Author’s response to reviews

Title: Genetic Association of Human Corticotropin-Releasing Hormone Receptor 1 (CRHR1) with Internet Gaming Addiction in Korean Male Adolescents

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Version: 1 Date: 14 Oct 2018

Author’s response to reviews:

Response to Reviewers

We greatly appreciate the reviewers’ thorough review and believe that the revisions recommended have substantially strengthened our manuscript. Below we include the verbatim comments of the reviewers and present our detailed responses. The associated revisions are also presented below and appear in red font in the revised manuscript.

Reviewer # 1

1. Abstract. Methods. Just say you performed chi-square tests, citing SPSS at this point is irrelevant.

Response: We have revised the text in the Abstract section according to your comment as follow.

In the Abstract section:

Line 31-34: The DAT1 and DRD4 genes were genotyped by polymerase chain reaction, and the NET8, CHRNA4, and CRHR1 genes were genotyped by pyrosequencing analysis. We performed a Chi-square test to examine the relationship of these five candidate genes to IGA. The DAT1 and DRD4 genes were genotyped by polymerase chain reaction, and the NET8,
CHRNA4, and CRHR1 genes were genotyped by pyrosequencing analysis. We performed a Chi-square test to examine the relationship of these five candidate genes to IGA.

Line 31-34: The DAT1 and DRD4 genes were genotyped by polymerase chain reaction, and the NET8, CHRNA4, and CRHR1 genes were genotyped by pyrosequencing analysis. We performed a Chi-square test to examine the relationship of these five candidate genes to IGA.

2. Results. Don't say you found differences between IGAs and non-IGAs and what was the significance on the chi-square, specify that having the AA genotype and the A allele were associated with higher odds to belong to the IGA group (p=0.016 and p=0.021, respectively) than to the non-IGA group.

Response: We found your comments extremely helpful and have revised the Abstract accordingly as follow.

In the Abstract section:

Line 34-36: Results: Having the AA genotype and the A allele of the CRHR1 gene (rs28364027) was associated with higher odds of belonging to the IGA participant group (p=.016 and p=.021, respectively) than to the non-IGA group.

3. Background. 2nd para. Cortisol triggers negative feedback in the HPA axis, not "The cortisol triggers negative feedback on the HPA axis". A bit further, shown to be higher, not greater.

Two paragraphs ahead, The majority of studies, not "Majority studies". 6th para. "CHR pathways in IGA have been investigated" should be "CHR pathways in IGA has been conducted". 7th paragraph, it seems that Norepinephrine lost its Transporter, please add.

Response: Thank you for your precise comments. We corrected the text according to your comments as follow.

In the Background section:

Line 81-83: Cortisol triggers negative feedback in the HPA axis [14]. In a recent study, serum cortisol levels were shown to be higher in excessive Internet game users than in non-excessive users [15].

Line 93-94: The majority of studies conducted to evaluate the association between genetic factors and IA or IGA have focused on genes of the dopamine system. A 2007 study found that Taq1A1 allele variant on the dopamine D2 receptor (DRD2) gene was more prevalent in an excessive online gaming group than in a control group.
Although research has been conducted on change in CRH pathways in relation to IGA [15,16], few studies have analyzed genetic factors related to CRH systems.

Specifically, we adopted an approach involving gene selection from stress-related genetic variants whose association with IGA has not been previously examined, including Dopamine Receptor D4 (DRD4), DAT1, Norepinephrine Transporter 8 (NET8), CHRNA4, and CRHR1 genes.

4. Methods. 2.2.2. DNA Extraction. Thawing instead of dissolving would be better.

Discussion, 3rd para., towards the end: "...with addiction diseases such as alcohol dependence and nicotine addiction" should read "...with addictive disorders, like alcohol and nicotine use disorders". Last paragraph, just before Conclusions, change "race-specific" with ethnicity-related.

Response: We revised the text in accordance with your comments as follow.

In the Method section:

Line 178-180: After thawing the frozen blood at room temperature, the DNA was extracted from whole blood using a QIAamp Blood Kit (Qiagen, Hilden, Germany) in accordance with manufacturer instructions.

In the Discussion section:

Line 294-296: On the other hand, our results are consistent with the findings of previous studies of Korean populations with addictive disorders--like, alcohol and nicotine use disorders [54, 55].

Line 389-391: In addition, studies of varying racial and ethnic populations are warranted to better understand potential race/ethnicity-related genetic associations with IGA.

Reviewer # 2

1. 5 genes were tested for correlation, but no corrections for multiple comparisons were made. If corrections would be made using the Bonferroni method, no results would remain statistically significant. Therefore it is unclear whether this manuscript has any significant results.

Response: We have carefully considered this comment. As you pointed out, the Bonferroni method has not been used to correct for multiple comparisons in our study. In fact, if we were to apply Bonferroni correction to our thresholds for p values, the detected associations might become nonsignificant. Nevertheless, we described the associations as significant because the
present study is the first to explore genetic predisposition for Internet gaming addiction (IGA). As a pilot study, the results have unique meaning even without Bonferroni correction because no relationship between the HPA axis and genetic predisposition for IGA has been previously reported. In addition, previous studies have not applied the Bonferroni method to multiple comparisons involving a small sample and about 5 SNPs, and the p value was specified as 0.05 [Choi et al., 2014; Malhotra et al., 2016; Nagaya et al., 2018]. In some cases, Bonferroni correction could even increase the likelihood that real effects would be missed [Choi et al., 2014]. Therefore, we set the p value as <0.05 to indicate that the results are initial clues to genetic susceptibility to IGA. In response to your comment, we stated that no correction for multiple comparisons was made in the Method section, and we also included this fact as a limitation of the study in the Discussion section as follow.

* References cited in the response


In the Method section:

Line 227-230: Also, the χ2-tests were carried out to identify differences in genetic polymorphism, including genotype and allele frequency, between the IGA and non-IGA groups. An uncorrected p value of < .05 was adopted as the threshold for statistical significance.

In the Discussion section:

Line 385-387: Finally, we did not correct for multiple comparisons, and therefore the associations between variants in the CRHR1 gene and IGA reported here may be limited to nominal significant associations.

2. The abstract states that CRHR1 polymorphism plays an important role in IGA susceptibility. However, this is a genetic study that tries to discover correlation not causation. Also, the CRHR1 AA-genotype was present in 92.2% of the "IGAs" and 82 % of the "non-IGAs". Therefore it would not seem to be of vital importance, as almost everyone carries it and only a small proportion of the carriers get affected. Maybe the AG-genotype is protective? Of course, from a genotype correlation study no-one could say.
Response: As you commented, this study was intended not to reveal causality but to identify correlations. In addition, the CRHR1 AA genotype difference between the two groups did not seem to be meaningful, although the statistical difference was significant. We hypothesized that genetic variations of the selected five genes (all well known to be addiction-related) would exist between individuals with and without IGA, and we examined the CRHR1 AA genotype difference between the two groups. Based on our correlational results, we concluded that CRHR1 polymorphism may play an important role in IGA susceptibility. In the Abstract, we rephrased “CRHR1 plays an important role” as “CRHR1 may play an important role”. Although a statistically significant difference was found, in fact, most participants carried the CRHR1 AA genotype, and we considered two possible explanations for this finding. First, most of our participants had been involved in Internet gaming for an average duration of 7.12 years (7.64±2.42 years for the IGA group and 6.82±2.38 years for the non-IGA group). That is, being in the non-IGA group did not necessarily mean that participants had not engaged in Internet gaming; rather, they had been engaged in Internet gaming less than the IGA group members. For these reasons, the CRHR1 AA phenotype difference might not have been so obvious. Second, most studies examining genetic polymorphism have reported statistically significant differences in the genotype of a specific gene even though the differences were seemingly small. For example, Kim et al (2011) reported that the frequency of the rs28364027 AA genotype was significantly higher in alcohol-dependent males than in females (92.8% for males vs 86.5% for females). Furthermore, the A allele of the gene was significantly more frequent in alcohol-dependent patients than in controls (97.8% for patients vs 94.7% for controls). In another study, Xie et al (2016) found differences in the frequency of rs2240158 in the GRIN3B gene in heroin addicts. The C allele frequency of rs2240158 was significantly higher in the subjects with heroin poisoning than in the controls (87.3% for patients vs 81.2% for controls). Gerra et al (2007) reported that heroin addicts had less frequent G alleles in the 36G> T polymorphism of the OPRK1 gene (89.62% for patients vs 95.71% for controls). In the case control studies that have been commonly used in genetic association research, a higher frequency of the genotypes or the alleles in subjects is considered to be a risk factor for disease [Hirschhorn et al., 2002]. A change in a single base pair of 3 billion paired nucleotides of the human genome can result in a large change at the functional level [Katara, 2014]. Potential variables such as gene-to-gene or gene-to-environment interactions may affect manifestation of a disease. Considering these points, we believe that the results of this study are meaningful as initial evidence of genetic associations with IGA.

* References cited in the response


In the Abstract section:

Conclusion: These results indicate that polymorphism of CRHR1 may play an important role in IGA susceptibility in the Korean adolescent male population. These findings provide a justification and foundation for further investigation of genetic factors related to IGA.

3. Some might have concerns on deciding that 15-18 year old children can already be classified as addicts, since their personality development is still not finished. Also, the study used cut-off scores of > 38 in the KADO-scale as IGA, although the authors themselves state that scores of 38-49 usually comprise only overuse and potential risk of IGA. How would the results look, if higher cut-off points would be used (say > 49)

Response: The KADO IGA scale has been used across Korea to assess the severity of Internet game overuse since its development and has shown high reliability and validity levels. According to the KADO scale, a score of 49 or above indicates high IGA risk, and a score of 38 or above indicates potential IGA risk that may cause some problems in daily life. In our study, we included both the high IGA risk group (≥49) and the potential IGA risk group (38-48) in the IGA group because they shared similar IGA characteristics such as daily life disturbance, withdrawal, tolerance, and preference for the virtual game world. Furthermore, most previous studies comparing excessive Internet (game) users and ordinary users included both the high risk and potential risk groups in the addiction or overuse group. Regarding the term “IGA,” various terms have been used to describe the phenomenon of IGA and its associated negative outcomes; these terms include “online game addiction,” “pathologic online gaming,” “problematic internet game use,” “internet gaming disorder,” and “internet game overuse.” To avoid confusion, we primarily use the term “Internet gaming addiction” or IGA in our study because we believe that it is clearer and it is commonly used in the research literature. We have added a more detailed explanation of the KADO scale in the Method section as follow.

In the Method section:

Korean adolescents with high IGA risk and with potential IGA risk share most core characteristics of Internet-related addiction, such as daily life disturbance, withdrawal, tolerance, and preference for the virtual world [34, 36]. Based on KADO scale cut-off scores, our
study participants were assigned to either the non-IGA group (scores < 38) or the IGA group (scores > 38). The scale’s Cronbach’s alpha in the current study was 0.93.

* Added references:


4. The manuscript states that subjects were recruited from a sports center. If this is not a center for e-sports, one might think that participants recruited from a sports center might not include the worst cases for IGA (who are likely playing at home, not doing sports). It also seems like quite a coincidence that roughly 50% of the participants had IGA. The recruitment/sampling methods need more description to explain for this.

Response: We recruited participants from nine high schools in a South Korean city and collected data (by means of questionnaires and blood sampling) in a centrally located, easy-to-access public sports center. More specifically, we visited each high school to explain the study's purpose and procedures, distributed a study flyer, and invite interested students to participate; we also directed them to visit the public sports center later if they were interested in participating. We wished to collect data in a stable environment, and the sports center has a quiet private room suitable for data collection. Also, after data collection, we provided health counseling and physical fitness measurement as rewards for study participation, and the sports center was a suitable location to do so. Regarding the high rate of IGA in our study, we recruited participants using convenience and snowball sampling methods to acquire a similar number of participants in the IGA and non-IGA groups for statistical comparison of genetic differences between the groups. To apply snowball sampling, we considered that the developmental characteristics of adolescents would include affinity for their peer groups. Thus, we asked participants to invite friends who were Internet game users to accompany them to the data collection site. We added explanations of our recruitment and sampling methods in the Method section as follow.

In the Method section:

Line 136-142: The 242 participants were 15- to 18-year-old boys recruited from nine high schools in a South Korean city using convenience and snowball sampling methods. We visited each high school to explain the study’s purpose and procedures and invited interested students to participate. We also asked interested students to visit the city’s public sports center—a hub for data collection—on a specific date for collection of study data. To maximize the sample size, we asked the students recruited to invite Internet game-using peers to come with them to the sport center, where we screened them for study eligibility.
Participants first completed a questionnaire in a private room at the sports center and then provided a blood sample. After completing data collection, we provided participants with health counseling and physical fitness measurements as rewards for participating in our study.

5. I could not access the demographic information that was said to be in reference 29. I could only access the abstract for that publication, which states that it had 68 participants? It would seem unlikely to contain information for the over 200 participants described here? This information would be important since, as the authors state, CRHR has been associated with alcohol dependency and no data is shown for this demographic (although it is stated that there are no differences). One might think that there could be differences in other addictive behaviours with IGA and non-IGA subjects. Either that IGA subjects have tendency for other addictions as well, or that IGA subjects do not have the time to get into other addictions, as their time is spent gaming.

Response: In the original manuscript, we mistakenly cited an incorrect reference with respect to our participants’ general characteristics and to describe daily sleep time. We now cite a journal article describing the general characteristics of our total sample of 230, which is designated as reference 31. General characteristics such as age, BMI, perceived academic performance, cigarette smoking, and alcohol consumption did not differ significantly between the IGA and non-IGA groups. Only daily sleep time and Internet gaming-related characteristics (daily gaming time, gaming duration, and IGA score) significantly differed between the two groups. We have revised the text to provide a more detailed explanation of the participants’ demographic characteristics and issues related to sleep time differences in the Results and Discussion sections, respectively as follow.

In the Results section:

Participants’ demographic characteristics, with the exception of daily sleep time, did not significantly differ between the IGA and non-IGA groups. The mean age of the participants was 16.63 years, with ages ranging from 15 to 18 years, and their mean body mass index (BMI) was 21.91 kilograms per square meter (kg/m²), with values ranging from 15.4 to 36.7 kg/m². About one-quarter of the participants reported that they currently smoked cigarettes and drank alcoholic beverages; the percentage was similar in the IGA and non-IGA groups. Perceived academic performance was also similar in the two groups. However, daily sleep time was significantly shorter in the IGA group, with about one-third of the participants indicating that they slept less than 6 hours per day. Regarding Internet gaming-related characteristics, participants’ daily Internet gaming time averaged 171.96 minutes (227.29 minutes in the IGA group and 113.66 minutes in the non-IGA group), their average duration of Internet gaming was 7.21 years (7.64 years in the IGA group and 6.82 years in the non-IGA group), and their average IGA score was 36.50 (46.05 in the IGA group and 26.43 in the non-IGA group). All these Internet gaming-related characteristics significantly differed between the IGA and non-IGA groups. Additional details of the participants’ demographic and Internet gaming-related characteristics are provided in our previous study [31].
In the Discussion section:

Line 336-342: According to the literature, the CRHR1 gene is well known to be related to alcohol dependency, but in our study, alcohol consumption and even smoking rate did not differ between the IGA and non-IGA groups. Among the other covariates, daily sleep time was significantly shorter with IGA than without IGA. However, previous research has provided little evidence that the CRHR1 gene is related to sleep behavior; rather, circadian clock and serotonin transporter genes have mainly been related to sleep phenotypes [69-71]. Thus, our findings suggest that the CRHR1 gene is a promising candidate for IGA risk research.

* Added references:


6. I think it has not been well shown that Internet gaming is a stress event, as is said on line 301. Internet gaming can have a lot of different forms, some more stressful than others. Also the fact that being away from gaming COULD cause withdrawal symptoms does not for a fact mean that these withdrawal symptoms cause significant stress (or happen to all with IGA or overly use of the Internet).

Response: We expanded the explanation of the mechanisms underlying Internet gaming-induced stress and addictive symptoms, including craving, immersion, and withdrawal, and we cited relevant references. Although Internet games can take many forms, such as violent, non-violent, game-alone, group games, and MMORPGs, and the associated stress level would differ, gaming behaviors have been consistently reported to increase stress levels in the literature with few exceptions. Our responses to your comments on this point have been incorporated into the Discussion section as follow.

In the Discussion section:

Line 343-355: Regarding the genetic differences between individuals with and without a certain addiction, researchers have emphasized the framework of gene-environment (G x E) interaction to explain those variations. For example, an interaction between genetic polymorphism and stressful life events was tentatively identified as a predictor or moderator and triggered alcohol use disorders [14, 28, 29], nicotine dependence [72], and cannabis use [73]. Although a variety
of stressors are known to be important risk factors for addiction [12, 13], some researchers have argued that addiction symptoms or addictive behaviors such as cravings [74,75], immersion [76, 77], withdrawal [78], pathologic gambling [16], and game playing [61, 62, 79] could also produce biological stress responses by activating the HPA axis or sympathetic nervous system [15, 16, 74, 80, 81]. Specifically, studies of Internet/online gaming have reported that the activity of the HPA axis and sympathetic nervous system were increased during and after gaming [15, 61, 62, 79, 80] and even in the resting state in prolonged game users [30, 31].

* Added references:


7. Line 307 states that excessive immersion causes stress but does not give any references.

Response: We have cited additional references regarding immersion and added a statement that excessive immersion causes stress within the Discussion section as follow.

In the Discussion section:

Line 348-352: Although a variety of stressors are known to be important risk factors for addiction [12, 13], some researchers have argued that addiction symptoms or addictive behaviors such as cravings [74,75], immersion [76, 77], withdrawal [78], pathologic gambling [16], and game playing [61, 62, 79] could also produce biological stress responses by activating the HPA axis or sympathetic nervous system [15, 16, 74, 80, 81].

* Added references:


8. Line 328 states that behavioural treatments could be tailored for certain genotypes in the future. I don't think we have any implications of this yet?

Response: The medical approach is changing from treatment of the general population to specific target groups. Studies have reported that it is important to improve the efficiency and effectiveness of treatment by providing the most suitable intervention and individualized information for each patient [Celis-Morales et al., 2015; Marsaux et al., 2015]. Although this trend is still in the early stages, intervention studies based on individual subjects’ genotype have been recently reported [Hietaranta-Luoma et al., 2014; Marsaux et al., 2015]. According to these studies, when subjects receive feedback based on individual genetic information rather than general interventions, there is a greater improvement in the subject’s behavior. Also, studies have applied genotypes to the development of preventive interventions for disease and have found that the risk of disease can be reduced through early intervention with subjects with genetic vulnerability [Rothbaum et al., 2014]. Altogether, these studies suggest that genotypes can be applied to behavioral therapy with considerable effectiveness. However, no IGA intervention has been tailored to individuals with a certain genotype. Thus, we have deleted the sentence regarding genetically targeted behavioral treatments for IGA from the Discussion section.

* References cited in the response


9. Line 347 states that early intervention is needed to prevent adverse GENETIC consequences. What would these genetic consequences be?

Response: We expanded the explanation of genetic consequences in the Conclusion section as follow.

In the Conclusion section:

Line 399-403: In addition, early assessment and intervention for adolescents engaging in excessive Internet gaming are needed to prevent adverse genetic consequences, including epigenetic changes resulting in cardio-metabolic health outcomes (e.g., cardiovascular disease, Type 2 diabetes, and hypertension) and psychiatric distress in adulthood [84, 85].

* Added references:


Reviewer # 3

1. In the introduction section you haven't mentioned the DSM-V criteria for internet addiction.
Response: We added information on the DSM-V criteria for Internet gaming disorder to the Background section as follow.

In the Background section:

Line 55-65: For these reasons, Internet gaming disorder (IGD), also referred to as IGA, was formally recognized as a potential mental health disorder and was included in section III of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) as a condition warranting further study [4]. In the DSM-V, the classification of IGD is similar to that of pathological gambling and contains nine descriptive criteria: preoccupation; withdrawal; tolerance; loss of control; giving up other activities; continuation; deception; escape; and loss of significant relationships, job, or educational or career opportunities. However, lack of standardized definitions and diagnostic criteria for IGD has hampered progress on research of this condition [1, 2]. Thus, additional research is necessary to establish clear criteria for IGD diagnosis. In this paper, we focus on IGA rather than IGD because it is somewhat narrower in scope and is commonly referred to in the research literature.

* Added reference:


2. Methods were extensively explained and that section is well written.

Response: We appreciate your comment.

3. Between the lines 73 and 76 in the introduction, you have given two contradictory information about the relationship between serum cortisol level and addiction. However you haven't given an adequate explanation for these contradictory data.

Response: Regarding the contradictory information, our intention is to point out that recent studies of the relationship between addiction behavior and the hypothalamic-pituitary-adrenal (HPA) axis have shown inconsistent results but that addiction is clearly related to the HPA axis, whether positively or negatively. To clarify this point, lines 82-86 have been revised as follow.

In the Background section:

Line 82-86: In a recent study, serum cortisol levels were shown to be higher in excessive Internet game users than in non-excessive users [15]. In another study, serum cortisol levels were negatively correlated with severity of pathological gambling [16]. Although these findings are not entirely consistent, addiction is commonly related to HPA axis activity in study results.
4. In line 132; you have stated "Students with a diagnosed medical condition were also excluded". Can you give a broader description for the exclusion criteria. If they were diagnosed and treated with any medical condition (psychiatric or not) were they included in the study. What were the medical condition that was adequate for the exclusion?

Response: In our study, we excluded those students who were diagnosed as having a medical condition, including any kind of physical or psychiatric distress, and those taking medications that might affect the physiology and genetic stability of the HPA axis. We have expanded the description of the exclusion criteria in the Method section as follow.

In the Method section:

Line 148-150: We excluded students with a diagnosed medical condition, including any kind of physical or psychiatric distress, and those taking medications that might affect HPA axis physiology and/or genetic stability (e.g., β-blockers or sedatives).

5. In the line 135; you have stated "Study data were collected in a public sports center." Were the subjects part of that sports center or were they selected according to their high school? Was the sports center a hub for the interviews and taking blood samples or were the subjects selected according to their sport center membership? Please clarify the confusion mentioned above.

Response: In our study, the public sports center was selected for data collection, including questionnaire completion and blood sampling, because it was centrally located and easy to access, provided a stable environment, and had a quiet private room suitable for data collection. We recruited participants from nine high schools in a South Korean city. More specifically, we visited each high school to explain the study's purpose and procedures, distributed a study flyer, and invited interested students to visit the public sports center later if they were interested in participating. We expanded the explanation of our recruitment and sampling methods in the Method section as follow.

In the Method section:

Line 136-142: The 242 participants were 15- to 18-year-old boys recruited from nine high schools in a South Korean city using convenience and snowball sampling methods. We visited each high school to explain the study’s purpose and procedures and invited interested students to participate. We also asked interested students to visit the city’s public sports center—a hub for data collection—on a specific date for collection of study data. To maximize the sample size, we asked the students recruited to invite Internet game-using peers to come with them to the sport center, where we screened them for study eligibility.

6. In the line, 217 you have stated that "Details of the participants’ demographic and Internet gambling-related characteristics are described elsewhere." You should specify "elsewhere".
Response: This study was a part of a larger research effort that examined the role of the autonomic nervous system in development of IGA among adolescent males. Thus, the participants’ characteristics were reported in our previous study, which was listed as reference number 31. To clarify this matter, we changed the term “elsewhere” to “in our previous study,” and we added a more detailed description of the participants’ demographic and Internet gaming-related characteristics in the Results section as follow.

In the Results section:

Line 234-249: Participants’ demographic characteristics, with the exception of daily sleep time, did not significantly differ between the IGA and non-IGA groups. The mean age of the participants was 16.63 years, with ages ranging from 15 to 18 years, and their mean body mass index (BMI) was 21.91 kilograms per square meter (kg/m2), with values ranging from 15.4 to 36.7 kg/m2. About one-quarter of the participants reported that they currently smoked cigarettes and drank alcoholic beverages; the percentage was similar in the IGA and non-IGA groups. Perceived academic performance was also similar in the two groups. However, daily sleep time was significantly shorter in the IGA group, with about one-third of the participants indicating that they slept less than 6 hours per day. Regarding Internet gaming-related characteristics, participants’ daily Internet gaming time averaged 171.96 minutes (227.29 minutes in the IGA group and 113.66 minutes in the non-IGA group), their average duration of Internet gaming was 7.21 years (7.64 years in the IGA group and 6.82 years in the non-IGA group), and their average IGA score was 36.50 (46.05 in the IGA group and 26.43 in the non-IGA group). All these Internet gaming-related characteristics significantly differed between the IGA and non-IGA groups. Additional details of the participants’ demographic and Internet gaming-related characteristics are provided in our previous study [31].

7. As you have explained between the lines 247 and 250; and throughout your manuscript, racial variations for genetic polymorphisms is one of the main limitations of your study. However you haven’t given broad enough explanation and literature information on the subject.

Response: In general, genetic polymorphism exists not only between racial/ethnic groups but also within a racial/ethnic group (Luczak et al., 2017). Thus, racial/ethnic variations with respect to genetic polymorphism is one of the main limitations in all genetic studies. For this reason, we recruited a homogeneous sample for our study (a Korean-only ethnic group of male gender in a specific age range from 15 to 18 years). In accordance with your comments, to better explain racial/ethnic variations for specific genes, we have expanded the related explanations and the discussion of the literature on subjects with various racial/ethnic backgrounds. Furthermore, we have clearly stated that our sample was relatively homogeneous to minimize racial/ethnic variation in the study. We have made these revisions and cited additional references in the Method and Discussion sections as follow.

* Reference cited in the response

In the Method section:

Line 145-148: The study sample was limited to males belonging to only one ethnic group—Korean—because IGA is more common among male than female adolescents [9] and because there may be gender and ethnicity differences in genetic polymorphism [33, 34].

In the Discussion section:

Line 266-272: In our study, we investigated the influence of gene polymorphism on IGA risk for five stress-related candidate genes: the DRD4 VNTR, DAT1 VNTR, NET8 (rs5569), CHRNA4 (rs1044396), and CRHR1 (rs28364027). We selected a gender- and age-controlled sample to minimize the impact of confounding variables on our genetic findings; in addition, our study was conducted with ethnically homogeneous participants. Notably, we found significant differences in the genotypes and allele frequency of rs28364027 between the IGA and non-IGA groups, but not in those of the other candidate genes.

Line 273-286: Specifically, we found no association between 48 bp VNTR polymorphism in the DRD4 gene and IGA. Our findings are seemingly quite different from study results for Western populations. In Western studies, the 48 bp VNTR polymorphism in the DRD4 gene has been associated with a number of addictive disorders, including substance abuse [39, 40, 41], alcohol dependence [42, 43], and smoking [44]. In particular, the 7-repeat allele of the DRD4 48 bp VNTR polymorphism has been reported as posing a risk for addictive disorders in Western populations [39, 40, 42, 44]. However, in Asians, including the Korean participants in our study, the DRD4 gene has not been associated with addictive disorders [45-47]. The rarity of the 7-repeat allele in Asian populations, including the Japanese, Chinese, and Taiwanese, may have contributed to the inconsistency between our findings and Western study results [34, 48]. On the whole, the genotype distribution of the DRD4 gene has shown considerable variation by ethnicity. Further research is needed to clarify whether the genotype and allele frequency of the DRD4 gene polymorphism influences IGA in particular races and ethnicities.

Line 307-310: For example, the CC genotype of rs1044396 in the CHRNA4 gene was found at a much higher frequency in individuals exhibiting addictive behavior and showed relatively consistent results across ethnicities [25, 58-60].

Line 374-378: The strengths include well-ascertained stress-related phenotypes and a relatively homogeneous sample in terms of age, gender, and Korean-only ethnicity. Moreover, our findings provide a justification and foundation for further investigation of genetic factors related to IGA.

* Added references:


8. In the line 273, you have stated "difference was not significant". This statement is a misuse, which should be corrected with "There was not a significant difference". However if you apply this change all the meaning in that paragraph will change. Do you want to argue that there is actually difference but your statistical analysis couldn't find a significance, in such case 230 subjects should have been plenty to find a statistical significance if there were any. Do you want to argue that if you had more subjects there would have been statistical significance; which would be highly debatable? If you change your statement to "There was not a significant difference" and make changes accordingly, you should argue why there was not a significant difference, instead of a difference that was not significant.

Response: We have changed the statement from “The difference was not significant” to “there was not a significant difference.” We have also changed the explanation regarding why there was not a significant difference accordingly. That is, we have explained the IGA group’s having a more frequent CC variant of rs1044396 than the non-IGA group but not significantly more frequent in several ways: we discussed the possibility of a weak HPA axis effect induced by heavy Internet gaming, our participants’ demographic characteristics, and the possible effect of
the relatively small sample size. We also described our point of view on this matter in the Discussion section as follow.

In the Discussion section:

Line 310-327: Similarly, our study indicated that the CC genotype of rs1044396 was present at a higher frequency in the IGA group than in the non-IGA group, but there was not a significant difference. The IGA group’s having a more frequent CC genotype of rs1044396 than the non-IGA group but not significantly so can be explained in several ways. First, prolonged and excessive Internet gaming might activate the HPA axis [61, 62], resulting in CC genotype of rs1044396; however, this pathway did not seem to cause a significant change in our Korean population. Second, our participants’ demographic characteristics might have contributed to the statistical non-significance. Our participants were restricted to male adolescents in high school, whereas Montag et al. [25] employed male and female university students with and without IA in identifying the candidate gene of IA—the CHRNA4 gene. In Montag’s study, the CC genotype of rs1044396 in the CHRNA4 gene occurred significantly more frequently in the IA group—specifically, this effect was driven by female gender [25]. Third, our study had a relatively small sample, which may have increased the possibility of false-negative results. Because ours is the first study to reveal an association between variants in the CHRNA4 gene and IGA, we cannot be certain that statistically significant differences in polymorphism would have been found in a larger sample. Therefore, replication studies with larger samples are needed to determine whether CHRNA4 gene polymorphism contributes to IGA.

* Added references:
