findings: We conducted a double-blind randomized trial of trust game, a monetary decision-making experiment, with ninety-nine human adult males who decided how much to trust an anonymous partner after a four-day administration of minocycline. Our previous pilot trial indicated a positive effect of minocycline, while the underlying mechanisms were not clarified. Therefore, in this trial with larger samples, we additionally measured the effects of anxiety and personality. The monetary score in trust game was significantly lower in the minocycline group. Interestingly, participants' ways of decision-making were significantly shifted; cooperativeness, one component of personality, proved to be the main modulating factor of decision-making in the placebo group, on the other hand, the minocycline group was mainly modulated by state anxiety and trustworthiness.

Conclusions/Significance: Our results suggest that minocycline led to more situation-oriented decision-making, possibly by suppressing the effects of personality traits, and furthermore that personality and social behaviors might be modulated by microglia. Early-life events may activate human microglia, establish a certain neuro-synaptic connection, and this formation may determine each human's personality and personality- oriented social behaviors in later life. To explore these mechanisms, further translational research is needed.

Trial Registration: UMIN clinical trial center UMIN000004803

Citation: Kato TA, Watabe M, Tsuboi S, Ishikawa K, Hashiya K, et al. (2012) Minocycline Modulates Human Social Decision-Making: Possible Impact of Microglia on Personality-Oriented Social Behaviors. PLoS ONE 7(7): e40461. doi:10.1371/journal.pone.0040461

Editor: Frank Krueger, George Mason University/Krasnow Institute for Advanced Study, United States of America

Received April 1, 2012; **Accepted** June 7, 2012; **Published** July 13, 2012

Funding: This work was financially supported by Grant-in-Aid from the Japan Society for the Promotion of Science (JSPS) to Dr. Kato, Dr. Watabe, Dr. Hashiya, Dr. Monji, Dr. Utsumi and Dr. Kanba, and a JSPS Research Fellow Grant to Dr. Tsuboi. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: All the authors have declared that no competing interests exist.

* E-mail: takahiro@npsych.med.kyushu-u.ac.jp (TAK); motokiw@gmail.com (MW)

◔ These authors contributed equally to this work.

Introduction

Microglia are one of the glial cells with immunological/inflammatory functions, and contribute to various brain pathologies; not only in neurodegenerative diseases [1,2,3] but also in psychiatric disorders such as schizophrenia and autism [4,5,6]. Minocycline, a tetracycline antibiotic, is known as the most famous microglial inhibitor [7], which has recently been applied to brain diseases such as stroke and neurodegenerative diseases [8,9]. In addition, minocycline has been suggested to be an effective drug for psychiatric disorders [10,11]. These reports suggest that inhibiting microglial activation may modulate human social and mental activities, and rodent studies have indicated this possibility [12,13].

Rodent microglia have recently been shown to monitor synaptic reactions via direct-touching not only in pathological brain but also in normal brain [14,15,16,17], and have proved to play important roles in normal brain development such as synaptic pruning [18]. Neurons and neuronal networks including synapses have been dominantly believed to play crucial roles in human social/mental activities. The above-mentioned evidence indicates

that human microglia may modulate various social/mental functions, while microglial social/mental roles continue to remain unresolved especially in healthy humans.

There is no known drug with the specific effect of modulating microglia. Therefore, using minocycline, a tetracycline antibiotic and the most famous microglial inhibitor, is one of the best alternative approaches to clarify microglial functions on human social/mental activities. One human study suggests that minocycline attenuates the subjective reward effects of dextroamphetamine [19], while, to our knowledge, the effects of minocycline on human social/mental activities are not well understood.

Crockett et al have revealed that serotonin modulates behavioral reactions to unfairness, via a monetary decision-making game with healthy volunteers who were administered tryptophan-depleted amino acid which induces lower serotonin levels [20]. In order to measure human social/mental activities, these monetary decision-making experiments have been actively applied because such experiments enable the analysis of the interaction between social/mental activities and actual social behaviors [21,22]. Pharmacology-based neuro-economic research is showing that human social behaviors are modulated by neurotransmitters such as serotonin and oxytocin [20,23,24,25]. In addition, a significant link has recently been reported between the dopamine D4 receptor gene and fairness preference in ultimatum game [26]. However, the pharmacological interaction of social decision-making beyond neurotransmitters remains to be clarified [27].

As a first step in this direction, we recently conducted a pilot experiment with trust game, one of the decision-making experiments, with minocycline [28]. The forty-nine participants, healthy adult humans, made a monetary decision about whether or not to trust an anonymous partner after a four-day oral administration of minocycline or placebo. The minocycline group showed a strong and positive correlation between their scores in trust game and their pre-evaluation scores in others' trustworthiness, but the placebo group did not. These pilot data have suggested that inhibitory effects of microglial activation may sharpen a sense of trust in social behavior, and this effect would enhance situation-oriented behaviors according to immediate social situations. In trust game, a player's optimal decision depends on his/her prediction about the other player's decision. Thus, social environment, including the other's behavior, determines what behavior the player should take. In our actual life, however, our decisions are determined not only by social environment but also by our fundamental mental factors such as temperament and character (i.e. personality), which are independent from situational factors and may strongly impact on decision-making. These factors may act as a "noise" in trust game and during similar human decision-making situations [29]. Our pilot data demonstrated that only the minocycline group showed situation- oriented decision-making, which suggests that microglia may be inducing the "noise" consistently and inhibiting microglial activation could reduce this "noise" effect. However, the underlying mechanisms of "noise" were not clarified in our previous trial [28], thus the next appropriate step is the measurement of the effects of not only trustworthiness but also anxiety and personality.

Therefore, to clarify the microglial "noise" effect during human social decision-making, we newly explored whether anxiety and personality as a "noise" influences outcomes of trust game on humans with minocycline or placebo. To improve the small sample size and the weaker statistical power of our previous trial, we newly conducted the trial with larger samples (about one hundred participants).

Methods and Materials

The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Checklist S1, Protocol S1 and Protocol S2. This double-blind randomized study was approved by the Kyushu University Ethical Committee under the administration of the UMIN clinical trial center (**UMIN000004803**). All the participants of the present experiment, which was conducted in December 2010, were unique to this study and differ from the previous participants who enrolled in an earlier experiment in March 2010 under the administration of the UMIN clinical trial center (UMIN000003281: published in Watabe et al. Psychopharmacology 2012). Flow diagram of this study is listed on **Figure 1**. All participants gave written informed consent to participate after a complete description of the study. Participants were administered minocycline or placebo for four days, after which they played a trust game with an anonymous partner.

Subjects

Participants were recruited by advertisements on campus. Inclusion criterion was as follows; healthy adult males from 20 to 30 years old who can obtain informed consent. Exclusion criteria were the following five items; 1) those who have had side effects to antibiotics including minocycline, 2) those who have severe heart, liver or kidney disease, 3) those who have a tendency to develop allergies, and 4) those who have been diagnosed with psychiatric disorders. Their mental and physical health was confirmed by interview with a psychiatrist (TAK). All the participants were qualified for this study (**Table S1**).

Drug Administration

Participants received a sheet describing their detailed dosing schedule. They were then asked to write the exact time of every dosing, and to submit every capsule package as evidence of dosing. Participants started to take a capsule in the evening of the first day and twice daily (morning and evening) for four days afterward. On the day of the game experiment (the fifth day), they were instructed to take the last capsule three-hours prior to the appointment time for the experiment so that all participants played the trust game under the similar drug effect. Each capsule contained 100 mg minocycline (in the treatment group) or 100 mg lactose (in the placebo group). This minocycline dose (200 mg/day) is within the range of the usual daily dose used for treatment of infections [30], and this dose has also been applied in recent clinical trials [10,19]. Participants were randomly assigned to the treatment group or to the placebo group in advance, with a double-blind procedure.

Procedure

Prior to drug administration, participants completed a set of questionnaires (details in **Scales**). After four days of drug administration, participants were interviewed by physicians regarding side effects, other medications, and adherence to the drug administration protocol. They then played a trust game [21] and responded to the same set of questionnaires they had completed before administration.

Trust Game

Figure 2 shows the structure of trust game. In this two-player game, each player was initially given 1300 JPY (nine hundred JPY had been used in our previous trial [28], but to let participants recognized clearer incentive and make their decisions more seriously, we used 1300 JPY (about 15 USD) in this new trial so

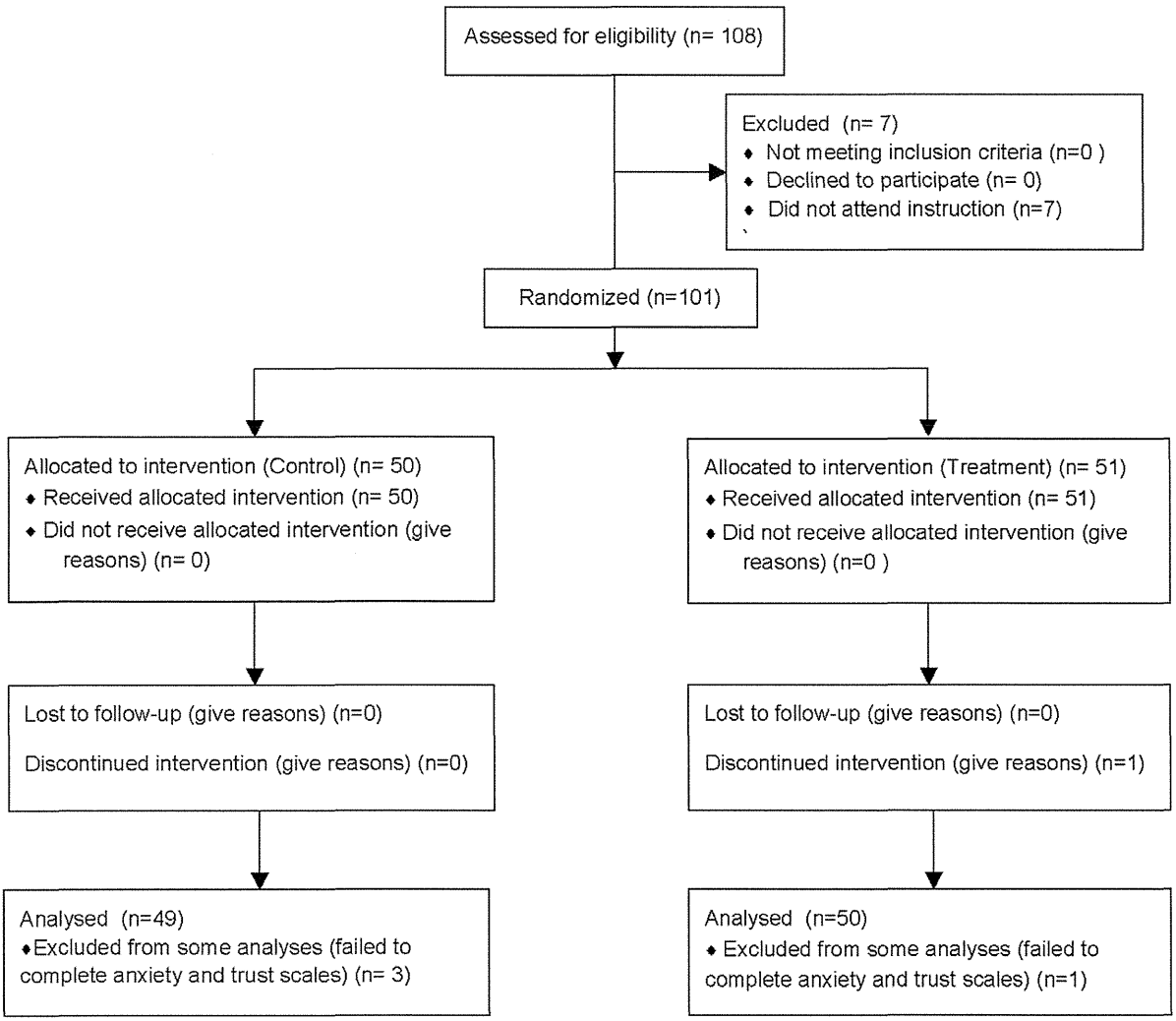

Figure 1. Flow Diagram of This Study.
doi:10.1371/journal.pone.0040461.g001

that we can obtain more reliable behavioral data). The first player then decided how much of the 1300 JPY to give to the second player. The second player then went to another room, where the amount of money given to him by the first player was tripled. The second player then decided whether to split his money equally with the first player or to take all of his money. In this experiment, all the participants were actually assigned to be the first player. The first player's decision as to how much money to give to the second player is thought to be the first player's level of trust in his partner. The amount of money given was expected to be a behavioral measure of the first player's trustfulness.

In this experiment, participants had no information about the partner except that he was male. The participants thus were likely to have made their decisions based primarily on how much they trusted others in general. All the participants' partner was actually a research confederate and always the same person, a 22-year-old Japanese male. In order to control the participant's impression of the partner, the partner acted and talked exactly in the same way throughout all the experimental sessions.

Scales

Our previous trial showed the positive correlation between participants' scores in trust game and their pre-evaluation scores in others' trustworthiness, while we did not examined other psychological factors and thus the underlying mechanisms were not clarified [28]. Therefore, we examined the effects of anxiety and personality, in addition to the trust scores, in this trial. The following self-rated questionnaires were completed by the participants at pre- and post-treatment.

Temperament and Character Inventory (TCI)

TCI is based on the seven-factor model of temperament and character in personality [31]. According to TCI model, personality is classified into temperament, which consists of Novelty Seeking (NS), Harm Avoidance (HA), Reward Dependence (RD), Persistence (PS), and character, which consists of Self-Directedness (SD), Cooperativeness (C), Self-Transcendence (ST) with a four-point Likert type scale. We used a Japanese version with 125 questions (TCI-125) [32], which was kindly provided for use in this study from the HUMAN CAPITAL CONSULTING Corporation, Tokyo, Japan.

State-Trait Anxiety Inventory (STAI)

This anxiety scale with 20 questions consists of two factors; state anxiety, which refers to relatively unstable emotional threat to

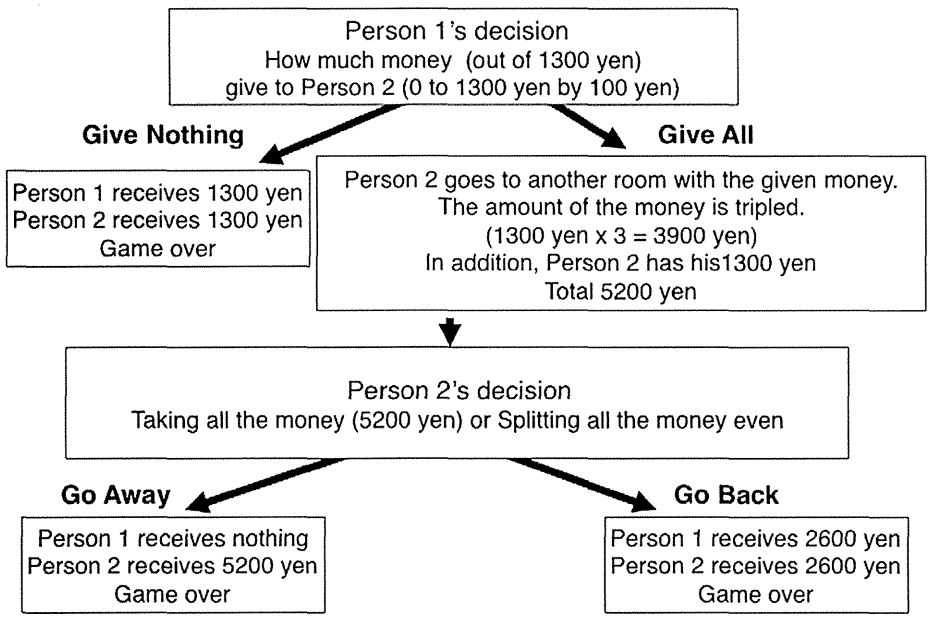

Figure 2. Trust Game Structure.
doi:10.1371/journal.pone.0040461.g002

current situations, and trait anxiety, which refers to relatively stable emotional threat consistently felt in daily life [33].

General Trust Scale (GTS)

GTS consists of six questions with a seven-point Likert type scale developed by Yamagishi and Yamagishi [34]. This scale measures respondents' estimation of others' trustworthiness. The reliability and validity of GTS have been confirmed across many countries [35]. According to past research on GTS, the major confounders of general trust are culture, sex and education level [34]. To eliminate the effects of these confounders, we recruited a homogenous sample as possible. As a result, all the participants were Japanese males and who had collage/university level educations so that we could test the effect of general trust without these confounders.

Data Analysis

Ninety-nine Japanese males, out of 101 entries, completed our experiments (mean age 21.52 years, SD 1.65 years), and analysis was conducted on this data. Among the participants, four (three in the minocycline condition, one in the control condition) failed to complete the questionnaires of STAI and GTS, so the analyses including these two scales were performed with the data of the 95 participants. All of the data analyses were performed with SPSS ver.19.

Results

In our previous trial, the statistical power was 0.766, and the statistical power in the present trial is 0.847. Therefore, the present trial exceeds the suggested efficient power of 0.8. The following analyses are shown with this more appropriate power.

Behavior in Trust Game

We compared the mean amount of participants' monetary offers in trust game by a t-test (**Table 1**). The monetary score in trust game was significantly lower in the minocycline group compared to the placebo group ($t(97) = 2.08$, $p<.05$). This result is consistent with our pilot study [28].

Effects of Minocycline on Personality, Anxiety and Trust

The effects of minocycline on personality, anxiety and trust were evaluated with the seven subscales of TCI, the two subscales of STAI, and GTS. We performed ANOVA with a repeated measure; the scores of the subscales as the dependent variable, and drug condition (Minocycline vs. Control), repeated measure of the subscales' scores (*Before* vs. *After* treatment) and their interaction as independent variables (**Table 1**).

There was no significant interaction term on each of the subscale of TCI. The main effect of time (*Before* vs. *After* treatment) was significant for *Persistence*. The score of *Persistence* is higher *After* (mean score = 13.09, SD = .237) than *Before* (mean score = 13.46, SD = .236). No effect was found on the rest of the items. These results indicate that participants' personality itself was not significantly affected by minocycline.

On STAI, interaction effect and main effect were significant on *Before-After* for state anxiety. Compared to the control group, the state anxiety score increased steeply in the minocycline group. According to simple main effect test, the score after the treatment was significantly higher in the minocycline group than in the control group ($p<.001$). Thus, this result may explain the cause of the lower trusting behavior for minocycline group in trust game. We found no significant effect on trait anxiety.

On GTS, there were no main or interaction effects.

Effects of Minocycline on Decision-Making Style

Next, to examine the effects of minocycline on decision-making style, we performed a multiple linear regression analysis of the amount of money offered (monetary score) in trust game as the dependent variable, and subscales of TCI, STAI and GTS as independent variables by conditions (**Table 2**). We revealed that state anxiety ($\beta = -.795$, $t = -4.42$, $p = .001$) and trust ($\beta = .321$, $t = 2.35$, $p = .023$) have significant effects in the minocycline group ($R^2 = .288$, $F(3,46) = 9.30$, $p = .001$) while only cooperation scale of

Table 1. Behavior in Trust Game, and Effects of Minocycline on Personality, Anxiety and Trust.

Category	Subcategory	Before Treatment		After Treatment		Before-After	Control-Minocycline	Interaciton
		Control	Minocycline	Control	Minocycline			
Monetary Score in Trust Game (%)	–	N/A	N/A	61.38 (32.43)	48.77 (27.70)	N/A	$t(97) = 2.08$, $p<.05$	N/A
TCI (from 1 to 20)	**Self-Transcendence**	10.69 (2.05)	10.82 (2.19)	10.52 (2.47)	10.57 (2.63)	*ns.*	*ns.*	*ns.*
	Cooperative-ness	14.98 (1.79)	14.98 (1.89)	14.80 (1.91)	14.79 (1.98)	*ns.*	*ns.*	*ns.*
	Self-Directedness	12.87 (0.20)	12.71 (0.22)	12.62 (1.98)	12.22 (2.09)	*ns.*	*ns.*	*ns.*
	Persistence	12.86 (2.14)	13.54 (2.53)	13.31 (2.34)	13.76 (2.25)	*ns.*	*ns.*	*ns.*
	Reward Dependence	14.07 (1.88)	14.28 (2.38)	14.10 (1.75)	14.10 (2.21)	*ns.*	*ns.*	*ns.*
	Harm Avoidance	13.51 (2.47)	13.30 (2.37)	13.55 (2.50)	13.26 (2.56)	*ns.*	*ns.*	*ns.*
	Novelty Seekng	12.97 (1.69)	12.99 (1.76)	12.94 (1.77)	13.02 (1.62)	*ns.*	*ns.*	*ns.*
STAI (from 1 to 4)	**State Anxiety**	2.04 (0.45)	2.00 (0.53)	2.11 (0.51)	2.28 (0.57)	$F(1, 93) = 18.60$ $p<.01$	*ns.*	$F(1, 93) = 6.57$ $p<.05$
	Trait Anxiety	2.33 (0.52)	2.21 (0.56)	2.27 (0.51)	2.27 (0.58)	*ns.*	ns.	ns.
General Trust Score (from 1 to 7)	-	4.31 (1.06)	4.51 (1.12)	4.41 (1.07)	4.53 (1.04)	*ns.*	*ns.*	*ns.*

We performed t-test on the behavior (monetary score) in trust game, and the average scores are shown in the Table. The effects of minocycline on personality, anxiety and trust were evaluated with the seven subscales of TCI, the two subscales of STAI, and GTS. We performed ANOVA with a repeated measure; the scores of the subscales as the dependent variable, and drug condition (Minocycline vs. Control), repeated measure of the subscales' scores (*Before* vs. *After* treatment) and their interaction as independent variables. As four participants (three for control, one for minocycline group) failed to complete the questions of STAI and GTS, 95 sets of data were analyzed. Significant and/or marginal effects are shown in the Table. Results were expressed as means (S.D.).

doi:10.1371/journal.pone.0040461.t001

TCI ($\beta = .486$, $t = 2.58$, $p = .013$) was significant in the control group ($R^2 = .092$, $F(3,42) = 2.51$, $p = .078$). Our novel finding in the present study is that the effect of state anxiety was stronger than that of trustworthiness. In sum, for the minocycline group, the less state anxiety and the more trustful, the more trusting behavior; while for the control group, the more cooperativeness, the more trusting behavior.

Discussion

As a first step to explore how microglia modulates human social/mental activities, we showed the novel effect of minocycline, the most famous inhibitor of microglial activation, on human monetary decision-making in trust game. Our previous trial, with smaller sample size and weaker statistical power, indicated the positive effect of minocycline on trust game, while the significant results were limited. In the present trial, we newly revealed that the monetary score in trust game was significantly lower in the minocycline group. Another novel finding was that minocycline treatment itself did not change personality, while, surprisingly participants' ways of decision-making were significantly shifted; cooperativeness, one component of personality, was the main modulating factor of decision-making in the placebo group, on the other hand, the minocycline group was mainly modulated by state anxiety and trustworthiness, both of which are known to be mainly dependent on real-time environments such as present social situation. In addition, the effect of state anxiety was stronger than that of trustworthiness. These results suggest that minocycline led to more situation-oriented decision-making, supporting our "noise reduction" hypothesis [28]; participants' personality may act as a "noise" during human social decision-making and minocycline may mimic personality- oriented behaviors.

Impact of Microglia on Personality-Oriented Social Behaviors

The novel effects of minocycline may explain the unknown role of microglia in social/mental activities. Until now, no study has reported microglial activities in healthy human subjects, while microglia have proved to play important roles in normal brain by communicating with neurons via releasing mediators and synaptic direct contact in rodent studies [14,15,16]. Therefore, human microglia may perform actively even in healthy brains, and inhibiting microglial activation with minocycline may create a shift from personality-oriented behaviors to situation-oriented behaviors by modulating neuro-synaptic-microglial networks. Rodent microglia play essential roles in synaptic pruning [18], which has pointed to the cryptic roles of microglia in human brain development. A recent study suggests that rodent microglial activation by infections during early developmental periods last, and these pre-activated microglia will be re-activated rapidly compared to normal state microglia [36]. Another study has suggested that microglia have a crucial role in the process of early-life memory in rats [37]. Early-life events can significantly modulate normal learning-dependent cytokine activity within the hippocampus, via a specific, enduring impact on brain microglial function, and preventing microglial activation by minocycline during learning prevents memory impairment in neonatally infected rats. Microglia are known to be activated not only by infection but also by physical and psychological stress in rodent studies [12,38,39,40]. In addition, Wei et al. reported that early life stress inhibits expression of a novel innate immune pathway in the developing hippocampus in pups [41]. Based on these recent findings, we suggest the possible existence of the following mechanism on personality and social behaviors; early-life environmental experiences such as psychological stress and traumatic events may activate human microglia, establish a certain neuro-synaptic-microglial connection, which may be memorized unconsciously as a primer for an extended period, and this formation in the human brain may determine each human's personality and personality- oriented social behaviors in later life (**Figure 3**). In addition, we can interpret the present results as follows; the control group's personality- oriented behaviors could be formulated by microglial priming effects, and the minocycline group's situation- oriented behaviors may be induced by suppressing microglial contribution to social behaviors. Further studies are needed to clarify contributions of microglia to human development including personality formation, and social/mental activities in later life.

Clinical Implication

Minocycline has been suggested to be an effective drug for psychiatric disorders [10,11]. Disturbed decision-making is a common symptom of various psychiatric disorders [42,43], and this disturbance is treated by psychotropic drugs such as antipsychotics and antidepressants, which have proved to inhibit microglial activation from *in vitro* studies [44,45,46,47,48]. In addition, a recent study suggests that effort-based decision-making in rat is modulated by estradiol [49], a sex hormone, which also has inhibitory effects on microglial activation [50,51]. These data support our minocycline results, and indicate that psychiatric

Table 2. Multiple Regression Analysis on Behavior in Trust Game.

Independent Variable	Control Group	Minocycline Group
	Beta	Beta
Cooperativeness (TCI)	.486*	
Reward Dependence(TCI)	−.281	
Self-Directedness (TCI)	−.284	
State Anxiety (STAI)		−.583**
General Trust		.321*
	N = 46, R^2 = .092, F(3, 42) = 2.51, p<.10	*N = 49, R^2 = .288, F(2, 46) = 9.30, p<.001*

Note: *p<.05,
**p<.01.
We performed a multiple linear regression analysis of the amount of money offered in trust game as the dependent variable, and subscales of TCI, STAI and GTS as independent variables by conditions. Remarkable effects are shown in the Table.
doi:10.1371/journal.pone.0040461.t002

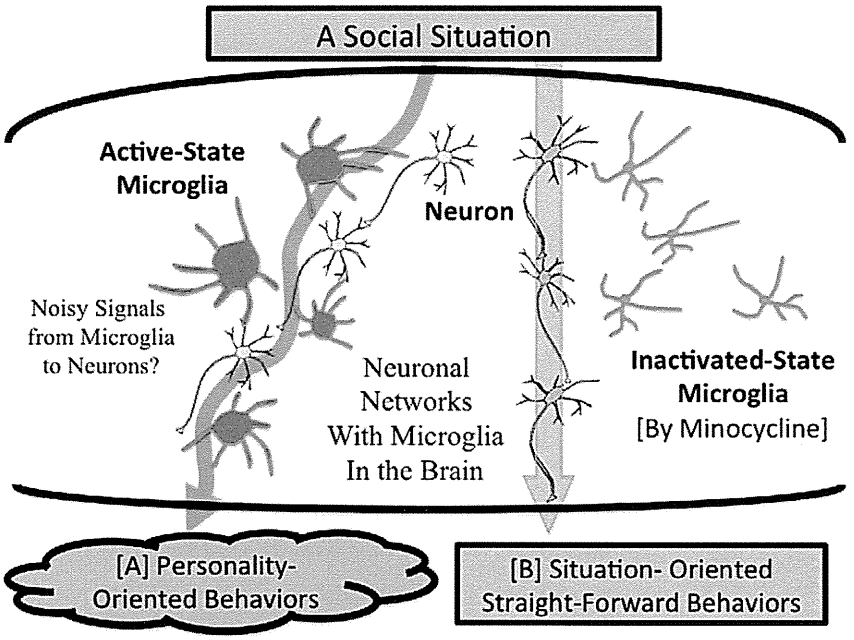

Figure 3. Possible Impact of Microglia on Personality and Social Behaviors. Early-life environmental experiences such as psychological stress and traumatic events may activate human microglia, establish a certain neuro-synaptic-microglial connection, which is memorized unconsciously as a primer for an extended period, and this formation in the human brain determines each human's personality and personality-oriented social behaviors in later life. In sum, neuronal networks with active microglia may induce noisy-decision-making, which is equivalent to personality- oriented behaviors (A). On the other hand, decision-making with neuronal dominant networks may induce straightforward behaviors, which are less affected by personality (B). In the present study, the control group's personality- oriented behaviors could be formulated by microglial priming effects (A), and the minocycline group's situation- oriented behaviors may be induced by suppressing microglial contribution to social behaviors (B).
doi:10.1371/journal.pone.0040461.g003

treatments may modulate microglial contribution to disturbed decision-making in social behaviors. To develop our results and these perspectives, animal based decision-making experiments with minocycline (or other microglial inhibitors) and histological analysis of microglia are called for in the near future. In addition, clinical trials of social decision-making experiments focusing on microglia should be attempted.

Limitation

First, this study did not examine the dose-dependent effects of minocycline. Second, this study was conducted only with adult males, while there may be a difference when players are female. Third, we did not measure microglia activity in the brain via imaging methods, while minocycline may inhibit some brain regional activities which are thought to be linked to trust and social decision-making [52,53]. Therefore, brain imaging studies are needed to clarify these regional activation mechanisms. Finally, other possible minocycline effects should be taken into account. Apart from inhibiting microglial activation, minocycline also has been reported to interact with brain glutamate and dopamine neurotransmission [54,55] and to have direct effects on neuronal cell line, PC12 [56]. Some reports suggest positive links between microglia, glutamate and dopamine interaction [57,58]. Further research should be performed to clarify this interaction mechanism. No specific inhibitor of microglia exists to date, therefore we selected minocycline as the most appropriate and safest drug to be used in humans at present. When a safe, specific inhibitor of microglial activation is developed, microglial human function will be clarified more effectively.

Conclusion

Based on the results of the present human social decision-making experiment, we have proposed a novel microglial contribution to personality and social behaviors. Our present study may shed new light on microglial roles in the social and mental life of healthy humans and also of people with psychiatric disorders. To explore these perspectives, further *in vitro/in vivo* studies and translational research are needed.

Ethics Statement

This double-blind randomized study was approved by the Kyushu University Ethical Committee under the administration of the UMIN clinical trial center (**UMIN000004803**). All participants gave written informed consent to participate after a complete description of the study.

Supporting Information

Table S1 Participants List.
(XLS)

Checklist S1 CONSORT Checklist.
(DOC)

Protocol S1 Trial Protocol.
(DOC)

Protocol S2 Japanese Version of Trial Protocol.
(DOC)

Acknowledgments

We thank Prof. Toshinori Kitamura for his kind advice in the usage of TCI. We thank Kaori Ishibashi, Lui Aoki, Fumika Hideshima, Hideki Horikawa, Yoshihiro Seki, Mina Sato-Kasai and Yusuke Yamauchi for their assistance, and are indebted to all our participants.

Author Contributions

Conceived and designed the experiments: TAK MW. Performed the experiments: TAK MW ST KI. Analyzed the data: MW. Contributed reagents/materials/analysis tools: TAK MW KH AM HU SK. Wrote the paper: TAK MW.

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Resequencing and Association Analysis of *PTPRA*, a Possible Susceptibility Gene for Schizophrenia and Autism Spectrum Disorders

Jingrui Xing[1], Chenyao Wang[1], Hiroki Kimura[1], Yuto Takasaki[1], Shohko Kunimoto[1], Akira Yoshimi[1], Yukako Nakamura[1], Takayoshi Koide[1], Masahiro Banno[1], Itaru Kushima[1], Yota Uno[1], Takashi Okada[1], Branko Aleksic[1]*, Masashi Ikeda[2], Nakao Iwata[2], Norio Ozaki[1]

1 Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan, **2** Department of Psychiatry, School of Medicine, Fujita Health University, Toyoake, Aichi, Japan

Abstract

Background: The *PTPRA* gene, which encodes the protein RPTP-α, is critical to neurodevelopment. Previous linkage studies, genome-wide association studies, controlled expression analyses and animal models support an association with both schizophrenia and autism spectrum disorders, both of which share a substantial portion of genetic risks.

Methods: We sequenced the protein-encoding areas of the *PTPRA* gene for single nucleotide polymorphisms or small insertions/deletions (InDel) in 382 schizophrenia patients. To validate their association with the disorders, rare (minor allele frequency <1%), missense mutations as well as one InDel in the 3'UTR region were then genotyped in another independent sample set comprising 944 schizophrenia patients, 336 autism spectrum disorders patients, and 912 healthy controls.

Results: Eight rare mutations, including 3 novel variants, were identified during the mutation-screening phase. In the following association analysis, L59P, one of the two missense mutations, was only observed among patients of schizophrenia. Additionally, a novel duplication in the 3'UTR region, 174620_174623dupTGAT, was predicted to be located within a Musashi Binding Element.

Major Conclusions: No evidence was seen for the association of rare, missense mutations in the *PTPRA* gene with schizophrenia or autism spectrum disorders; however, we did find some rare variants with possibly damaging effects that may increase the susceptibility of carriers to the disorders.

Citation: Xing J, Wang C, Kimura H, Takasaki Y, Kunimoto S, et al. (2014) Resequencing and Association Analysis of *PTPRA*, a Possible Susceptibility Gene for Schizophrenia and Autism Spectrum Disorders. PLoS ONE 9(11): e112531. doi:10.1371/journal.pone.0112531

Editor: Namik Kaya, King Faisal Specialist Hospital and Research center, Saudi Arabia

Received April 11, 2014; **Accepted** September 30, 2014; **Published** November 13, 2014

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

Funding: Funding for this study was provided by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan; the Ministry of Health, Labor and Welfare of Japan; Grant-in-Aid for "Integrated research on neuropsychiatric disorders" carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan; Grant-in-Aid for Scientific Research on Innovative Areas, "Glial assembly: a new regulatory machinery of brain function and disorders"; and Grant-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network) from the Ministry of Education, Science, Sports and Culture of Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: Dr. Aleksic is a PLOS ONE Editorial Board member, and that does not alter the authors' adherence to PLOS ONE Editorial policies and criteria.

* Email: branko@med.nagoya-u.ac.jp

Introduction

Schizophrenia (SCZ) is a genetically heterogeneous disorder with heritability estimated at up to 80% [1]. In recent years, although research projects such as large-scale genome-wide association studies (GWAS) have focused on common variants, they have failed to explain the majority of the heritability of SCZ [2,3]. Subsequently, great interest has been drawn to rare (minor allele frequency, MAF <1%) missense mutations as potentially important contributing factors to the 'missing heritability' [4,5]. The concept of Autism Spectrum Disorders (ASD) has been defined in the newly released Diagnostic and Statistical Manual of Mental Disorders version 5 (DSM-5) to include previous diagnoses of autistic disorder, Asperger's syndrome and PDD-NOS (pervasive developmental disorders not otherwise specified) [6]. Both SCZ and ASD are recognized as neurodevelopmental disorders, and are reported to have a major overlap of genetic risk, especially from *de novo*, deleterious mutations, [7–10]although further research concerning implicated loci and/or genetic risk factors (i.e., copy number variants [CNV], insertion/deletions, and single nucleotide variants) is required.

The human protein tyrosine phosphatase receptor type A (*PTPRA*) gene encodes the enzyme receptor-type tyrosine-protein phosphatase alpha (RPTP-α), a member of the protein tyrosine phosphatase (PTP) family that is involved in numerous neurodevelopmental processes related to the pathogenesis of SCZ and ASD such as myelination, radial neuronal migration, cortical cytoarchitecture formation and oligodendrocyte differentiation [11–14]. Moreover, RPTP-α is also functionally involved in the *neuregulin 1* (*NRG1*) signaling pathway, which regulates neurodevelopment as well as glutamatergic and gamma-aminobutyric acid–ergic neurotransmission [15–17]. The *NRG1* gene, together with two other genes in the same pathway—*ERBB4*, which encodes a downstream tyrosine kinase receptor[16–18], and *PTPRZ1*, which encodes an *ERBB4*-associated protein tyrosine phosphatase[19]—have been reported to be associated with SCZ [20–22].

Multiple lines of biological evidence implicate the *PTPRA* gene in the etiology of SCZ or ASD. Previous linkage studies conducted in 270 Irish high-density families ($p = .0382$) and an inbred, Arab Israeli pedigree of 24 members (LOD score = 2.56 at 9.53 cM) have pointed to the area that harbors the gene [23,24]. A GWAS comprising 575 cases and 564 controls of the Japanese ethnicity showed an association between polymorphisms within the *PTPRA* gene and SCZ (best uncorrected $p = .002$), albeit not at the level of genome-wide significance [25]. This result was followed by a replication study of 850 cases and 829 controls, which further confirmed the association ($p = .04$, $p = .0008$ for pooled analysis of first and second stages) [26]. Patients carrying copy number variations (CNVs) within the gene have been reported to suffer from autism, or have delayed language and speech development or stereotypical behaviors [27]. Reduced *PTPRA* expression levels have been observed in postmortem brains from patients with SCZ when compared to brains from healthy controls (13% decrease; $p = .018$). In the same study, a significant difference in the expression of mRNA levels of one alternative splicing variant within the gene ($p = .024$) was discovered in an expression analysis using lymphoblastoid cell lines (LCL) derived from 28 patients with SCZ and 20 healthy controls [26]. *Ptpra* knockout mice have been shown to exhibit neurodevelopmental deficiencies and schizophrenic-like behavioral patterns that are thought to model certain aspects of the disorder in humans. In addition, loss of *Ptpra* function in mice also leads to reduced expression of multiple myelination genes, [26] a phenomenon commonly associated with SCZ [28–32] and ASD [33–35] in human patients.

Given the aforementioned studies suggesting the association between *PTPRA* and SCZ/ASD, we decided to sequence the exonic areas of the gene in search for rare, protein-altering mutations that may further strengthen the evidence implicating *PTPRA* as a risk gene for these neurodevelopmental disorders.

Materials and Methods

Participants

Two independent sample sets were used in this study (Table 1). The first set, comprising 382 SCZ patients (mean age = 53.6±14.2; male = 56.5%;), was sequenced for missense rare variants, including single nucleotide polymorphisms (SNPs), small InDels and splicing site variations. The second, larger set, comprising 944 SCZ patients (mean age = 50.4±15.6, male = 58.7%), 336 ASD patients (mean age = 19.3±10.0, male = 77.1%), and 912 controls (mean age = 39.1±15.9, male = 44.5%), was used for association analysis of variants detected in the first phase.

All participants in this study were recruited in the Nagoya University Hospital and its associated institutes. Patients were included in the study if they (1) met DSM-5 criteria for SCZ or ASD and (2) were physically healthy. Controls were selected from the general population and had no personal or family history of psychiatric disorders (first-degree relatives only based on the subject's interview). The selection was based on the following: (1) questionnaire responses from the subjects themselves during the sample inclusion step; or (2) an unstructured diagnostic interview conducted by an experienced psychiatrist during the blood collection step. All subjects were unrelated, living in the central area of the Honshu island of Japan, and self-identified as members of the Japanese population. The Ethics Committees of the Nagoya University Graduate School of Medicine approved this study. Written informed consent was obtained from all participants. In addition, the patients' capacity to consent was confirmed by a family member when needed. Individuals with a legal measure of reduced capacity were excluded.

Resequencing and Data Analysis

The human *PTPRA* gene is located at Chromosome 20: 2,844,830–3,019,320 and has a total of 28 exons (Ensembl release 73; Genome assembly: GRCh37; Transcript: ENST00000380393) (Fig. 1). We included only coding regions and 3′UTR (exons 8–28) (Fig. 2). Genomic DNA was extracted from whole blood or saliva using QIAGEN QIAamp DNA blood kit or tissue kit (QIAGEN Ltd. Hilden, Germany). Primers for 10 amplicons ranging from lengths of 700 to 3000 bps covering all the target exons were designed with the Primer-BLAST tool by NCBI (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and tested for validity with UCSC In-Silico PCR (http://genome.ucsc.edu/cgi-bin/hgPcr). The Takara LA taq Kit (Takara Bio Inc. Shiga, Japan) was used for PCR amplification, and products were cleaned up with Illustra Exonuclease I and Alkaline Phosphatase (GE Healthcare & Life Science, Little Chalfont, United Kingdom). After that, Sanger sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, United States). Upon the initial discovery, for all variants, we used Sanger sequencing to confirm the detection.

Table 1. Profiles of participants in the resequencing and association sample sets.

	Sequencing	Association Study				Total
	Schizophrenia	Schizophrenia	ASD	Control	Total	
Total	382	944	336	912	2192	2574
Male	216 (56.5%)	554 (58.7%)	259 (77.1%)	406 (44.5%)	1037 (47.3%)	1253 (48.7%)
Female	166 (43.5%)	369 (39.1%)	77 (22.9%)	503 (55.2%)	1131 (51.6%)	1297 (50.4%)
Mean Age (years)	53.6±14.2	50.4±15.6	19.3±10.0	39.1±15.9	44.9±18.7	42.3±18.7

Note: Some samples in the association study group were not identified by sex.

doi:10.1371/journal.pone.0112531.t001

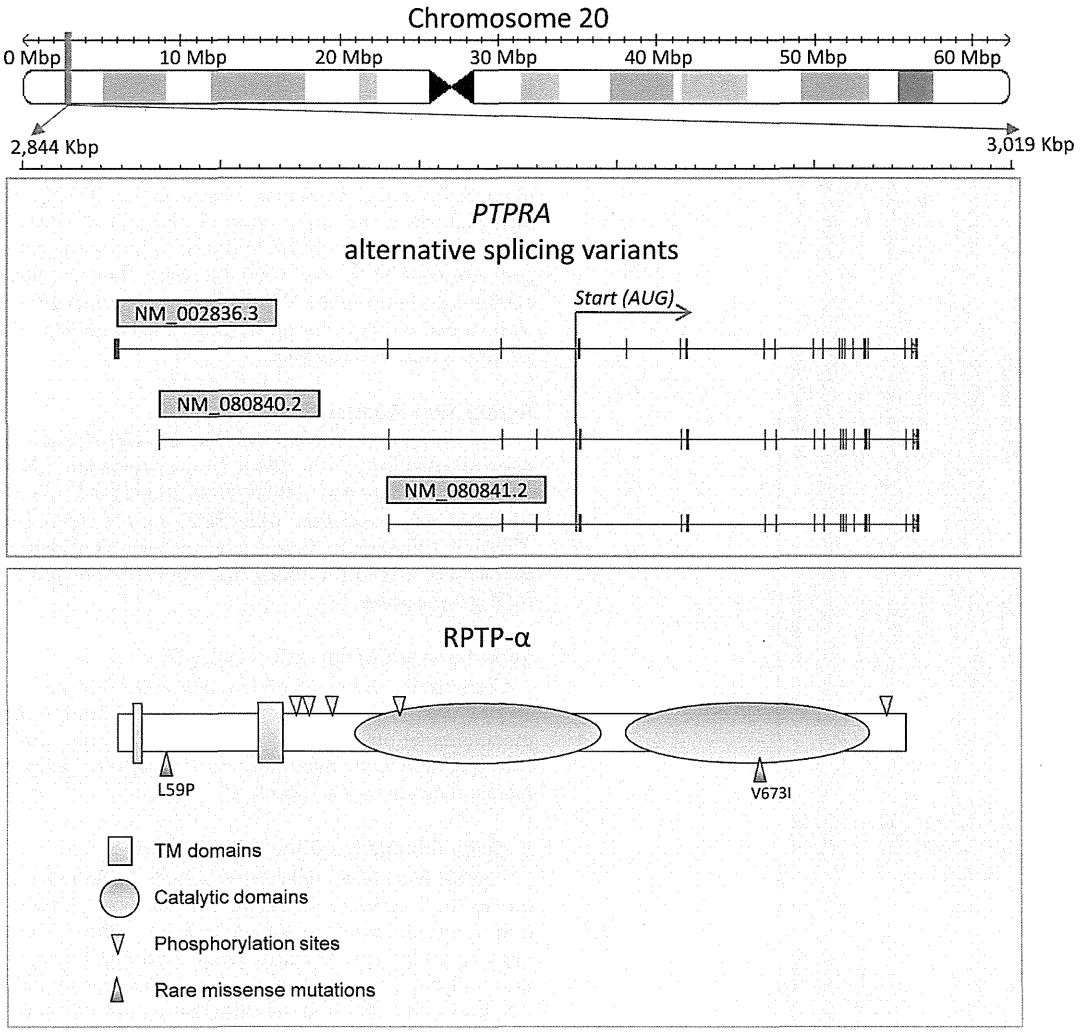

Figure 1. Structure of the *PTPRA* gene, RPTP-α, and position of discovered rare missense mutations.
doi:10.1371/journal.pone.0112531.g001

Sequenced samples were read on an Applied Biosystems 3130xL Genetic Analyzer. Mutation detection was performed with Mutation Surveyor (Softgenetics, State College, PA, USA). The mutation calls were then revalidated for confidence.

Association Analysis

Missense and 3'UTR mutations with MAF<1% were picked up for the association stage. Due to the altering effects that splice site variants have on the structure of mRNAs, and consequently the production of the protein, [36,37] they were also included in the association analysis if they met the MAF criteria.

Custom TaqMan SNP genotyping assays were designed and ordered from Applied Biosystems. Allelic discrimination analysis was performed on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, California, United States). Differences in allele and genotype frequencies of the mutations were compared between SCZ patients/controls and ASD patients/

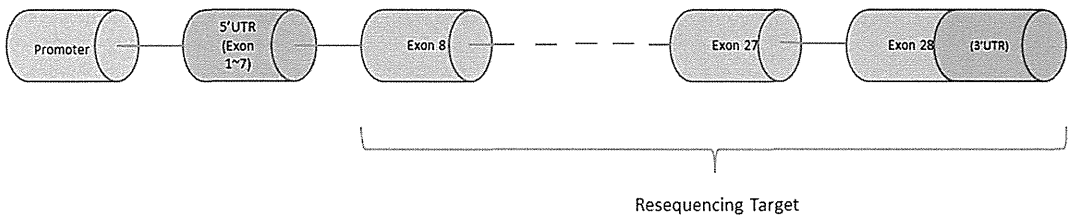

Figure 2. Targeted sequencing areas of the *PTPRA* Gene.
doi:10.1371/journal.pone.0112531.g002

Table 2. Rare exonic mutations identified during the resequencing stage.

Genomic Position [a]	Exon	Base Pair Change [b]	AA Change [c]	Frequency	dbSNP Reference	1000 Genomes	ESP Variant Server
20:3016327	Exon 25	171999G>GA	673V>VI	1/382	rs61742029	Registered	Registered
20:2945609	Exon 9	107281T>TC	59L>LP	2/382	Not Registered	Not Registered	Not Registered
20:3018948	3'UTR	174620_174623 het_dupTGAT	—	1/382	Not Registered	Not Registered	Not Registered
20:3019013	3'UTR	174685A>AT	—	2/382	Not Registered	Not Registered	Not Registered
20:2945649	Exon 9	124753A>AG	Synonymous	4/382	rs138210276	Registered	Registered
20:3005207	Exon 21	160879G>GA	Synonymous	1/382	rs150908061	Registered	Registered
20:3017902	Exon 27	173574G>GT	Synonymous	2/382	rs375917163	Not Registered	Not Registered
20:3017903	Exon 27	173575C>CA	Synonymous	2/382	Not Registered	Not Registered	Not Registered

Notes:
[a]: Based on NCBI build 37.1.
[b]: Based on NCBI Reference Sequence NC_000020.10.
[c]: Based on NCBI Reference Sequence NP_001099043. AA: amino acid.
All mutations are heterozygous.
doi:10.1371/journal.pone.0112531.t002

controls using Fisher's exact test (one-tail), with a threshold of significance set at $p<0.05$.

Results

Mutation Screening Step

Eight rare mutations consisting of 2 missense SNPs, 4 synonymous SNPs and 2 variations located in the 3'UTR area were identified within the target exons (Table 2), 4 of which were not previously reported in dbSNP Build 139 (http://www.ncbi.nlm.nih.gov/projects/SNP/), the 1000 Genomes Project (http://www.1000genomes.org), or the NHLBI Exome Sequencing Project (ESP) Variant Server (http://evs.gs.washington.edu/EVS/). All detected mutations were heterozygous.

Association Analysis

Two missense mutations, rs61742029, which had been previously observed only in the Han Chinese population, L59P, a novel variant, as well as the 174620_174623dupTGAT mutation were validated for association with SCZ and/or ASD in stage 2 (Table 3). Although we were unable to detect significance with our sample sets, it is worth noting that L59P was only present in the SCZ patient group.

Evolutionary Conservation Analysis

Conservation status of rs61742029 and L59P in 11 common species was investigated using Mutation Taster (http://www.mutationtaster.org/). Results showed that the amino acids corresponding to the mutations in RPTP-α were highly conserved among different species (Table 4).

In Silico Functional Effects Prediction

Possible functional implications brought by amino acid changes due to the 2 missense mutations were analyzed with PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), PMut (http://www.ngrl.org.uk/Manchester/page/pmut) and SIFT (http://sift.jcvi.org/). (Table 5) According to the results, the mutation L59P, which was only observed in schizophrenia patients, was predicted to be mostly benign, while rs61742029 showed a high probability of pathogenicity in PolyPhen-2.

3'UTR Motif Prediction

174620_174623dupTGAT, a small duplication discovered in the 3'UTR area, was predicted by RegRNA 2.0 (http://regrna2.mbc.nctu.edu.tw) to be located within a human Musashi Binding Element (MBE), an evolutionarily conserved region shown to affect neural cell differentiation through its mRNA translation regulator properties [38].

Clinical Information of the Carriers of Mutation L59P and 174620_174623dupTGAT

The patient carrying the *PTPRA* L59P mutation was a male diagnosed with SCZ at the age of 19. The patient was born in 1947 had a normal course of development during childhood. In early 1966, he started to suffer from auditory hallucinations, and soon withdrew into an indoor lifestyle. His family reported him being irritated when visited, as well as behaving improperly in public. He was promptly diagnosed and admitted to a psychiatry ward in the same year, and spent the rest of his life living in a hospital. A remarkable improvement was observed in his positive symptoms after admission and administration of antipsychotic drugs; however, he remained secluded, hardly communicating with people around him. At the time of his enrollment in the study, he was 162 cm tall

Table 3. Association analysis results of two rare missense mutations and one 3′UTR variant.

Mutation	Genotype Counts (Resequencing) [a]	Genotype Counts (Association)			P Value [b]	
		SZ	ASD	Ctrl	SZ	ASD
171999G>GA, 673V>VI	**0/3/379**	**0/2/942**	**0/2/334**	**0/4/908**	**0.3276**	**0.2829**
101281T>TC, 59L>L/P	0/2/380	0/0/944	0/0/336	0/0/912	1.0000	1.0000
174620_174623het_dupTGAT	0/1/381	0/0/944	0/0/336	0/1/911	0.4914	1.0000

Notes:
[a]: Homozygote of minor allele/heterozygote/homozygote of major allele.
[b]: Calculated using Fisher's exact test, one-tailed.
Ctrl: healthy controls.
doi:10.1371/journal.pone.0112531.t003

and weighed 48 kg. No comorbid physical or mental illnesses were present. He had 3 children, among whom, one daughter had a history of mental disorder. The patient succumbed to pneumonia in the second half of 2013. In a computerized axial tomography (CAT) scan of the head taken a few weeks prior to patient's death, diffuse neocortical atrophy was observed.

The other patient carrying the L59P mutation was a female diagnosed with SCZ at the age of 34. No childhood development abnormalities were reported, but she was noted to have a history of irritability/aggressive tendencies in high school. Since onset, she had experienced auditory hallucinations and persecutory delusions, as well as continued irritability and aggression. Despite the efficacy of antipsychotic drugs on her positive symptoms, the patient suffered numerous relapses throughout her course of illness due to poor insight and lack of adherence to treatment. At the time of recruitment, she was 61 years old, with a chronic condition of diabetes and no comorbid mental conditions. She died in 2012 at the age of 62.

The patient carrying the *PTPRA* 174620_174623dupTGAT mutation was a male diagnosed with SCZ and comorbid intellectual disability at the age of 27, while he was enrolled in our study. He had a normal conception and birth, born to a 28-year-old father and 27-year-old mother. His father died when he was 3. Delayed intellectual development was observed since his childhood, with reports of illiteracy, hyperactivity, poor concentration and low performance at school. He subsequently dropped out of high school in his first year and started attending a technical school. After graduation, not being able maintain a steady position, he changed part-time jobs frequently. He presented at onset with hallucinations, persecutory delusions, and psychomotor excitement, and was subjected to involuntary commitment due to harmful behavior to others as a result of his delusions. At the time of admission, he was 168 cm tall and weighed 74 kg, with a Wechsler Adult Intelligence Scale (WAIS) score of 49 (Verbal IQ = 57, Performance IQ = 48); he also suffered from stuttering (anarthria literalis). After remission under antipsychotic treatment, he was discharged; however, lack of insight or compliance persisted. It was reported that his mother had a history of panic attacks, and one of his maternal relatives was also diagnosed with SCZ.

Discussion

To our knowledge, this is the first study that systematically screened all coding regions and 3′UTR of the *PTPRA* gene for rare variants in SCZ patients and assessed the association of identified mutations in such a study with SCZ/ASD.

Main Findings

In this study, we sequenced the encoding regions, splicing sites, and 3′UTR region of the *PTPRA* gene in 382 SCZ patients using the Sanger sequencing method, and discovered 8 rare variants. We then conducted association analysis in a much larger sample set for the 2 rare, missense mutations and one 3′UTR InDel identified during the mutation-screening phase in order to investigate their relationship with SCZ and/or ASD.

We were unable to detect a statistically significant association for any of the 3 mutations; this may be attributed partially to the low frequency of rare mutations in the population. However, according to our estimation using CaTS, the power calculator for two-stage association studies (http://www.sph.umich.edu/csg/abecasis/CaTS/), it would require a sample size of around 25,000 cases and controls for the study to obtain possible significance [39,40]. Also, L59P was only detected among SCZ patients in our sample, which infers possible connection of this mutation to the disorder. The evolutionary conservation status of the locus also indicates its biological importance.

Recent studies have discussed the limited impact of protein-coding variants detected in exome resequencing projects, attributing it partly to the fact that most associated variants alter gene expression rather than protein structure. These findings may help explain the lack of association for the 2 missense mutations we detected, while hinting that 174620_174623dupTGAT, predicted to be located within an expression-regulating element, may have a more significant effect. [10]

Additionally, an increasing amount of evidence suggests that genetic risks for SCZ and ASD may not be conferred by the effects of individual variants alone, but also the amplifying interactions between multiple susceptibility loci [41–44]. Thus it may be interesting to sequence the mutation carriers for additional related variants in future.

Limitations

Several limitations should be considered when interpreting the results of our study. The single candidate gene paradigm for a gene with less than robust ties to schizophrenia may have been one of the reasons leading to negative results. Besides, the Sanger method it employed predetermined its relatively small sample size and detection power in contrast with next generation resequencing. In addition, we did not have lymphoblastoid cell lines (LCLs) from the mutation carriers for expression analysis or blood samples from their family members for pedigree study. Therefore, we were unable to follow up the results with further biological evidence. Moreover, some potentially interesting regions of the *PTPRA* gene, such as the promoter, 5′UTR, and most of the intronic