Variation rs6971 in the Translocator Protein Gene (TSPO) Is Associated with Aggressiveness and Impulsivity but Not with Anxiety in a Population-Representative Sample of Young Adults

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Abstract

Background Expression of the 18 kDa translocator protein, originally identified as a peripheral benzodiazepine receptor, has been found to be altered in several psychiatric disorders. A common single nucleotide polymorphism rs6971 in the TSPO gene leads to an amino-acid substitution, Ala147Thr, which dramatically alters the affinity with which TSPO binds drug ligands. As cholesterol also binds TSPO in the same transmembrane domain, it is suggested that this substitution may impair the ability of TSPO to bind or import cholesterol, and hence may affect steroid synthesis and HPA function.

Methods The analysis was carried out on older birth cohort (n=655) of the longitudinal Estonian Children Personality, Behaviour and Health Study sample. Anxiety, aggressive behavior, impulsiveness, and history of stressful life events were self-reported in various data collection waves.

Psychiatric assessment of lifetime prevalence of anxiety disorders was carried out at age 25 by experienced clinical psychologists. *TSPO* rs6971 was genotyped in all participants.

Results *TSPO* rs6971 was not associated with self-reported levels of anxiety or lifetime prevalence of anxiety disorders. However, subjects homozygous for the minor A allele displayed the highest aggressiveness and dysfunctional impulsivity scores. The positive, adaptive aspect of impulsivity was sensitive to stressful life events as AA genotype was associated with functional impulsivity only when the subjects had experienced a low number of SLEs during childhood.

Conclusions *TSPO* rs6971 polymorphism may be related to development of aggressiveness and impulsivity by adulthood, regardless of the subjects' gender.

Keywords: TSPO, benzodiazepine receptor, aggressiveness, impulsivity, anxiety

1. Introduction

The 18 kDa translocator protein (TSPO) has become a highly promising candidate for explaining the basis of different psychiatric impairments. TSPO is a five transmembrane domain protein localized mainly in the outer mitochondrial membrane of steroid-synthesizing tissues, including the brain.

TSPO was identified decades ago in a search for peripheral tissue binding sites for benzodiazepines (diazepam), and was formerly called the peripheral benzodiazepine receptor (Braestrup & Squires, 1977). Benzodiazepines are one of the most widely available class of drugs that have been prescribed to treat patients with anxiety, convulsions, and insomnia. Subsequent studies showed that the central benzodiazepine receptor (CBR) and the peripheral benzodiazepine receptor (PBR) were distinct proteins that differed in tissue distribution, pharmacology, and cellular and subcellular localization (Anholt et al., 1986; Patel & Marangos, 1982). CBRs are primarily expressed in neurons and coupled to GABAa receptors, regulating the GABA-regulated chloride channels (e.g., Tallman et al., 1978), while PBRs have a more versatile distribution (e.g., Richards & Mohler, 1984). PBRs have been located in glial cells (e.g., astrocytes, microglia; Junck et al., 1989; Schoemaker et al., 1982), endothelial cells, tanycytes, and some populations of neurons (Lee et al., 2016; Notter et al., 2018). PBR is primarily located on the outer membrane of mitochondria (playing a role in maintaining the mitochondrial membrane potential) and found to be abundant in steroid-synthesizing cells (Papadopoulos et al., 2006). PBR was renamed as 18 kDa Translocator Protein (TSPO) in 2006 by the HUGO Gene Nomenclature Committee (Papadopoulos et al., 2006) reflecting its putative function in protein or ligand transport/translocation (Bonsack & Sukumari-Ramesh, 2018).

Microglia and macrophages are the predominant cell types expressing TSPO in diseased brains; in addition, astrocytes can also express TSPO in humans (Cosenza-Nashat et al, 2009). As the resident brain macrophages, microglia function as immune sentries, and they become activated in both acute and chronic conditions in a context-dependent manner (Cosenza-Nashat et al, 2009). *In vivo* and *in vitro* administration of TSPO ligands results in suppression of microglial activation, including inhibition of cytokine expression (Choi et al., 2002; Veiga et al., 2007). TSPO is a widely used biomarker of neuroinflammation, but its non-selective cellular expression pattern implies roles beyond inflammatory processes (Notter et al., 2020).

TSPO mRNA levels have been found to be decreased in the lymphocytes of clinically diagnosed anxious patients (Nudmamud et al., 2000). TSPO expression levels are altered in several psychiatric disorders in which anxiety is the main symptom. A decreased number of platelet PBRs/TSPO receptors has been reported among patients with panic disorder (Marazziti et al., 1994), posttraumatic stress disorder (Gavish et al., 1996), generalized social phobia (Johnson et al., 1998), and following a suicide attempt (Marazziti et al., 2005). In addition, a decreased number of PBRs/TSPO receptors has been found in lymphocytes of patients with generalized anxiety disorder (Ferrarese et al., 1990; Rocca et al., 1992).

TSPO plays an important role in the synthesis of neurosteroids (e.g., allopregnanolone, pregnanolone), thereby representing a putative novel target for anxiolytic compounds (Nothdurfter et al., 2012). Steroids are known to act on neuronal GABAa and other receptors, thereby affecting neurotransmission and neuronal function (Papadopoulos et al., 2015). Stimulation of TSPO has been shown to have therapeutic use as anxiolytic by inducing allopregnanolone production in the brain in animal models (Papadopoulos et al., 2018). Endogenous allopregnanolone influences aggression levels in rodents, with an inverted U-shaped

dose–response curve - moderate doses evoke aggression, whereas low or high doses reduce aggressive behavior (Miczek et al., 2002). In humans, reduced levels of allopregnanolone in the peripheral blood or cerebrospinal fluid have been found to be associated with anxiety disorders and impulsive aggression (among others) and TSPO ligands have been found to increase neurosteroidogenesis and have anxiolytic effects (reviewed by Schüle et al., 2014).

The common single nucleotide polymorphism rs6971 in the TSPO gene leads to an amino-acid substitution, Ala147Thr, which dramatically alters the affinity with which TSPO binds drug ligands (Owen et al., 2012). This functional polymorphism confers a deletion of the C-terminus on TSPO's fifth transmembrane domain, reducing cholesterol entry into mitochondria, consequently blunting production of steroid precursors (Lacapere & Papadopoulos, 2003; Li & Papadopoulos, 1998; Prossin et al., 2018). This polymorphism has been linked to anxiety-related clinical conditions (Costa et al., 2009b: n=372; Nakamura et al., 2006: n=269).

Anxiety-related disorders are often characterized by impaired social behaviors including excessive aggression and violence (Neumann et al., 2010). Namely, it is reactive aggression that is associated with anxiety (Fite et al., 2010). It may be that the worry and stress of high anxiety scorers may prime some individuals towards engaging in reactive aggression. This could be a function of the sympathetic nervous system being more active and/or it could represent a defensive desire to protect oneself from perceived stresses and dangers (Book et al., 2019). Aggressiveness may be an advantageous quality, but can also have undesirable consequences when displayed in an inappropriate situation. Excessive and uninhibited aggressive behavior is an associated symptom of many psychiatric disorders and can manifest throughout the life span,

from attention-deficit hyperactivity disorder (ADHD) in children and adolescents, to domestic violence in adults, to dementia in older adults (Liu et al., 2013).

One of the characteristic components of uncontrolled aggression is enhanced impulsivity (Nelson & Trainor, 2007; Nestor, 2002). As reviewed by Moeller et al. (2001), impulsivity has been defined as swift action without forethought or conscious judgment, behavior without adequate thought, and the tendency to act with less forethought than do most individuals of equal ability and knowledge. Impulsivity also includes a tendency to live on the spur of the moment, lacking a careful planning of short and long-term goals (Patton et al., 1995). Impulsivity has been shown to predict trait aggression and episodes of aggressive behaviors in forensic patients (Bousardt et al., 2016; Tonnaer et al., 2016). Also in the general population, there have been consistent findings linking impulsivity facets (e.g., lack of premeditation, lack of perseveration, sensation seeking) and indices of aggression (reviewed by Garofalo et al., 2016). Impulsivity is moderately heritable (up to 50%) as are disorders with which it is associated (Bevilacqua & Goldman, 2013).

Based on previous results, we tested the hypothesis that the minor allele of the *TSPO* rs6971 (Thr substitution) might be related to higher levels of anxiety and occurrence of anxiety disorders in general population. Since stimulation of TSPO has also been shown to induce allopregnanolone production in the brain and levels of allopregnanolone have been associated with aggressive behavior and impulsive aggressiveness, we also analyzed the effect of *TSPO* rs6971 on aggressive behavior and impulsiveness. Emerging evidence suggests that by reducing both cholesterol binding/transport and subsequent cortisol production, this *TSPO* functional polymorphism may enhance susceptibility to environmental stress

(Colasanti et al., 2013; Prossin et al., 2018). In addition, we examined the possible interaction between the *TSPO* genotype and stressful life events (SLEs).

2. Materials and Methods

2.1. Study Population

The analysis was carried out on older birth cohort of the Estonian Children Personality, Behaviour and Health Study (ECPBHS) sample. This is the original Estonian sample of the European Youth Heart Study (1998/99) which was subsequently incorporated into the longitudinal ECPBHS. All the subjects are of European descent. The principles of formation of the original sample has been described in detail in Harro et al. (2001). In brief, this is a representative birth cohort sample of the Tartu city and county with a school as the sampling unit. All schools of Tartu County, Estonia, that agreed to participate (54 of the total of 56) were included into the sampling using the probability proportional to the number of students of the respective age groups in the school, and 25 schools were selected. All children from grades 3 (younger birth cohort) and 9 (older birth cohort) were invited to participate.

The total number of subjects in the older birth cohort during the initial data collection wave in 1998/99 was 593 (mean age=15.4, SD=0.6). The follow-up studies for the older cohort took place in 2001 (n=479, including 62 additional subjects, mean age=18.4, SD=0.9), 2008 (n=541, mean age=24.7, SD=0.7) and 2016 (n=504, mean age=33.5, SD=0.7). ECPBHS is population representative, while 79.1% of subjects of the randomized regional sample participated in the original sampling. The study was approved by the Ethics Review Committee

on Human Research of the University of Tartu. Written informed consent was obtained from all the participants, and in case of minors, also from their parents.

2.2. Measures

2.2.1. Anxiety

The Spielberger State Anxiety Inventory (STAI-S; Spielberger et al., 1983) used at 25 and 33 years and the Spielberger Trait Anxiety Inventory (STAI-T) at ages 18, 25 and 33 to assess self-reported levels of anxiety. Internal consistency coefficients for the scale have ranged from .86 to .95; test-retest reliability coefficients have ranged from .65 to .75 over a 2-month interval (Spielberger et al., 1983). Psychiatric assessment of lifetime prevalence of anxiety disorders based on DSM-IV was carried out in both birth cohorts at age 25 by experienced clinical psychologists using the Mini-International Neuropsychiatric Interview (M.I.N.I.5.0.0; Sheehan et al.., 1998; Estonian version: Shlik et al., 1999). The prevalence of affective, anxiety and substance use disorders in the sample has been published elsewhere (Laas et al., 2014, 2015).

2.2.2. Aggressive Behavior

During the last data collection wave at age 33, aggressive behavior was self-reported by Illinois Bully Scale and by Buss-Perry Aggression Questionnaire (Laas et al., 2017).

Illinois Bully Scale is an 18-item scale with three subscales, Bully, Fight, and Victim, assessing the frequency of bullying behavior, fighting, and victimization by peers (Espelage & Holt, 2001). Cronbach's alpha coefficients have been previously found to be 0.87 for the total scale, 0.71 for

victims, 0.77 for bullying, and 0.76 for the fighting subscales; all of which are satisfactory (Akbari Balootbangan & Talepasand, 2015). Subjects were asked to recall the times spent in primary school assessing the frequency of listed behaviors in 5-point scale ranging from 'never' to 'very often'.

The 29-item self-report Buss-Perry Aggression Questionnaire (Buss & Perry, 1992) assesses 4 aspects of aggressive behavior: Physical aggression, Verbal aggression, Anger, and Hostility. The internal consistency coefficients have been determined as follows: Physical Aggression, α = .85; Verbal Aggression, α = .72; Anger, α = .83 and Hostility, α = .77, with the internal consistency being α = .89 (Buss & Perry, 1992). Participants rated each statement on a 5-point Likert Scale (uncharacteristic=1, characteristic=5).

The subscales of Illinois Bully Scale and Buss-Perry Aggression Questionnaire are weakly to moderately correlated (r=0.14 – 0.6; see Kiive et al., 2017). Thus, the shared covariance indicates that to certain extent they measure the same underlying concept but since there is also unique variance, each subscale captures a specific aspect of aggressiveness.

2.2.3. Impulsiveness

All measures were self-reported using the Adaptive and Maladaptive Impulsivity Scale (AMIS) and Barratt Impulsiveness Scale, 11th version (BIS-11). AMIS follows the concept of functional and dysfunctional impulsivity (Dickman, 1990). It comprises subscales measuring fast decision-making and excitement seeking (functional or adaptive impulsivity, Cronbach's α = .74) and disinhibition and thoughtlessness (dysfunctional or maladaptive impulsivity, Cronbach's α = .85) and was used at ages 18, 25 and 33 (Laas et al., 2010; Paaver et al., 2008). BIS-11 (Patton et al.,

1995; Paaver et al., 2007) was used for measuring impulsiveness at ages 25 and 33. Internal consistency coefficient (α) for the BIS-11 have been determined to be in the range of .79 - .83 depending on the population (Patton et al., 1995).

2.2.4. Stressful Life Events

History of stressful life events (SLEs) was self-reported in all follow-up studies (Laas et al., 2015). Subjects were divided into low (two or fewer events) and high (three or more events) SLE exposure groups. The list of adverse life events varied across measurement times and consisted of 10–17 (dependent on the study wave) stressful experiences, including parental death and divorce/separation, unemployed parent, parental alcoholism, poverty, poor living conditions, poor health, accidents and traumas, physical abuse, emotional abuse, severe burden/serious concerns, suicidal attempts, leaving home for several days without telling anyone, depression of a close relative, and suicide attempt or committed suicide of a close relative. The events were recorded as dichotomous variables (present or not present) and were then counted to form the number of experienced adverse life events.

2.2.5. Genotyping

Genomic DNA was extracted from venous blood samples using Qiagen QIAamp® DNA Blood Midi Kit. The real-time polymerase chain reaction (RT-PCR) for genotyping the *TSPO* rs6971 polymorphism was performed using a TaqMan Pre-Designed SNP Genotyping Assay (Applied Biosystems; Foster City, CA, USA) C___2512465_20 containing primers and fluorescent probes. Genotyping reactions were performed in a total volume of 10 µl with ~25 ng of template

DNA. RT-PCR reaction components and final concentrations were as follows: 1:5 5 x HOT FIREPol® Probe qPCR Mix Plus (ROX) (Solis BioDyne) and 1:20 80 x TaqMan Primers Probe. Context sequence [VIC/FAM] was as follows:

CCCCTACCTGGCCTGGCTGGCCTTC[A/G]CGACCACACTCAACTACTGCGTATG.

Reactions were performed on the Applied Biosystems ViiA™ 7 Real-Time PCR System. The amplification procedure consisted of an initial denaturation step at 95 °C for 12 min and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Positive and negative controls were added to each reaction plate. No inconsistencies occurred. Genotyping was performed blind to all phenotypic data. Allele frequencies agreed with National Center for Biotechnology Information database (*e.g.*, 1000 Genomes Project phase3 sequence data, European sample) and published reports. Genotype frequencies were in Hardy–Weinberg equilibrium. The frequency of the minor A allele was 0.27 (alleles are reported in the Forward orientation).

2.3. Statistical Analysis

Subjects were divided by *TSPO* rs6971 genotype into AA, AG, and GG groups. The effect of *TSPO* rs6971 genotype on lifetime prevalence of anxiety disorders was assessed by Pearson's chi-square test. Analysis of variance (ANOVA) was used to test the effects of *TSPO* rs6971 genotype on self-report measures of anxiety (STAI-S, STAI-T), aggressiveness (IBS, BPAQ) and impulsiveness (AMIS, BIS). For each model, F-values, two-sided raw *P* values, and effect sizes (η^2), as derived from the SPSS output, were reported. *TSPO* rs6971 interaction effects with SLE on the impulsiveness scores were also analyzed using ANOVA. Impulsiveness measures was defined as the dependent variable. SLE (as a binary variable, split by median) and

TSPO rs6971 genotype (as a categorical variable) were defined as fixed factors, and factor interactions were included in the model. Fisher's least significance difference method (LSD) was used in all *post hoc* comparisons. Contrasts were calculated for significant model effects. All *P* values are reported as 2-tailed, and raw results are considered significant at the $P < .05$ level. Statistical analyses were performed using IBM SPSS Statistics, Version 21.

3. Results

TSPO rs6971 polymorphism was not associated with self-reported levels of anxiety or lifetime prevalence of anxiety disorders.

When analyzing the effects of *TSPO* rs6971 polymorphism on aggressive behavior during school years (Figure 1), we observed that the subjects homozygous for the A allele were the ones reporting bullying other students the most [$F(2, 500)=3.9$, $p=0.022$, $\eta^2=0.015$]. *TSPO* rs6971 genotype was not associated with actual fighting [$F(2, 500)=0.4$, $p=0.688$, $\eta^2=0.001$] or being victimized [$F(2, 500)=0.3$, $p=0.708$, $\eta^2=0.001$] during the school years. AA homozygotes were also acting most aggressively by age 33 (Figure 2) according to the Buss Perry Aggression Questionnaire (BPAQ). The AA homozygotes had higher Verbal Aggression scores than G allele carriers [$F(2, 496)=4.1$, $p=0.016$, $\eta^2=0.016$]. There were no significant associations with the Physical Aggression score [$F(2, 496)=1.5$, $p=0.214$, $\eta^2=0.006$], Anger [$F(2, 496)=1.1$, $p=0.322$, $\eta^2=0.005$], Hostility [$F(2, 496)=0.1$, $p=0.93$, $\eta^2<0.001$] or the BPAQ total score [$F(2, 496)=1.1$, $p=0.32$, $\eta^2=0.005$].

Impulsive behavior measured using the Barratt Impulsiveness Scale (BIS) was also associated with the *TSPO* rs6971 polymorphism by age 33 (Figure 3), but not at earlier age (data not

shown). At age 33, AA homozygotes received higher total score than the G allele carriers [$F(2, 492)=3.1$, $p=0.046$, $\eta^2=0.012$].

In the case of AMIS scores, the Thoughtlessness score was associated with the *TSPO* rs6971 genotype by age 33 (Figure 4). AA homozygotes received higher Thoughtlessness scores compared with G allele carrier groups [$F(2, 499)=3.1$, $p=0.047$, $\eta^2=0.012$]. Similarly to the associations with the BIS results, *TSPO* rs6971 genotype did not affect the AMIS scores at ages 18 and 25 (data not shown).

In order to analyze whether the effect of the *TSPO* rs6971 genotype was dependent of the environmental stressors, the interaction with stressful life events (SLEs) was taken into account (Figure 5). It could be observed that *TSPO* rs6971 AA homozygotes had the highest Fast decision making and Excitement seeking scores (adding up to Functional impulsivity) by age 33, but only when the subjects had not experienced above average level of SLEs by age 15 [$F(2, 446)=3.6$, $p=0.036$, $\eta^2=0.015$ and $F(2, 446)=2.8$, $p=0.063$, $\eta^2=0.012$, respectively]. In the case of high number of SLEs by age 15, the AA homozygotes no longer displayed the highest Functional impulsivity scores. This interaction effect was only present in the case of SLEs reported at age 15. SLEs reported at later ages did not affect the association between *TSPO* rs6971 genotype and different measurements of impulsivity or aggressiveness. *TSPO* rs6971 genotype itself was not associated with the number of SLEs reported by subjects.

Analyzing the effects of *TSPO* rs6971 polymorphism on aggressiveness and impulsivity by gender yielded no interaction effect.

[insert Figure 1.]

[insert Figure 2.]

[insert Figure 3.]

[insert Figure 4.]

[insert Figure 5.]

4. Discussion

Our findings suggest that the *TSPO* rs6971 polymorphism may be related to development of aggressiveness and impulsivity by adulthood, regardless of the subjects' gender. In general, subjects homozygous for the minor A allele displayed the highest aggressiveness and dysfunctional impulsivity scores. The positive, adaptive aspect of impulsivity was sensitive to stressful life events as AA genotype was associated with functional impulsivity only when the subjects had experienced a low number of SLEs during childhood. These results support the notion that HPA dysregulation and differences in neurosteroids have a causal role in the development of aggressiveness and impulsivity, since variances in the TSPO gene are likely to alter steroid synthesis or its regulation. However, it should be noted that the finding is modest and the effect has been demonstrated in a relatively small sample, only present when uncorrected for multiple comparisons, and there is a possibility of another gene in linkage disequilibrium with the *TSPO* rs6971 polymorphism (e.g., rs138911) that might be contributing to the findings.

Neuromodulatory and hormonal abnormality appear to play a role in the increased likelihood of aggressive behavior (Pavlov et al., 2012). The minor allele of the *TSPO* rs6971 has been associated with reduced cholesterol entry into mitochondria, consequently blunting production of steroid precursors (Lacapere & Papadopoulos, 2003; Li & Papadopoulos, 1998; Prossin et al., 2018). The 147Thr allelic variant of *TSPO* rs6971 has been indicated to possibly

affect the synthesis of the neurosteroid pregnenolone in lymphomonocytes (Costa et al., 2009a). Stimulation of TSPO has also been shown to have therapeutic use as anxiolytic by inducing allopregnanolone production in the brain (Papadopoulos et al., 2018). Allopregnanolone is another neurosteroid that has been demonstrated to play a role in regulating mammalian aggressive behavior (Miczek et al., 2002; Pibiri et al., 2006; Pinna et al., 2003; Soma et al., 2008). Since steroid molecules are involved in several biological functions, this *TSPO* polymorphism could be associated with the susceptibility/protection to the diseases that have been correlated to a decreased/increased production of steroids (Costa et al., 2009a). The elucidation of neuroinflammatory mechanisms in psychiatric and neurological disorders is a highly active area of research that has the promise of establishing novel therapeutic strategies beyond classical nosologic boundaries (Miller et al., 2017; Mondelli et al., 2017; Notter et al., 2020; Pape et al., 2019).

Originally used to detect discrete neurotoxic damages, TSPO has generally turned into a biomarker of 'neuroinflammation' or 'microglial activation' (Notter et al., 2018). The common human polymorphism analyzed in the current paper - *TSPO* rs6971 (C/T; A/G), leading to a base substitution from alanine to threonine at position 147 in TSPO's fifth transmembrane domain - has been demonstrated to cause differences in affinity of TSPO-binding chemicals used for diagnostic imaging (Owen et al., 2012; Mizrahi et al., 2012). The minor T(A) allele (Thr substitution) has been determined to be the low affinity binder. Individuals homozygous for the minor are often excluded from PET imaging studies as the low binding limits accurate quantification (Notter et al., 2018). So, in addition to been linked to anxiety-related clinical conditions, this polymorphism has been used in guiding the development of suitable radioligands for positron emission tomography. The association between the rs6971 polymorphism and

mentioned clinical conditions has been explained on the basis of the lower affinity of cholesterol toward the TSPO in low affinity binders (Berroterán-Infante et al., 2019; Owen et al., 2017). Here, we extend these results by demonstrating that this variation in the TSPO gene – rs6971 – is associated with aggressiveness and impulsivity in general population by adulthood.

Some limitations should be considered here, starting with sample size since it is a major source of both false positive and false negative findings. We also did not apply the correction for multiple comparisons, since it would put limits to relevant analyses given the fixed sample sizes of longitudinal studies. Using Illinois Bully scale as a retrospect measure could also be considered as a limiting factor. All of the questionnaires were self-report instruments and therefore vulnerable to response bias. Until replicated in other samples, one should remain cautious about interpretations of the present findings. However, the strengths of the present study are its longitudinal design, rigorous questionnaire data collection performed in uniform conditions of the laboratory, and the fact that the sample has a strong representation of regional population. In addition, the early assessment of adolescent stressful environment can be considered as a strength, as it minimizes the possible recall bias. In order to validate the current findings, replication in a larger sample or assessing the genotype frequencies in a sample where aggressive/impulsive behavior has been problematic (e.g., offenders) is needed.

Aggression developed in response to competition for mates or vital resources and has been determined to have a very strong genetic component (Clark & Grunstein, 2004). However, heritability of aggression has been shown to change with time; whilst genetic factors and common environment were equally important in childhood, heritability became even more prominent in adulthood (Craig & Halton, 2009). In our longitudinal population-representative study, we also found the effect of *TSPO* rs6971 polymorphism to be fully observable in

adulthood, namely by early 30s. This is in line with a common genetic maturation hypothesis. Genetic factors are likely to be associated with cognitive processes (e.g., planning, decision-making, cognitive control) that are maturing during childhood. These processes are important for executive functioning, including mechanisms related to aggression (Séguin & Zelazo, 2005). Decision-making and cognitive control are important for developing strategies of action and thus be implied in the persistence or desistance of aggression (Paquin et al., 2017). By identifying genes and brain mechanisms that predispose people to the risk of acting impulsively and being aggressive – even if the risk is small – we may eventually be able to tailor prevention programs to those who need them most.

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Conflicts of Interest

The Author declares that there is no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available upon reasonable request from the corresponding author, MV. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

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FIGURE 1:

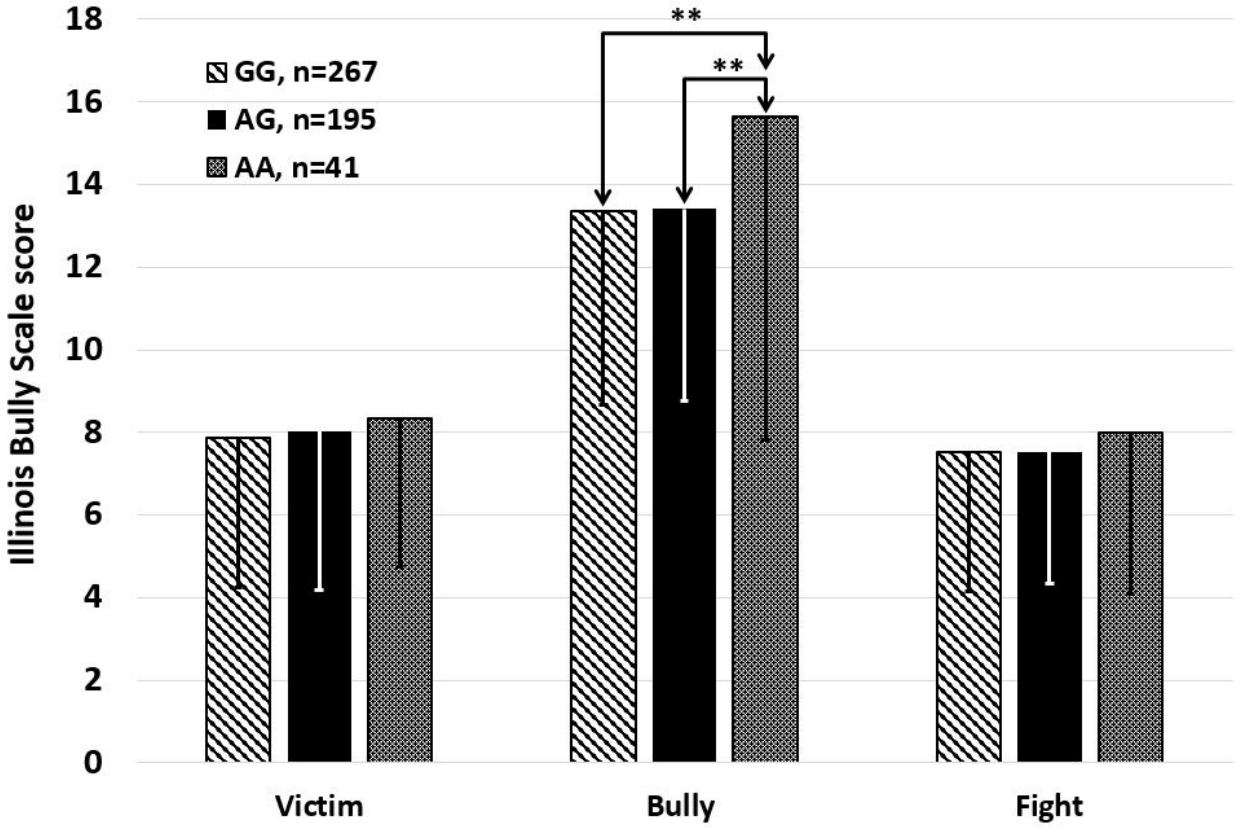


FIGURE 2:

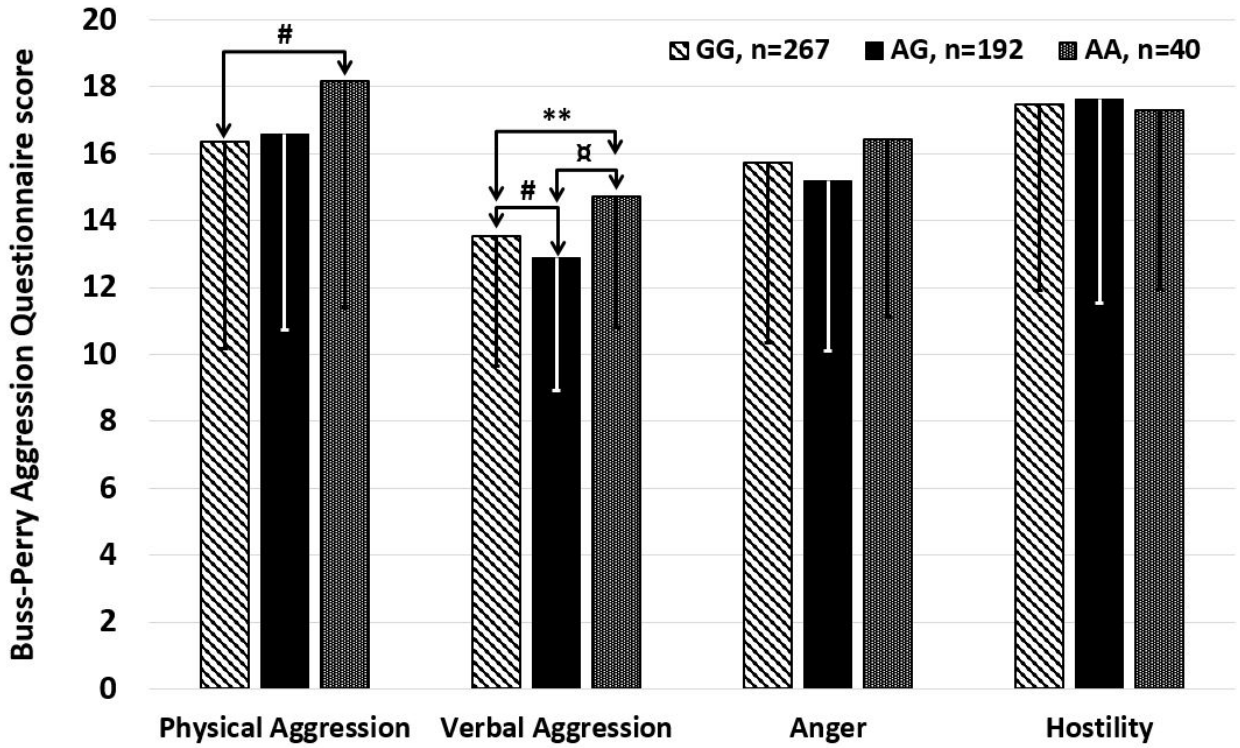
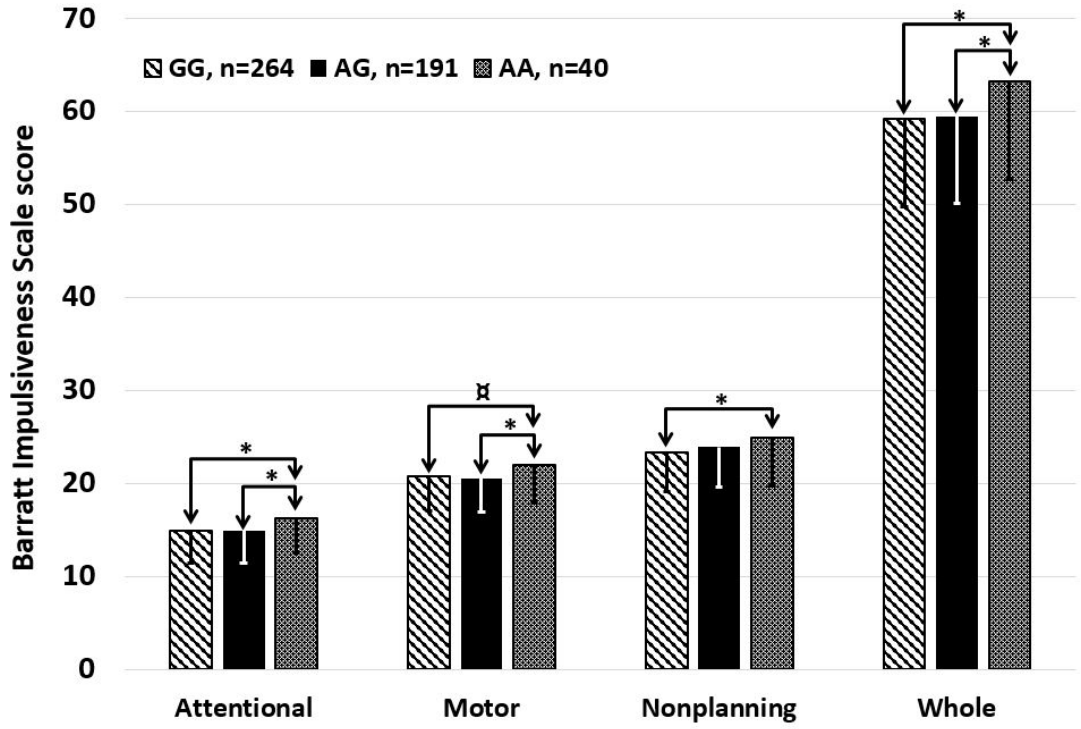


FIGURE 3:



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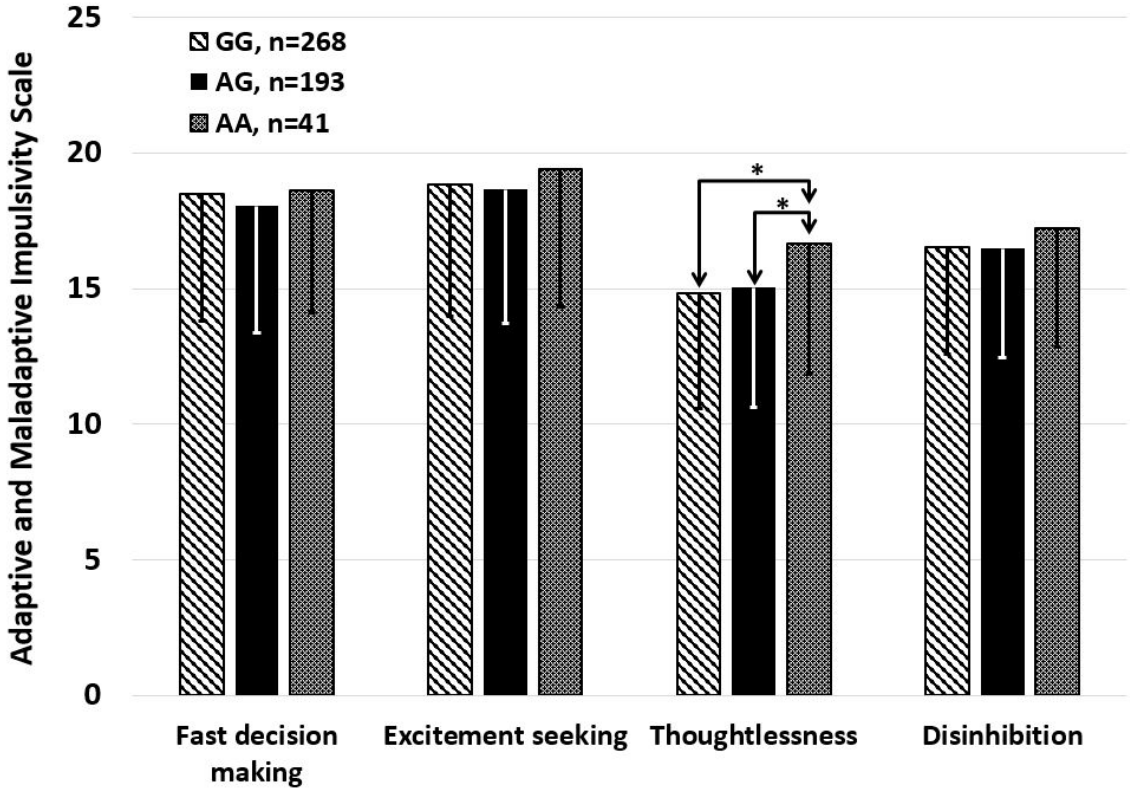

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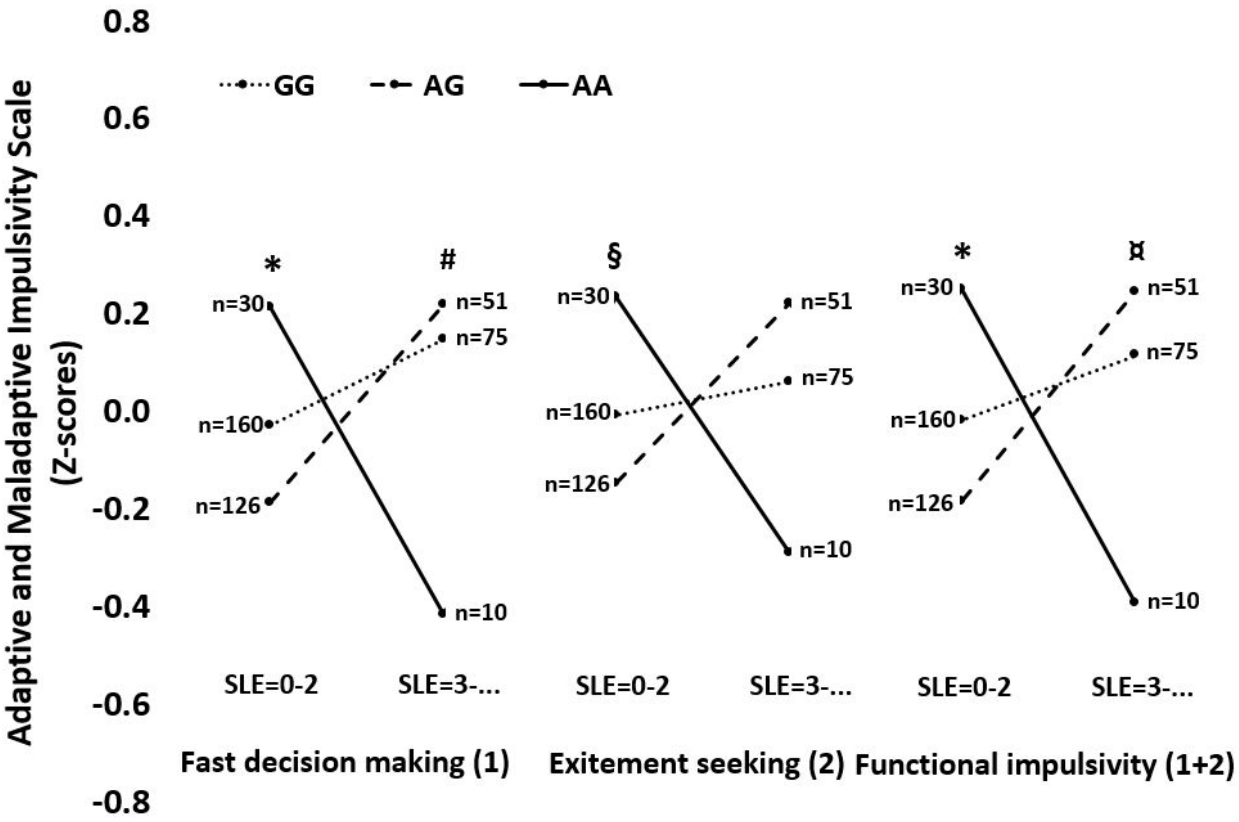

FIGURE CAPTIONS

Fig. 1. The effect of TSPO rs6971 polymorphism on the subscales of the Illinois Bully Scale (retrospective self-report data). N represents the number of subjects in the group. Vertical bars denote standard deviations. Post hoc: Fisher's least significant difference (LSD). Significant differences between groups (nominal p values): **p < 0.01.

Fig. 2. The effect of TSPO rs6971 polymorphism on the subscales of the Buss Perry Aggression Questionnaire at age 33. N represents the number of subjects in the group. Vertical bars denote standard deviations. Post hoc: Fisher's least significant difference (LSD). Significant differences between groups (nominal p values): **p < 0.01, ¤ p=0.077, #p = 0.079.

Fig. 3. The effect of TSPO rs6971 polymorphism on the subscales of the Barratt Impulsiveness Scale at age 33. N represents the number of subjects in the group. Vertical bars denote standard deviations. Post hoc: Fisher's least significant difference (LSD). Significant differences between groups (nominal p values): *p < 0.05, ¤ p=0.069.

Fig. 4. The effect of TSPO rs6971 polymorphism on the subscales of the Adaptive and Maladaptive Impulsivity Scale at age 33. N represents the number of subjects in the group. Vertical bars denote standard deviations. Post hoc: Fisher's least significant difference (LSD). Significant differences between groups (nominal p values): *p < 0.05.

Fig. 5. The effect of TSPO rs6971 polymorphism in interaction with stressful life events (SLE) on the functional impulsivity subscales (Z-scores) of the Adaptive and Maladaptive Impulsivity Scale at age 33. N represents the number of subjects in the group. Post hoc: Fisher's least significant difference (LSD). Significant differences between AA and AG genotype groups (nominal p values): *p < 0.05, § p = 0.054, # p = 0.08, ¤ p = 0.082.

Abstract

Background Expression of the 18 kDa translocator protein (TSPO), originally identified as a peripheral benzodiazepine receptor, has been found to be altered in several psychiatric disorders. A common single nucleotide polymorphism rs6971 in the TSPO gene leads to an amino-acid substitution, Ala147Thr, which dramatically alters the affinity with which TSPO binds drug ligands. As cholesterol also binds TSPO in the same transmembrane domain, it is suggested that this substitution may impair the ability of TSPO to bind or import cholesterol, and hence may affect steroid synthesis and HPA function.

Methods The analysis was carried out on older birth cohort (n=655) of the longitudinal Estonian Children Personality, Behaviour and Health Study sample. Anxiety, aggressive behavior, impulsiveness, and history of stressful life events were self-reported in various data collection waves.

Psychiatric assessment of lifetime prevalence of anxiety disorders was carried out at age 25 by experienced clinical psychologists. *TSPO* rs6971 was genotyped in all participants.

Results *TSPO* rs6971 was not associated with self-reported levels of anxiety or lifetime prevalence of anxiety disorders. However, participants homozygous for the minor A allele displayed the highest aggressiveness and dysfunctional impulsivity scores. The positive, adaptive aspect of impulsivity was sensitive to stressful life events as AA genotype was associated with functional impulsivity only when the participants had experienced a low number of SLEs during childhood.

Conclusions *TSPO* rs6971 polymorphism may be related to development of aggressiveness and impulsivity by adulthood, regardless of the participants' gender.

Keywords: TSPO, benzodiazepine receptor, aggressiveness, impulsivity, anxiety

1. Introduction

The 18 kDa translocator protein (TSPO) has become a highly promising candidate for explaining the basis of different psychiatric impairments. TSPO is a five transmembrane domain protein localized mainly in the outer mitochondrial membrane of steroid-synthesizing tissues, including the brain.

TSPO was identified decades ago in a search for peripheral tissue binding sites for benzodiazepines (diazepam), and was formerly called the peripheral benzodiazepine receptor (Braestrup & Squires, 1977). Benzodiazepines are one of the most widely available class of drugs that have been prescribed to treat patients with anxiety, convulsions, and insomnia. Subsequent studies showed that the central benzodiazepine receptor (CBR) and the peripheral benzodiazepine receptor (PBR) were distinct proteins that differed in tissue distribution, pharmacology, and cellular and subcellular localization (Anholt et al., 1986; Patel & Marangos, 1982). CBRs are primarily expressed in neurons and coupled to GABAa receptors, regulating the GABA-regulated chloride channels (e.g., Tallman et al., 1978), while PBRs have a more versatile distribution (e.g., Richards & Mohler, 1984). PBRs have been located in glial cells (e.g., astrocytes, microglia; Junck et al., 1989; Schoemaker et al., 1982), endothelial cells, tanycytes, and some populations of neurons (Lee et al., 2016; Notter et al., 2018). PBR is primarily located on the outer membrane of mitochondria (playing a role in maintaining the mitochondrial membrane potential) and found to be abundant in steroid-synthesizing cells (Papadopoulos et al., 2006). PBR was renamed as 18 kDa Translocator Protein (TSPO) in 2006 by the HUGO Gene

Nomenclature Committee (Papadopoulos et al., 2006) reflecting its putative function in protein or ligand transport/translocation (Bonsack & Sukumari-Ramesh, 2018).

Microglia and macrophages are the predominant cell types expressing TSPO in diseased brains; in addition, astrocytes can also express TSPO in humans (Cosenza-Nashat et al, 2009). As the resident brain macrophages, microglia function as immune sentries, and they become activated in both acute and chronic conditions in a context-dependent manner (Cosenza-Nashat et al, 2009). *In vivo* and *in vitro* administration of TSPO ligands results in suppression of microglial activation, including inhibition of cytokine expression (Choi et al., 2002; Veiga et al., 2007). TSPO is a widely used biomarker of neuroinflammation, but its non-selective cellular expression pattern implies roles beyond inflammatory processes (Notter et al., 2020).

TSPO mRNA levels have been found to be decreased in the lymphocytes of clinically diagnosed anxious patients (Nudmamud et al., 2000). TSPO expression levels are altered in several psychiatric disorders in which anxiety is the main symptom. A decreased number of platelet PBRs/TSPO receptors has been reported among patients with panic disorder (Marazziti et al., 1994), posttraumatic stress disorder (Gavish et al., 1996), generalized social phobia (Johnson et al., 1998), and following a suicide attempt (Marazziti et al., 2005). In addition, a decreased number of PBRs/TSPO receptors has been found in lymphocytes of patients with generalized anxiety disorder (Ferrarese et al., 1990; Rocca et al., 1992).

TSPO plays an important role in the synthesis of neurosteroids (e.g., allopregnanolone, pregnanolone), thereby representing a putative novel target for anxiolytic compounds (Nothdurfter et al., 2012). Steroids are known to act on neuronal GABAa and other receptors, thereby affecting neurotransmission and neuronal function (Papadopoulos et al., 2015). Stimulation of TSPO has been shown to have therapeutic use as anxiolytic by inducing

allopregnanolone production in the brain in animal models (Papadopoulos et al., 2018). Endogenous allopregnanolone influences aggression levels in rodents, with an inverted U-shaped dose–response curve - moderate doses evoke aggression, whereas low or high doses reduce aggressive behavior (Miczek et al., 2002). In humans, reduced levels of allopregnanolone in the peripheral blood or cerebrospinal fluid have been found to be associated with anxiety disorders and impulsive aggression (among others) and TSPO ligands have been found to increase neurosteroidogenesis and have anxiolytic effects (reviewed by Schüle et al., 2014).

The common single nucleotide polymorphism rs6971 in the TSPO gene leads to an amino-acid substitution, Ala147Thr, which dramatically alters the affinity with which TSPO binds drug ligands (Owen et al., 2012). This substitution changes the mitochondrial TSPO protein structure, decreases cholesterol transport into the mitochondria and subsequently reduces production of pregnenolone as compared to that occurring in the presence of the ancestral allele (Costa et al., 2009a; Prossin et al., 2018). This polymorphism has been linked to anxiety-related clinical conditions (Costa et al., 2009b: n=372; Nakamura et al., 2006: n=269).

Anxiety-related disorders are often characterized by impaired social behaviors including excessive aggression and violence (Neumann et al., 2010). Namely, it is reactive aggression that is associated with anxiety (Fite et al., 2010). It may be that the worry and stress of high anxiety scorers may prime some individuals towards engaging in reactive aggression. This could be a function of the sympathetic nervous system being more active and/or it could represent a defensive desire to protect oneself from perceived stresses and dangers (Book et al., 2019). Aggressiveness may be an advantageous quality, but can also have undesirable consequences when displayed in an inappropriate situation. Excessive and uninhibited aggressive behavior is an associated symptom of many psychiatric disorders and can manifest throughout the life span,

from attention-deficit hyperactivity disorder (ADHD) in children and adolescents, to domestic violence in adults, to dementia in older adults (Liu et al., 2013).

One of the characteristic components of uncontrolled aggression is enhanced impulsivity (Nelson & Trainor, 2007; Nestor, 2002). As reviewed by Moeller et al. (2001), impulsivity has been defined as swift action without forethought or conscious judgment, behavior without adequate thought, and the tendency to act with less forethought than do most individuals of equal ability and knowledge. Impulsivity also includes a tendency to live on the spur of the moment, lacking a careful planning of short and long-term goals (Patton et al., 1995). Impulsivity has been shown to predict trait aggression and episodes of aggressive behaviors in forensic patients (Bousardt et al., 2016; Tonnaer et al., 2016). Also in the general population, there have been consistent findings linking impulsivity facets (e.g., lack of premeditation, lack of perseveration, sensation seeking) and indices of aggression (reviewed by Garofalo et al., 2016). Impulsivity is moderately heritable (up to 50%) as are disorders with which it is associated (Bevilacqua & Goldman, 2013).

Based on previous results, we tested the hypothesis that the minor allele of the *TSPO* rs6971 (Thr substitution) might be related to higher levels of anxiety and occurrence of anxiety disorders in general population. Since stimulation of TSPO has also been shown to induce allopregnanolone production in the brain and levels of allopregnanolone have been associated with aggressive behavior and impulsive aggressiveness, we also analyzed the effect of *TSPO* rs6971 on aggressive behavior and impulsiveness. Emerging evidence suggests that by reducing both cholesterol binding/transport and subsequent cortisol production, this *TSPO* functional polymorphism may enhance susceptibility to environmental stress

(Colasanti et al., 2013; Prossin et al., 2018). In addition, we examined the possible interaction between the *TSPO* genotype and stressful life events (SLEs).

2. Materials and Methods

2.1. Study Population

The analysis was carried out on older birth cohort of the Estonian Children Personality, Behaviour and Health Study (ECPBHS) sample. This is the original Estonian sample of the European Youth Heart Study (1998/99) which was subsequently incorporated into the longitudinal ECPBHS. All the participants are of European descent. The principles of formation of the original sample has been described in detail in Harro et al. (2001). In brief, this is a representative birth cohort sample of the Tartu city and county with a school as the sampling unit. All schools of Tartu County, Estonia, that agreed to participate (54 of the total of 56) were included into the sampling using the probability proportional to the number of students of the respective age groups in the school, and 25 schools were selected. All children from grades 3 (younger birth cohort) and 9 (older birth cohort) were invited to participate.

The total number of participants in the older birth cohort during the initial data collection wave in 1998/99 was 593 (mean age=15.4, SD=0.6). The follow-up studies for the older cohort took place in 2001 (n=479, including 62 additional participants, mean age=18.4, SD=0.9), 2008 (n=541, mean age=24.7, SD=0.7) and 2016 (n=504, mean age=33.5, SD=0.7). ECPBHS is population representative, while 79.1% of participants of the randomized regional sample participated in the original sampling. The study was approved by the Ethics Review Committee

on Human Research of the University of Tartu. Written informed consent was obtained from all the participants, and in case of minors, also from their parents.

2.2. Measures

2.2.1. Anxiety

The Spielberger State Anxiety Inventory (STAI-S; Spielberger et al., 1983) used at 25 and 33 years and the Spielberger Trait Anxiety Inventory (STAI-T) at ages 18, 25 and 33 to assess self-reported levels of anxiety. Internal consistency coefficients for the scale have ranged from .86 to .95; test-retest reliability coefficients have ranged from .65 to .75 over a 2-month interval (Spielberger et al., 1983). Psychiatric assessment of lifetime prevalence of anxiety disorders based on DSM-IV was carried out in both birth cohorts at age 25 by experienced clinical psychologists using the Mini-International Neuropsychiatric Interview (M.I.N.I.5.0.0; Sheehan et al.., 1998; Estonian version: Shlik et al., 1999). The prevalence of affective, anxiety and substance use disorders in the sample has been published elsewhere (Laas et al., 2014, 2015).

2.2.2. Aggressive Behavior

During the last data collection wave at age 33, aggressive behavior was self-reported by Illinois Bully Scale and by Buss-Perry Aggression Questionnaire (Laas et al., 2017).

Illinois Bully Scale is an 18-item scale with three subscales, Bully, Fight, and Victim, assessing the frequency of bullying behavior, fighting, and victimization by peers (Espelage & Holt, 2001). Cronbach's alpha coefficients have been previously found to be 0.87 for the total scale, 0.71 for

victims, 0.77 for bullying, and 0.76 for the fighting subscales; all of which are satisfactory (Akbari Balootbangan & Talepasand, 2015). Participants were asked to recall the times spent in secondary school and assess the frequency of listed behaviors in 5-point scale ranging from 'never' to 'very often'.

The 29-item self-report Buss-Perry Aggression Questionnaire (Buss & Perry, 1992) assesses 4 aspects of aggressive behavior: Physical aggression, Verbal aggression, Anger, and Hostility. The internal consistency coefficients have been determined as follows: Physical Aggression, α = .85; Verbal Aggression, α = .72; Anger, α = .83 and Hostility, α = .77, with the internal consistency being α = .89 (Buss & Perry, 1992). Participants rated each statement on a 5-point Likert Scale (uncharacteristic=1, characteristic=5).

The subscales of Illinois Bully Scale and Buss-Perry Aggression Questionnaire are weakly to moderately correlated (r=0.14 – 0.6; see Kiive et al., 2017). Thus, the shared covariance indicates that to certain extent they measure the same underlying concept but since there is also unique variance, each subscale captures a specific aspect of aggressiveness.

2.2.3. Impulsiveness

All measures were self-reported using the Adaptive and Maladaptive Impulsivity Scale (AMIS) and Barratt Impulsiveness Scale, 11th version (BIS-11). AMIS follows the concept of functional and dysfunctional impulsivity (Dickman, 1990). It comprises subscales measuring fast decision-making and excitement seeking (functional or adaptive impulsivity, Cronbach's α = .74) and disinhibition and thoughtlessness (dysfunctional or maladaptive impulsivity, Cronbach's α = .85) and was used at ages 18, 25 and 33 (Laas et al., 2010; Paaver et al., 2008). BIS-11 (Patton et al.,

1995; Paaver et al., 2007) was used for measuring impulsiveness at ages 25 and 33. Internal consistency coefficient (α) for the BIS-11 have been determined to be in the range of .79 - .83 depending on the population (Patton et al., 1995).

2.2.4. Stressful Life Events

History of stressful life events (SLEs) was self-reported in all follow-up studies (Laas et al., 2015). Participants were divided into low (two or fewer events) and high (three or more events) SLE exposure groups. The list of adverse life events varied across measurement times and consisted of 10–17 (dependent on the study wave) stressful experiences, including parental death and divorce/separation, unemployed parent, parental alcoholism, poverty, poor living conditions, poor health, accidents and traumas, physical abuse, emotional abuse, severe burden/serious concerns, suicidal attempts, leaving home for several days without telling anyone, depression of a close relative, and suicide attempt or committed suicide of a close relative. The events were recorded as dichotomous variables (present or not present) and were then counted to form the number of experienced adverse life events.

2.2.5. Genotyping

Genomic DNA was extracted from venous blood samples using Qiagen QIAamp® DNA Blood Midi Kit. The real-time polymerase chain reaction (RT-PCR) for genotyping the *TSPO* rs6971 polymorphism was performed using a TaqMan Pre-Designed SNP Genotyping Assay (Applied Biosystems; Foster City, CA, USA) C___2512465_20 containing primers and fluorescent probes. Genotyping reactions were performed in a total volume of 10 µl with ~25 ng of template

DNA. RT-PCR reaction components and final concentrations were as follows: 1:5 5 x HOT FIREPol® Probe qPCR Mix Plus (ROX) (Solis BioDyne) and 1:20 80 x TaqMan Primers Probe. Context sequence [VIC/FAM] was as follows:

CCCCTACCTGGCCTGGCTGGCCTTC[A/G]CGACCACACTCAACTACTGCGTATG.

Reactions were performed on the Applied Biosystems ViiA™ 7 Real-Time PCR System. The amplification procedure consisted of an initial denaturation step at 95 °C for 12 min and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Positive and negative controls were added to each reaction plate. No inconsistencies occurred. Genotyping was performed blind to all phenotypic data. Allele frequencies agreed with National Center for Biotechnology Information database (*e.g.*, 1000 Genomes Project phase3 sequence data, European sample) and published reports. Genotype frequencies were in Hardy–Weinberg equilibrium. The frequency of the minor A allele was 0.27 (alleles are reported in the Forward orientation).

2.3. Statistical Analysis

Participants were divided by *TSPO* rs6971 genotype into AA, AG, and GG groups. The effect of *TSPO* rs6971 genotype on lifetime prevalence of anxiety disorders was assessed by Pearson's chi-square test. Analysis of variance (ANOVA) was used to test the effects of *TSPO* rs6971 genotype on self-report measures of anxiety (STAI-S, STAI-T), aggressiveness (IBS, BPAQ) and impulsiveness (AMIS, BIS). For each model, F-values, two-sided raw *P* values, and effect sizes (η^2), as derived from the SPSS output, were reported. *TSPO* rs6971 interaction effects with SLE on the impulsiveness scores were also analyzed using ANOVA. Impulsiveness measures was defined as the dependent variable. SLE (as a binary variable, split by median) and

TSPO rs6971 genotype (as a categorical variable) were defined as fixed factors, and factor interactions were included in the model. Fisher's least significance difference method (LSD) was used in all *post hoc* comparisons. Contrasts were calculated for significant model effects. All *P* values are reported as 2-tailed, and raw results are considered significant at the $P < .05$ level. Statistical analyses were performed using IBM SPSS Statistics, Version 21.

3. Results

TSPO rs6971 polymorphism was not associated with self-reported levels of anxiety or lifetime prevalence of anxiety disorders.

When analyzing the effects of *TSPO* rs6971 polymorphism on aggressive behavior during school years (Figure 1), we observed that the participants homozygous for the A allele were the ones reporting bullying other students the most [$F(2, 500)=3.9$, $p=0.022$, $\eta^2=0.015$]. *TSPO* rs6971 genotype was not associated with actual fighting [$F(2, 500)=0.4$, $p=0.688$, $\eta^2=0.001$] or being victimized [$F(2, 500)=0.3$, $p=0.708$, $\eta^2=0.001$] during the school years. AA homozygotes were also acting most aggressively by age 33 (Figure 2) according to the Buss Perry Aggression Questionnaire (BPAQ). The AA homozygotes had higher Verbal Aggression scores than G allele carriers [$F(2, 496)=4.1$, $p=0.016$, $\eta^2=0.016$]. There were no significant associations with the Physical Aggression score [$F(2, 496)=1.5$, $p=0.214$, $\eta^2=0.006$], Anger [$F(2, 496)=1.1$, $p=0.322$, $\eta^2=0.005$], Hostility [$F(2, 496)=0.1$, $p=0.93$, $\eta^2<0.001$] or the BPAQ total score [$F(2, 496)=1.1$, $p=0.32$, $\eta^2=0.005$].

Impulsive behavior measured using the Barratt Impulsiveness Scale (BIS) was also associated with the *TSPO* rs6971 polymorphism by age 33 (Figure 3), but not at earlier age (data not

shown). At age 33, AA homozygotes received higher total score than the G allele carriers [F(2, 492)=3.1, p=0.046, η^2=0.012].

In the case of AMIS scores, the Thoughtlessness score was associated with the *TSPO* rs6971 genotype by age 33 (Figure 4). AA homozygotes received higher Thoughtlessness scores compared with G allele carrier groups [F(2, 499)=3.1, p=0.047, η^2=0.012]. Similarly to the associations with the BIS results, *TSPO* rs6971 genotype did not affect the AMIS scores at ages 18 and 25 (data not shown).

In order to analyze whether the effect of the *TSPO* rs6971 genotype was dependent of the environmental stressors, the interaction with stressful life events (SLEs) was taken into account (Figure 5). It could be observed that *TSPO* rs6971 AA homozygotes had the highest Fast decision making and Excitement seeking scores (adding up to Functional impulsivity) by age 33, but only when the participants had not experienced above average level of SLEs by age 15 [F(2, 446)=3.6, p=0.036, η^2=0.015 and F(2, 446)=2.8, p=0.063, η^2=0.012, respectively]. In the case of high number of SLEs by age 15, the AA homozygotes no longer displayed the highest Functional impulsivity scores. This interaction effect was only present in the case of SLEs reported at age 15. SLEs reported at later ages did not affect the association between *TSPO* rs6971 genotype and different measurements of impulsivity or aggressiveness. *TSPO* rs6971 genotype itself was not associated with the number of SLEs reported by participants.

Analyzing the effects of *TSPO* rs6971 polymorphism on aggressiveness and impulsivity by gender yielded no interaction effect.

[insert Figure 1.]

[insert Figure 2.]

[insert Figure 3.]

[insert Figure 4.]

[insert Figure 5.]

4. Discussion

The findings suggest that the *TSPO* rs6971 polymorphism may be related to development of aggressiveness and impulsivity by adulthood, regardless of the participants' gender. In general, participants homozygous for the minor A allele displayed the highest aggressiveness and dysfunctional impulsivity scores. The positive, adaptive aspect of impulsivity was sensitive to stressful life events as AA genotype was associated with functional impulsivity only when the participants had experienced a low number of SLEs during childhood. These results support the notion that HPA dysregulation and differences in neurosteroids have a causal role in the development of aggressiveness and impulsivity, since variances in the TSPO gene are likely to alter steroid synthesis or its regulation. However, it should be noted that the finding is modest and the effect has been demonstrated in a relatively small sample, only present when uncorrected for multiple comparisons, and there is a possibility of another gene in linkage disequilibrium with the *TSPO* rs6971 polymorphism (e.g., rs138911) that might be contributing to the findings.

Neuromodulatory and hormonal abnormality appear to play a role in the increased likelihood of aggressive behavior (Pavlov et al., 2012). The minor allele of the *TSPO* rs6971 has been associated with reduced cholesterol entry into mitochondria, consequently blunting production of steroid precursors (Lacapere & Papadopoulos, 2003; Li & Papadopoulos, 1998; Prossin et al., 2018). The 147Thr allelic variant of *TSPO* rs6971 has been indicated to possibly

affect the synthesis of the neurosteroid pregnenolone in lymphomonocytes (Costa et al., 2009a). Stimulation of TSPO has also been shown to have therapeutic use as anxiolytic by inducing allopregnanolone production in the brain (Papadopoulos et al., 2018). Allopregnanolone is another neurosteroid that has been demonstrated to play a role in regulating mammalian aggressive behavior (Miczek et al., 2002; Pibiri et al., 2006; Pinna et al., 2003; Soma et al., 2008). Since steroid molecules are involved in several biological functions, this *TSPO* polymorphism could be associated with the susceptibility/protection to the diseases that have been correlated to a decreased/increased production of steroids (Costa et al., 2009a). The elucidation of neuroinflammatory mechanisms in psychiatric and neurological disorders is a highly active area of research that has the promise of establishing novel therapeutic strategies beyond classical nosologic boundaries (Miller et al., 2017; Mondelli et al., 2017; Notter et al., 2020; Pape et al., 2019).

Originally used to detect discrete neurotoxic damages, TSPO has generally turned into a biomarker of 'neuroinflammation' or 'microglial activation' (Notter et al., 2018). The common human polymorphism analyzed in the current paper - *TSPO* rs6971 (C/T; A/G), leading to a base substitution from alanine to threonine at position 147 in TSPO's fifth transmembrane domain - has been demonstrated to cause differences in affinity of TSPO-binding chemicals used for diagnostic imaging (Owen et al., 2012; Mizrahi et al., 2012). The minor T(A) allele (Thr substitution) has been determined to be the low affinity binder. Individuals homozygous for the minor are often excluded from PET imaging studies as the low binding limits accurate quantification (Notter et al., 2018). So, in addition to been linked to anxiety-related clinical conditions, this polymorphism has been used in guiding the development of suitable radioligands for positron emission tomography. The association between the rs6971 polymorphism and

mentioned clinical conditions has been explained on the basis of the lower affinity of cholesterol toward the TSPO in low affinity binders (Berroterán-Infante et al., 2019; Owen et al., 2017). Here, we extend these results by demonstrating that this variation in the TSPO gene – rs6971 – is associated with aggressiveness and impulsivity in general population by adulthood.

Some limitations should be considered here, starting with sample size since it is a major source of both false positive and false negative findings. We also did not apply the correction for multiple comparisons, since it would put limits to relevant analyses given the fixed sample sizes of longitudinal studies. Using Illinois Bully scale as a retrospect measure could also be considered as a limiting factor. All of the questionnaires were self-report instruments and therefore vulnerable to response bias. Until replicated in other samples, one should remain cautious about interpretations of the present findings. However, the strengths of the present study are its longitudinal design, rigorous questionnaire data collection performed in uniform conditions of the laboratory, and the fact that the sample has a strong representation of regional population. In addition, the early assessment of adolescent stressful environment can be considered as a strength, as it minimizes the possible recall bias. In order to validate the current findings, replication in a larger sample or assessing the genotype frequencies in a sample where aggressive/impulsive behavior has been problematic (e.g., offenders) is needed.

Aggression developed in response to competition for mates or vital resources and has been determined to have a very strong genetic component (Clark & Grunstein, 2004). However, heritability of aggression has been shown to change with time; whilst genetic factors and common environment were equally important in childhood, heritability became even more prominent in adulthood (Craig & Halton, 2009). In our longitudinal population-representative study, we also found the effect of *TSPO* rs6971 polymorphism to be fully observable in

adulthood, namely by early 30s. This is in line with a common genetic maturation hypothesis. Genetic factors are likely to be associated with cognitive processes (e.g., planning, decision-making, cognitive control) that are maturing during childhood. These processes are important for executive functioning, including mechanisms related to aggression (Séguin & Zelazo, 2005). Decision-making and cognitive control are important for developing strategies of action and thus be implied in the persistence or desistance of aggression (Paquin et al., 2017). By identifying genes and brain mechanisms that predispose people to the risk of acting impulsively and being aggressive – even if the risk is small – we may eventually be able to tailor prevention programs to those who need them most.

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Conflicts of Interest

The Author declares that there is no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available upon reasonable request from the corresponding author, MV. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

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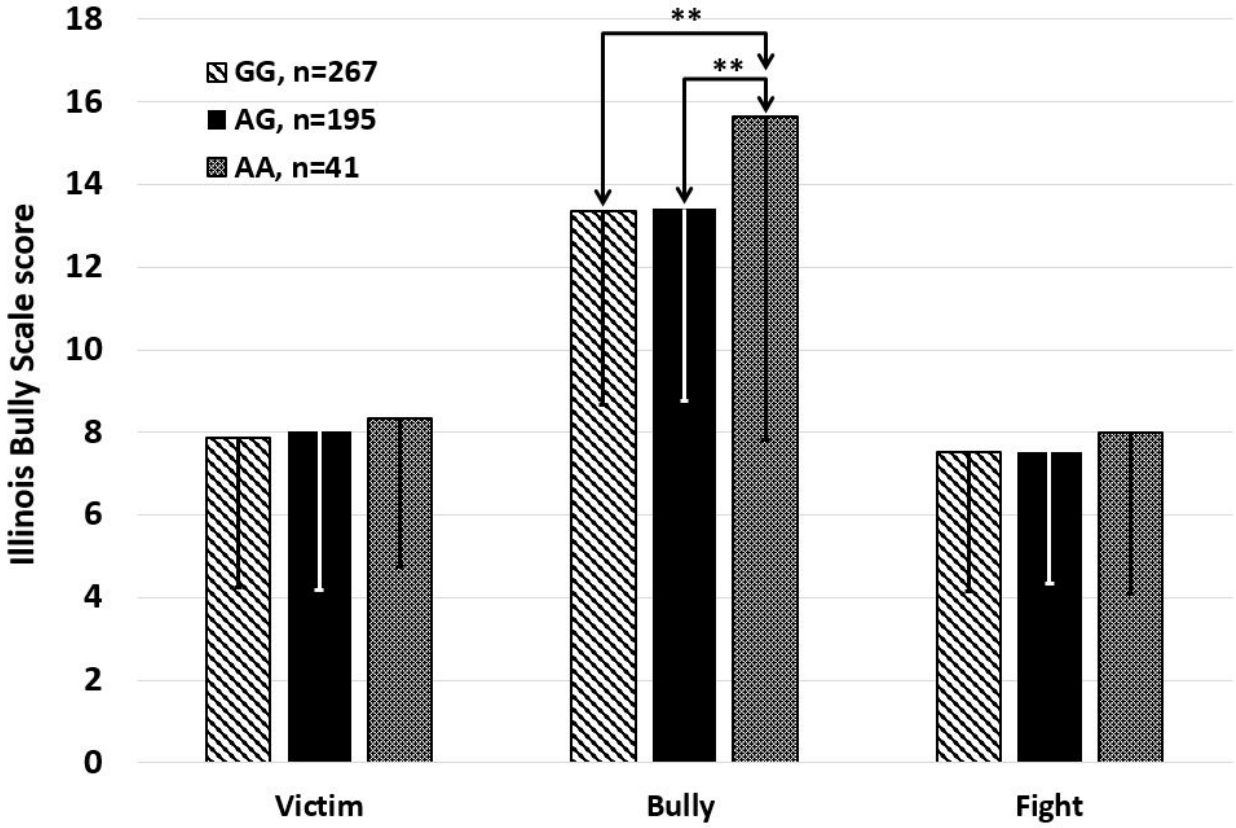
FIGURE 1:



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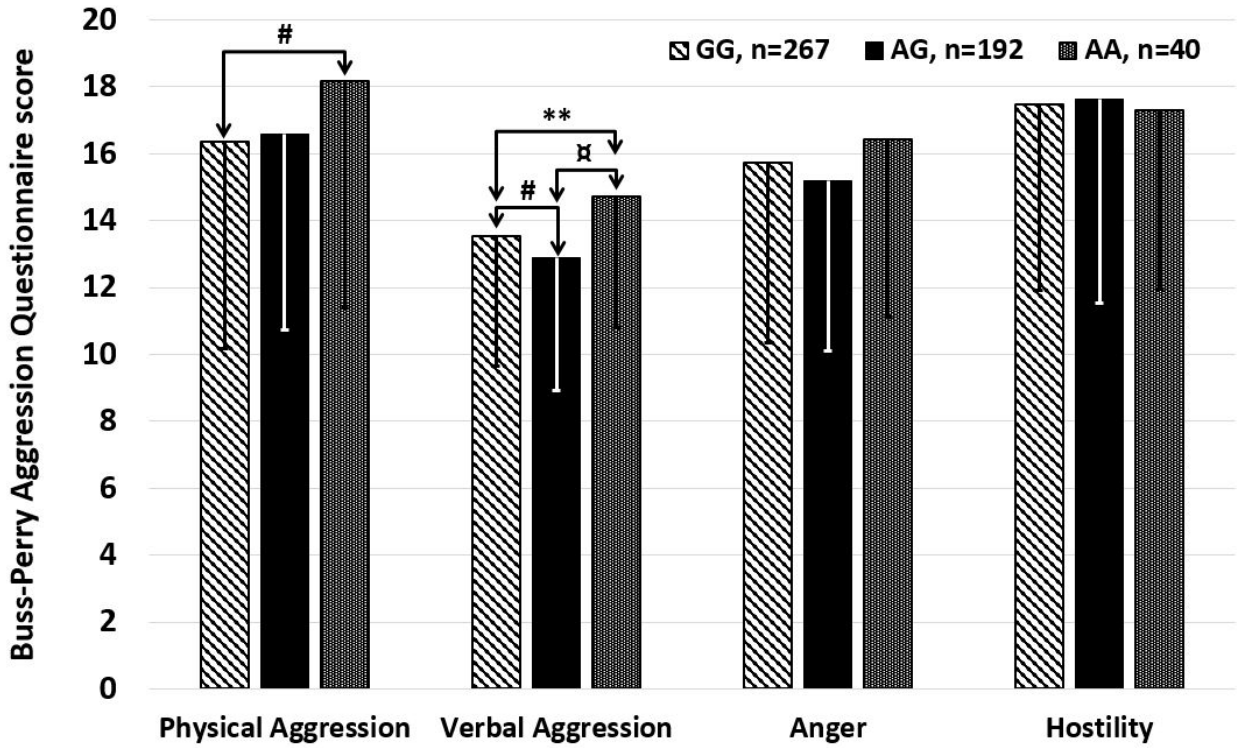

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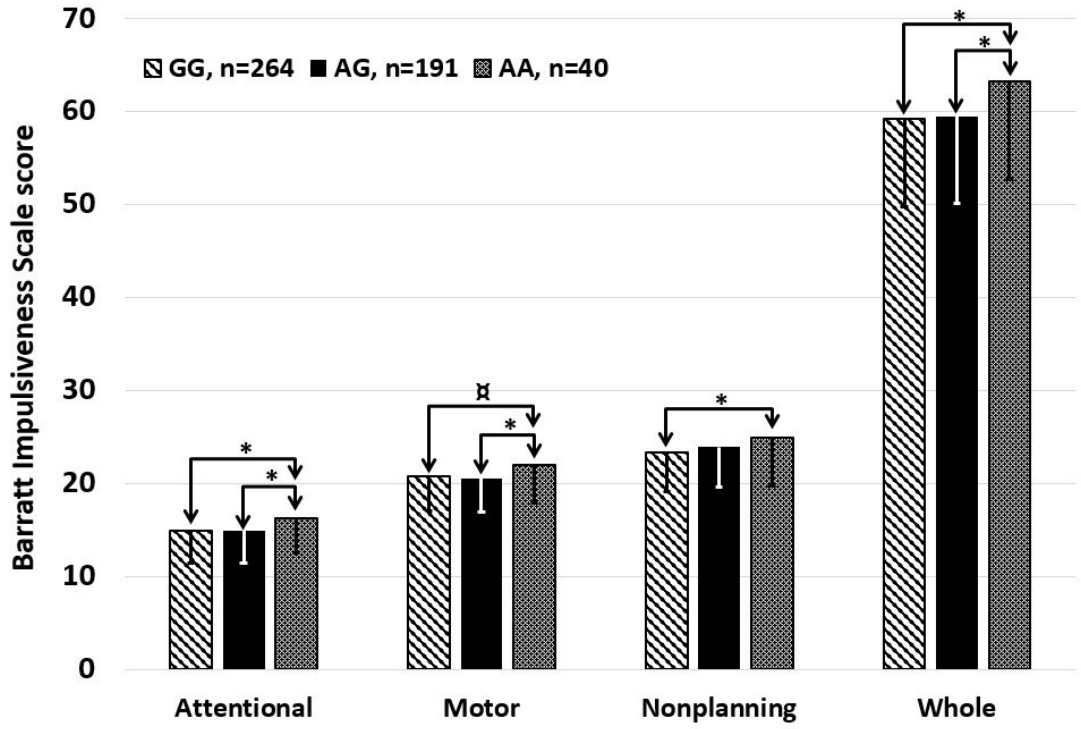

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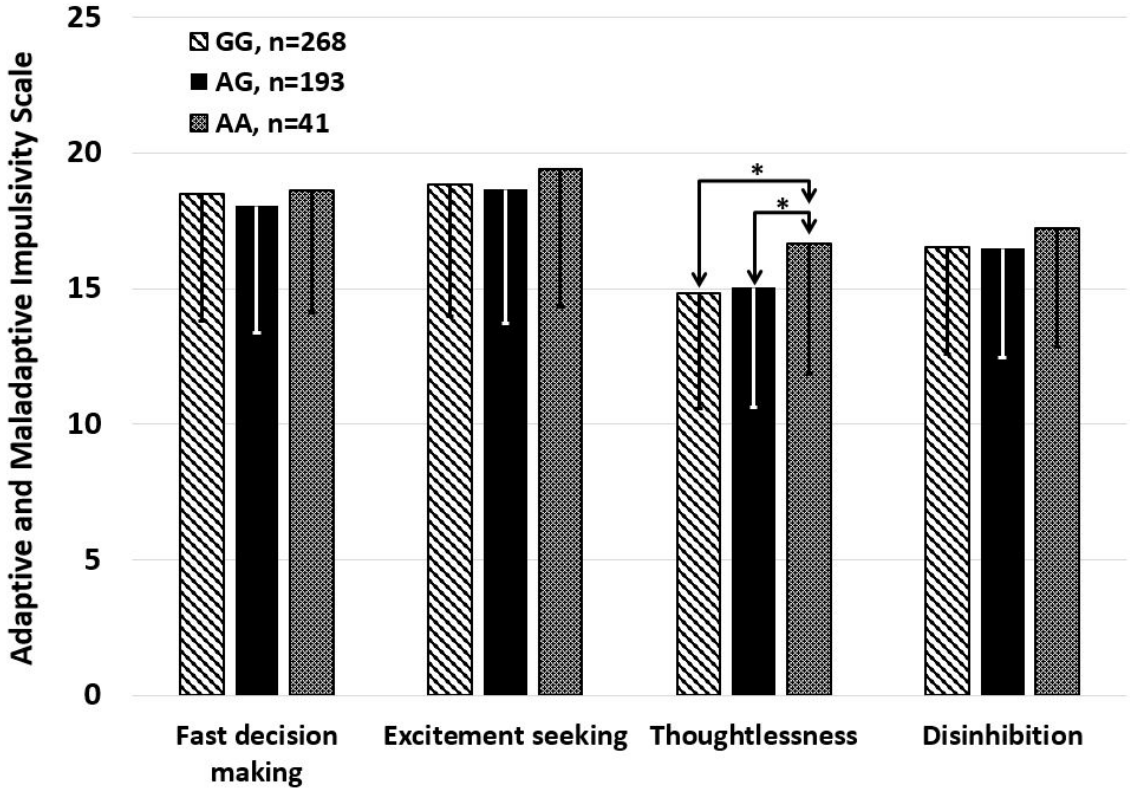

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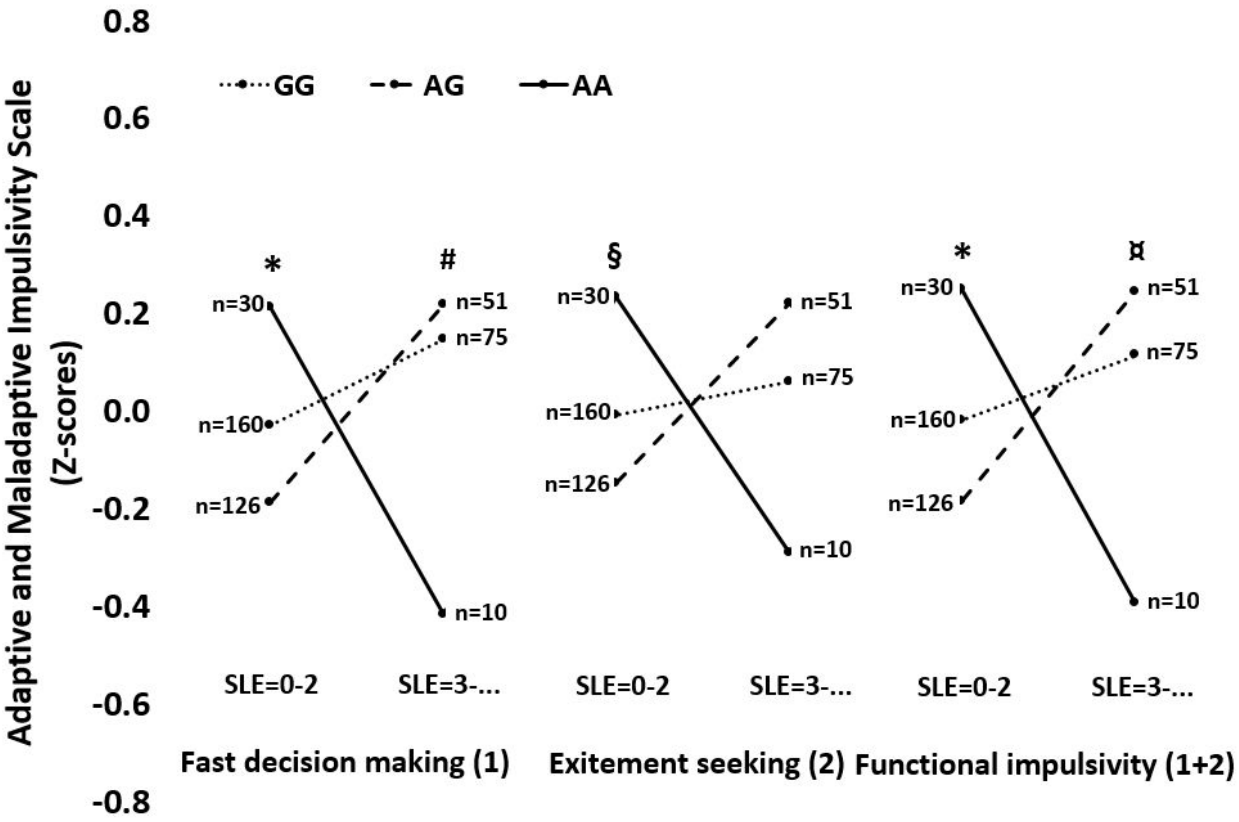


FIGURE CAPTIONS

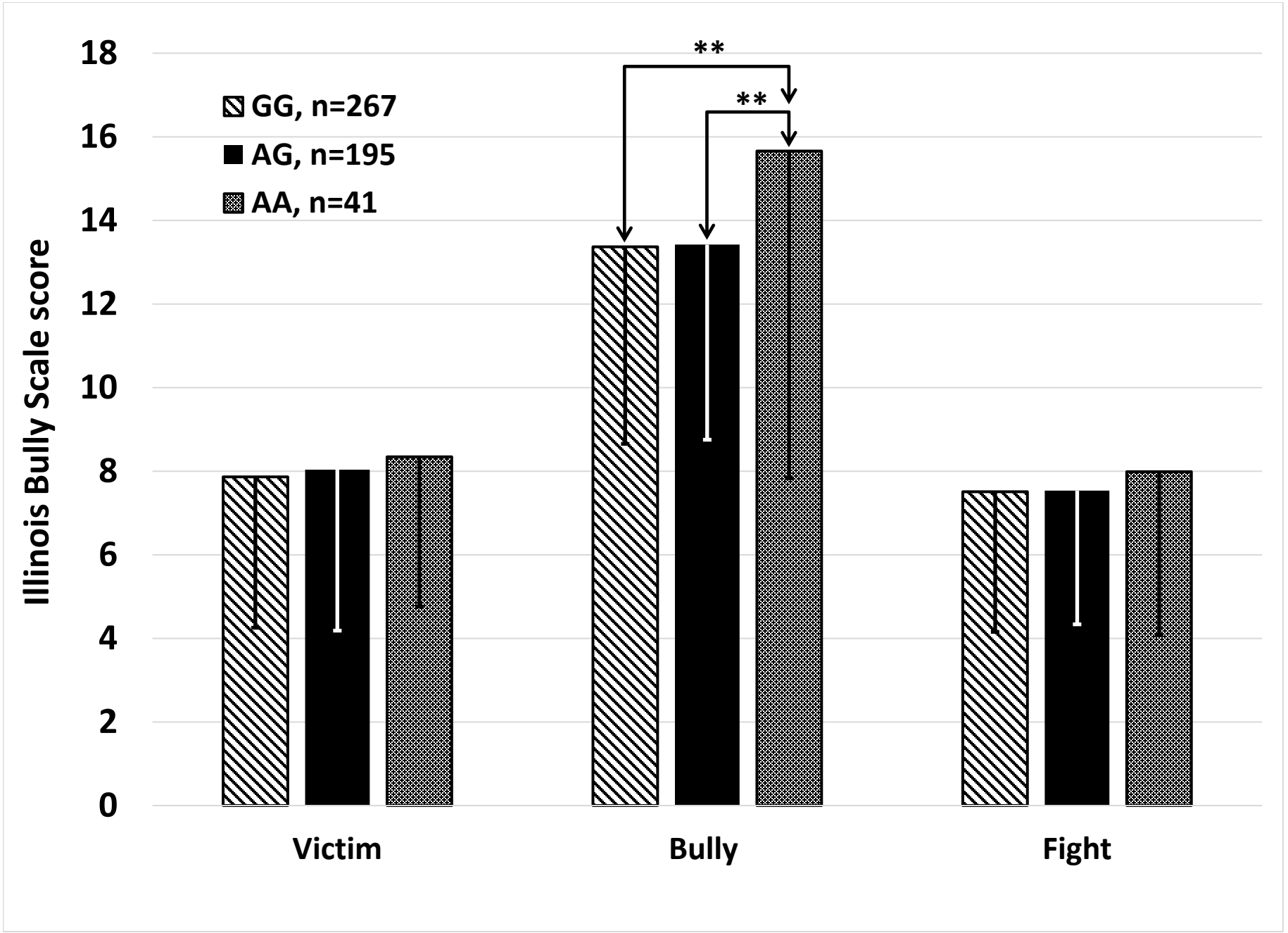
Fig. 1. The effect of TSPO rs6971 polymorphism on the subscales of the Illinois Bully Scale (retrospective self-report data). N represents the number of participants in the group. Vertical bars denote standard deviations. Post hoc: Fisher's least significant difference (LSD). Significant differences between groups (nominal p values): **p < 0.01.

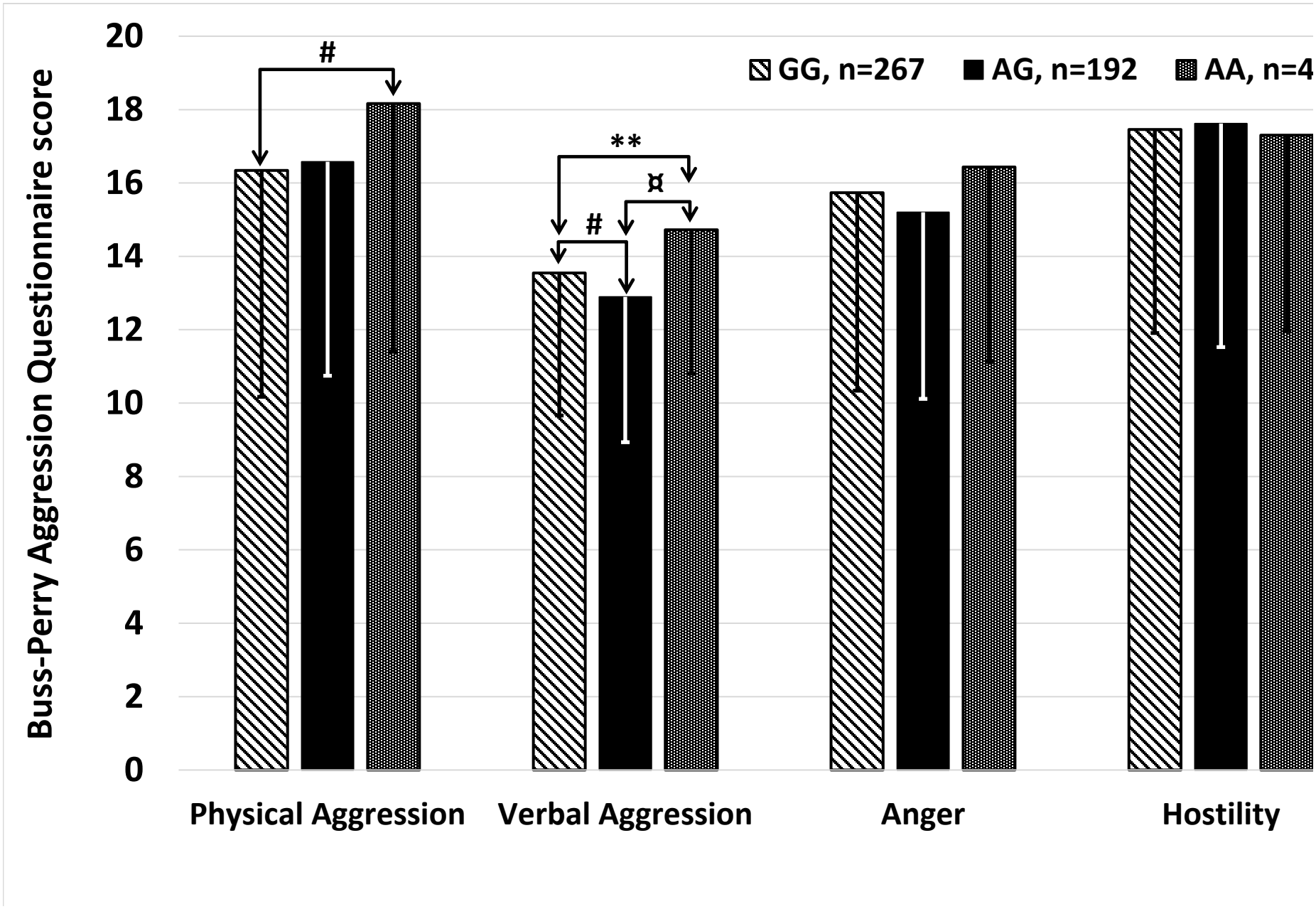
Fig. 2. The effect of TSPO rs6971 polymorphism on the subscales of the Buss Perry Aggression Questionnaire at age 33. N represents the number of participants in the group. Vertical bars denote standard deviations. Post hoc: Fisher's least significant difference (LSD). Significant differences between groups (nominal p values): **p < 0.01, ¤ p=0.077, #p = 0.079.

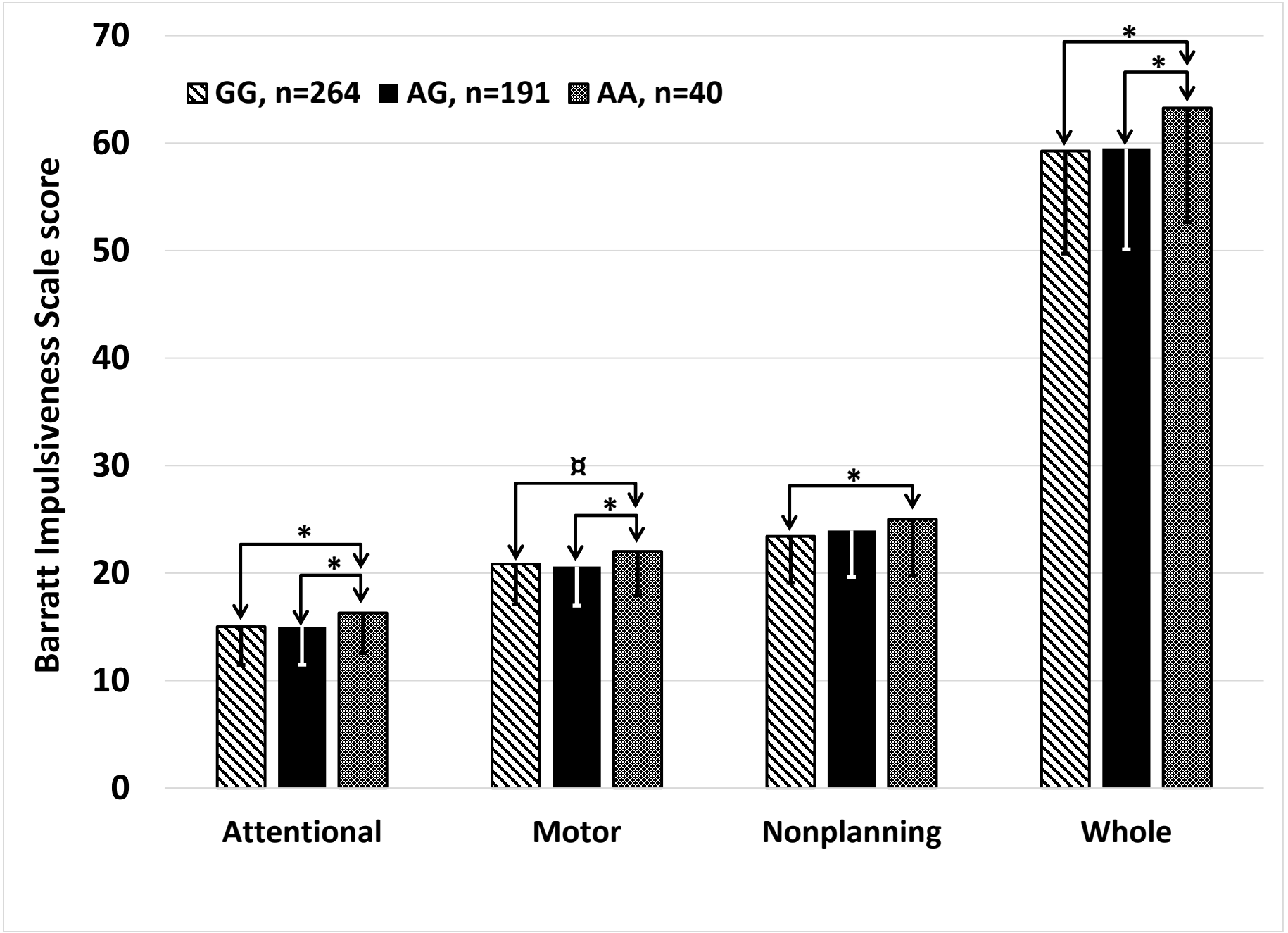
Fig. 3. The effect of TSPO rs6971 polymorphism on the subscales of the Barratt Impulsiveness Scale at age 33. N represents the number of participants in the group. Vertical bars denote standard deviations. Post hoc: Fisher's least significant difference (LSD). Significant differences between groups (nominal p values): *p < 0.05, ¤ p=0.069.

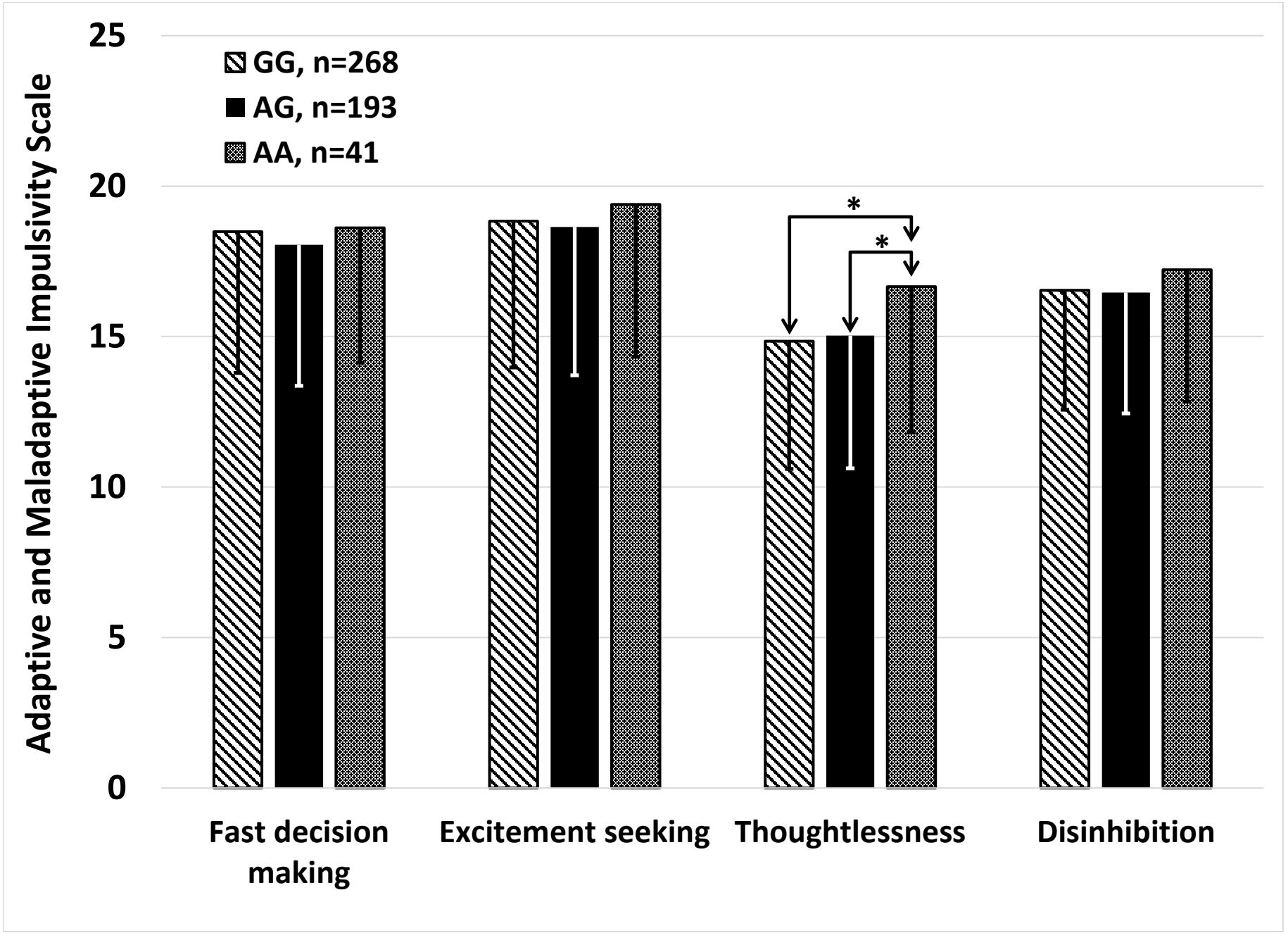
Fig. 4. The effect of TSPO rs6971 polymorphism on the subscales of the Adaptive and Maladaptive Impulsivity Scale at age 33. N represents the number of participants in the group. Vertical bars denote standard deviations. Post hoc: Fisher's least significant difference (LSD). Significant differences between groups (nominal p values): *p < 0.05.

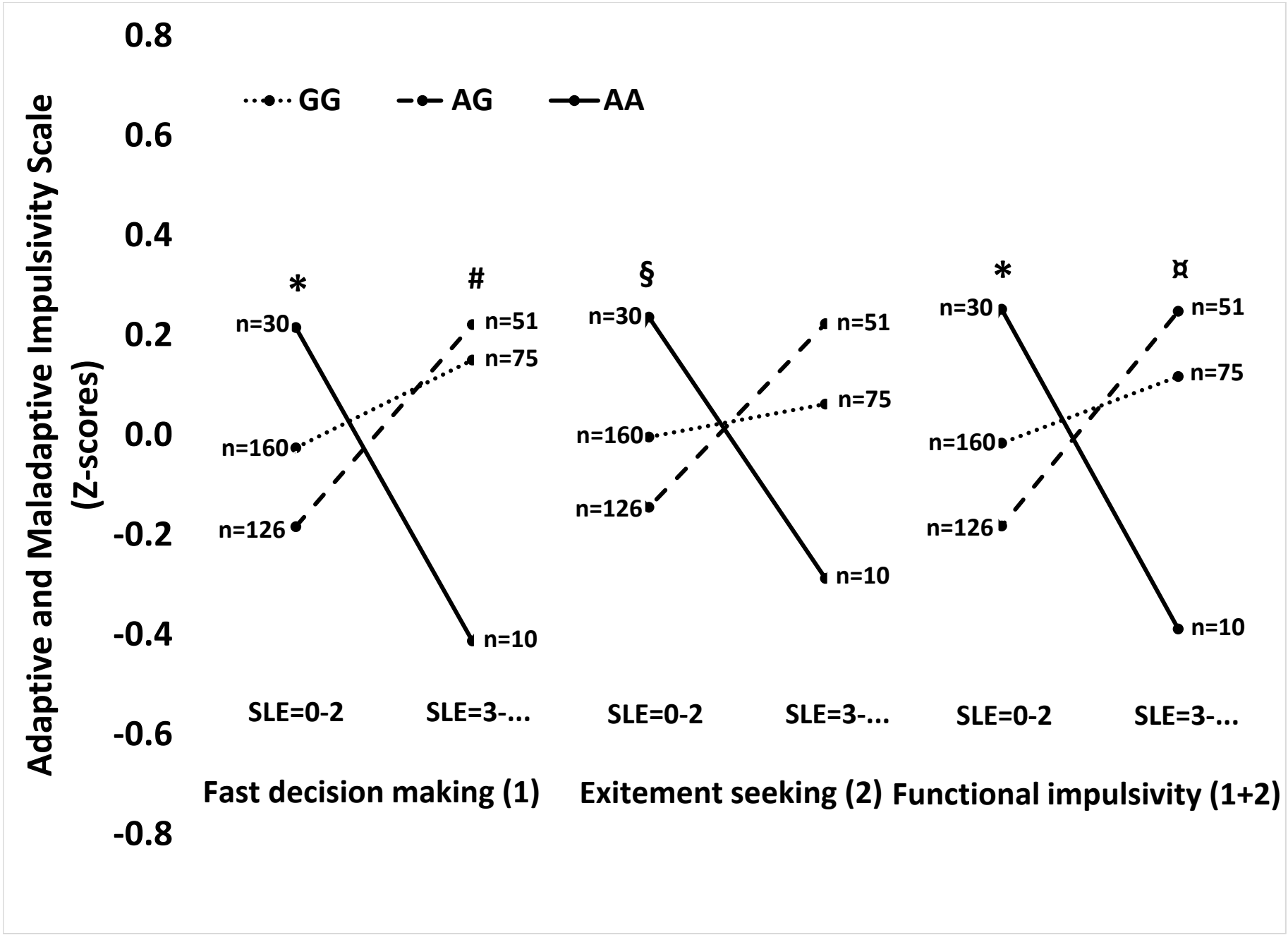
Fig. 5. The effect of TSPO rs6971 polymorphism in interaction with stressful life events (SLE) on the functional impulsivity subscales (Z-scores) of the Adaptive and Maladaptive Impulsivity Scale at age 33. N represents the number of participants in the group. Post hoc: Fisher's least significant difference (LSD). Significant differences between AA and AG genotype groups (nominal p values): *p < 0.05, § p = 0.054, # p = 0.08, ¤ p = 0.082.


18
16
14
12
10
8
6
4
2
0
Illinois Bully Scale score
GG, n=267
AG, n=195
AA, n=41
**
**
Victim
Bully
Fight


Buss-Perry Aggression Questionnaire score
20
18
16
14
12
10
8
6
4
2
0
GG, n=267
AG, n=192
AA, n=4
#
**
¤
#
Physical Aggression
Verbal Aggression
Anger
Hostility


70
60
50
40
30
20
10
0
Barratt Impulsiveness Scale score
GG, n=264
AG, n=191
AA, n=40
*
*
*
¤
*
*
*
*
Attentional
Motor
Nonplanning
Whole


Adaptive and Maladaptive Impulsivity Scale
25
20
15
10
5
0
GG, n=268
AG, n=193
AA, n=41
*
*
Fast decision making
Excitement seeking
Thoughtlessness
Disinhibition


Adaptive and Maladaptive Impulsivity Scale (Z-scores)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
GG
AG
AA
*
#
§
*
¤
n=30
n=51
n=75
n=160
n=126
n=10
SLE=0-2
SLE=3-...
Fast decision making (1)
Exitement seeking (2)
Functional impulsivity (1+2)