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Adriana S. Miu Date

Association between the Serotonin Receptor 1B Gene (*HTR1B*) and Childhood Aggression

By

Adriana S. Miu
Master of Arts

Clinical Psychology

_____
Irwin D. Waldman, Ph.D.
Advisor

_____
Jocelyne Bachevalier, Ph.D.
Committee Member

_____
Patricia A. Brennan, Ph.D.
Committee Member

Accepted:

_____
Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____
Date

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Adriana S. Miu
B.A., Stanford University, 2010

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A thesis submitted to the Faculty of the
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Abstract

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By Adriana S. Miu

Research has demonstrated that childhood aggression is highly heritable, but molecular genetic studies have not been successful in identifying susceptibility genes for aggression. These inconsistent findings may be due at least in part to grouping heterogeneous forms of aggression together, rather than examining reactive and proactive aggression separately. Given that reactive and proactive forms of aggression have both shared and unique behavioral and physiological characteristics, the two forms may also differ genetically. Further, some theoretically plausible candidate genes, such as serotonin receptor 1B gene (*HTR1B*), have not been well examined in the extant literature on childhood aggression. The present study investigated the relations between *HTR1B* and aggression in general, as well as reactive and proactive aggression specifically. In a sample of 514 children, we examined these relations using a gene-based association test to capture full genetic variation across *HTR1B*. We found significant associations between *HTR1B* and total, reactive, and proactive aggression, which provide support for the influence of *HTR1B* on both forms of childhood aggression. The current study also illustrated the importance of using a more comprehensive gene-based approach to examine the genetic influence on aggression. Further research is needed to independently replicate these findings and to elucidate the underlying neurobiological mechanisms accounting for *HTR1B*'s effects on reactive and proactive aggression.

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Overview

Although aggression has consistently been shown to be moderately to highly heritable (Miles & Carey, 1997; Rhee & Waldman, 2011), the specific genetic mechanisms underlying aggression are still largely unknown. Molecular genetic studies have attempted to identify specific genes that contribute to aggression, but these studies have failed to uncover loci that yielded consistent associations with this complex phenotype (Maxson, 2008). Nonetheless, many theoretically plausible candidate genes, such as the serotonin receptor 1B gene (*HTR1B*), have been implicated in neurobiological and animal models of aggression but understudied in the extant literature. Another reason for our difficulty in identifying susceptibility genes for aggression may be due to our operationalization of aggression. Researchers have identified several forms of aggression, including reactive and proactive aggression, which have different behavioral, physiological, and neurobiological characteristics. Despite these differences, the extant literature has mostly examined the association between genes and general aggression without taking into consideration the heterogeneity in this phenotype. For these reasons, the present study investigated whether the candidate gene *HTR1B* may be differentially related to two specific dimensions of aggression, reactive and proactive aggression.

Aggression is generally defined as any behavior directed toward another individual with the intent to harm the individual physically or psychologically (Bushman & Anderson, 2001). One in twelve high school students is threatened or injured with a weapon each year (APA, 2011) and youths account for a significant portion of violent

crime arrests (14%) (CDC, 2011), creating a serious public health concern. Not only is childhood aggression harmful toward others, but it also places aggressive children at higher risk for serious problems, such as academic failure (Hinshaw, 1992; Sutherland, Lewis-Palmer, Stichter, & Morgan, 2008), interpersonal problems (Dodge et al., 2003), anxiety and depression (Reef, van Meurs, Verhulst, & van der Ende, 2010), and criminal offenses (Huesmann, Dubow, & Boxer, 2009; Loeber, 1990). Given the devastating impact of childhood aggression on society, it is therefore important to understand the factors underlying aggression in the effort to reduce its impact.

Operationalization of Aggression

Some researchers have conceptualized aggression as consisting of two dimensions, reactive and proactive aggression, derived from factor analyses of aggression measures (Dodge & Coie, 1987; Poulin & Boivin, 2000; Raine et al., 2006). Reactive aggression is defined as an angry, often impulsive, overreaction to provocation or perceived threats to the self (Dodge & Coie, 1987), whereas proactive aggression is defined as premeditated and goal-directed aggression, such as thefts or intimidations without any prior provocation (Dodge & Coie, 1987). Although the distinction between reactive and proactive aggression has been made, there has been an ongoing debate regarding whether the two factors of aggression should be distinguished or viewed as reflections of one general aggression factor (Bushman & Anderson, 2001). Some researchers argue that because the two forms share many similarities, the distinction may not be meaningful (e.g., Bushman & Anderson, 2001; DeWall, Anderson & Bushman, 2011). On the other hand, other researchers have demonstrated many personality, physiological, and neurological differences that justify this distinction (e.g., Dodge &

Coie, 1987; Poulin & Boivin, 2000). By comparing reactive and proactive aggression, we may reduce heterogeneity in the operationalization of the aggression phenotype and improve our ability to identify genetic and environmental influences that are specific to one form of aggression and not another.

Reactive and proactive aggression have been found to share some similarities phenotypically and etiologically. Behavioral observation studies have estimated that among aggressive children, approximately half were found to exhibit both reactive and proactive aggression (Dodge, Lochman, Harnish, Bates, & Pettit, 1997; Pulkkinen, 1996; Vitaro, Gendreau, Tremblay, & Oligny, 1998). On parents' and teachers' reports, children's reactive and proactive aggression were also moderately correlated (r = .46 to .80) (Dodge & Coie, 1987; Poulin et al., 1997; Raine et al., 2006). These studies suggest that there is considerable phenotypic overlap between the two forms of aggression.

Despite these similarities, there are motivational, personality, physiological, and neurological differences between reactive and proactive aggression. Children exhibiting reactive aggression have been found to immediately react to provocations in order to soothe their anger and frustration despite anticipating negative consequences (Berkowitz, 1993; Dollard, Doob, Miller, Mowrer & Sears, 1939) whereas children engaged in proactive aggression have been found to expect rewards rather than punishment as a result of their aggression (Crick & Dodge, 1996; Marsee & Frick, 2007). Reactive aggression is often associated with personality traits such as "hot-bloodedness," impulsivity (Brendgen, Vitaro, Boivin, Dionne, & Pérusse, 2006), and neuroticism (Miller & Lynam, 2006), and children with reactive aggression were found to be at higher risk for low peer status, few close friends, and internalizing problems (Kempes, Matthys,

de Vries, & Van Engeland, 2005; Raine et al., 2006). In contrast, proactive aggression is characterized by psychopathic traits, such as "cold-bloodedness" and fearlessness (Kempes et al., 2005; Raine et al., 2006). Although reactive aggression is often associated with impaired interpersonal relationships, proactive aggression is actually associated with greater peer status and fewer school or internalizing problems (Kempes et al., 2005). Nonetheless, proactive aggression predicts more serious problems in the future, such as delinquency, oppositional defiant disorder, and conduct disorder (Vitaro, Gendreau, Tremblay, & Oligny, 1998).

Some physiological studies have indicated that children involved in reactive aggression exhibit heightened arousal and increased heart rate whereas children engaged in proactive aggression exhibit hypo-arousal, lower resting heart rate and lower stress, as indicated by lower skin conductance levels (Beauchaine, Katkin, Strassberg, & Snarr, 2001; Raine, Venables, & Williams, 1990; Scarpa, Raine, Venables, & Mednick, 1997; Scarpa & Raine, 2000). These opposing patterns of arousal are consistent with the hot-blooded versus cold-blooded personality differences between the two forms of aggression. Neurobiological studies have further revealed that these differences are apparent in amygdala reactivity. Individuals with reactive aggression have shown increased amygdala reactivity to threatening stimuli whereas individuals with proactive aggression have shown reduced amygdala reactivity (Blair, 2007, 2010). This difference in patterns of amygdala reactivity may explain why the hyper-aroused reactive group may tend to immediately react to provocations with aggression whereas the hypo-aroused proactive group does not. Although reactive and proactive aggression appear to have different behavioral, physiological, and neurobiological differences, more research is

needed to elucidate the etiological factors that may explain the differences in the two forms of aggression.

Etiology of Aggression

Researchers have examined genetic influences on aggression using quantitative and molecular genetic approaches. By contrasting the phenotypic similarity of monozygotic and dizygotic twins, researchers can estimate the heritability of a phenotype (DiLalla, 2002). Twin studies examining aggression have reported moderate to high heritability (~50%) for general aggression (Miles & Carey, 1997; Rhee & Waldman, 2011). Other twin studies have shown heritability estimates of ~30-40% for both reactive and proactive aggression (Baker, Raine, Liu, & Jacobson, 2008; Brendgen et al., 2006). These estimates suggest that genetic influences play an important role in the etiology of aggression. In addition, twin studies have indicated that each form of aggression is similarly heritable (a^2 = .39 for reactive and a^2 = .41 for proactive) and the genetic influences for each form are highly correlated (r =.87) (Brendgen et al., 2006; Vitaro & Brendgen, 2005). The genetic overlap between the two forms of aggression provides some evidence that the two forms are similar and that there may be overlapping underlying causes.

Although there is substantial evidence for the heritability of aggression, molecular genetic studies have yielded inconsistent results in identifying susceptibility genes for aggression. These inconsistencies may be due to the predominant operationalization of general aggression in the extant literature, despite the aforementioned differences between reactive and proactive aggression. It is thus necessary to compare both forms of aggression in molecular genetic studies of aggression. Furthermore, we may better

understand the etiology of aggression by examining candidate genes that have not yet been extensively studied in humans but have been implicated in the neurobiological pathways of aggression. Therefore, the current study examined the relations between one gene in the serotonergic system (i.e., the serotonin receptor 1B gene, *HTR1B*) and aggression, and compared the association of this gene with the two forms of aggression.

Serotonin and Aggression

Much research on the genetics of aggression has focused on the serotonergic system (Nelson & Trainor, 2007), which is particularly active in the amygdala and orbitofrontal cortex (OFC), areas involved in decision-making and behavioral inhibition (Carver, Johnson, & Joormann, 2008; Yang, Raine, Narr, Colletti, & Toga, 2009). Depletion in serotonin in the OFC has been associated with reduced behavioral inhibition (Carver et al., 2008; Yang et al., 2009) whereas depletion in the amygdala has been associated with hyperarousal to threatening stimuli (Cools et al., 2005; Hariri et al., 2005). Because the amygdala and the OFC are involved in processing and responding to potential threats, these two regions have been implicated in aggression (Nelson & Trainor, 2007). In addition to these brain regions, the serotonin is also involved in modulating aggression. Compared to non-aggressive individuals, aggressive individuals have been found to have lower baseline serotonin levels, indicated by lower levels of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA) (Brown et al., 1982, 1990; Linnoila et al., 1983; Raine, 2008). In contrast, drugs that increase the availability of serotonin were found to decrease aggression in children and adults (Brown et al., 1982, 1990; Virkkunen & Linnoila, 1993; Virkkunen, Goldman, Nielsen, & Linnoila, 1995;

Van Erp & Miczek, 2000; Raine, 2008). These studies provide evidence that the amount of serotonin may be inversely related to aggression.

Given its moderate to high heritability and the implicated role of the serotonergic system in aggression, serotonergic genes are strong candidate genes for aggression. Two serotonergic genes that have frequently been studied are the monoamine oxidase A gene (*MAOA*) and the serotonin transporter gene (5-*HTT*). The variable number of tandem repeats promoter polymorphism in *MAOA* significantly decreases the expression of the monoamine oxidase enzyme that degrades serotonin (Deckert et al., 1999), thereby influencing synaptic levels of serotonin. A similar effect is found in the serotonin transporter gene promoter repeat length polymorphism (*5HTTLPR*), which influences the effectiveness of serotonin reuptake as well as the levels of synaptic serotonin (Reif et al., 2007). Nevertheless, molecular genetic studies that have tried to replicate the associations between either *MAOA* or *5HTTLPR* and antisocial behavior have yielded mixed results (Reif et al., 2007; Buckholtz & Meyer-Lindenberg, 2008; Douglas et al., 2011; Ficks & Waldman, in preparation). It is important to examine other serotonergic genes that have not been extensively studied yet in humans, such as the serotonin receptor 1B gene **(*HTR1B*)**.

Expressions and Functions of Serotonin Receptor 1B Gene (*HTR1B*)

The serotonin receptor 1B gene (*HTR1B*) may be particularly involved in aggression because serotonin exerts its effect by binding to the HTR1B receptors that are produced by the gene. *HTR1B* is located on chromosome 6 (6q13) and is approximately 1500 base pairs long (HapMap, 2010). *HTR1B* encodes the HTR1B receptor that serves several functions. First, serotonin binds to HTR1B receptors, and changes to these

receptors due to genetic variation may alter the effect of serotonin binding on the cell (Olivier & Van Oorschot, 2005). Second, HTR1B receptors self-regulate the release of serotonin and thus influence the levels of serotonin available in the synapse to bind to HTR1B receptors (Olivier & Van Oorschot, 2005).

As part of the serotoninergic system, HTR1B receptors are also expressed in the orbitofrontal cortex (OFC), an area involved in behavioral inhibition and decision-making (Carver, Johnson, & Joormann, 2008), and in the amygdala, an area involved in emotional processing (Duan et al., 2003; Sari, 2004). Activating HTR1B receptors in the OFC with HTR1B receptor agonists has been found to inhibit activation in the OFC when needed for behavioral inhibition (Oliver et al., 2005), suggesting that HTR1B receptors may serve as a "brake" that facilitates behavioral inhibition. In support, reducing HTR1B receptor activation, either by depleting serotonin or by knocking out *HTR1B*, has resulted in behavioral disinhibition (Olivier, 1981; Olivier, Mos, & Rasmussen, 1990; Olivier &Young, 2002; Olivier & van Oorschot, 2005).

Given the important role of HTR1B receptors, changes in the gene, such as single nucleotide polymorphisms (SNPs) in the coding sequence and surrounding 5'- and 3'-untranslated regions (UTRs) in *HTR1B*, have been found to affect the function of HTR1B receptors and serotonin neurotransmission. One commonly studied SNP in the coding region, rs6296, is a synonymous SNP that changes from a G allele to a C minor allele (minor allele frequency = .35) but does not result in a functional change of the amino acid (Val) encoded. In individuals with the C minor allele of the rs6296 SNP, there were 20% fewer HTR1B receptors available for serotonin binding, such that fewer serotonin could bind to the receptor and resulted in reduced neurotransmission (Huang, Grailhe, Arango,

Hen, & Mann, 1999). Nevertheless, because the rs6296 SNP is a synonymous SNP that does not change the amino acid or protein encoded, it is likely that the effect on receptors may not actually be due to the rs6296 SNP itself, but may be due to other functional SNPs that are in linkage disequilibrium (LD) with rs6296 because of their close chromosomal proximity (Duan et al., 2003).

Other functional SNPs found in the promoter and 3'UTR regions have been shown to affect gene expression. Two functional promoter SNPs (rs11568817 and rs130058) were found to affect the binding site of the transcriptional factor and resulted in reduced transcriptional activity of the HTR1B promoter (Duan et al., 2003), which may subsequently affect the HTR1B receptor protein produced. These SNPs are in high linkage disequilibrium with rs6296, suggesting that the fewer HTR1B receptors observed in individuals with the rs6296 SNP may instead be due to these two promoter SNPs. Two other SNPs in the 3'UTR region are rs13212041 and rs6297, which disrupt the binding site for the microRNA miR-96 (Jensen et al., 2009). As a result, these two SNPs reduce the level of gene expression and affect the process of translating from RNA to HTR1B receptor protein (Baek et al., 2008; Selbach et al., 2008). These studies indicate that *HTR1B* polymorphisms influence HTR1B receptors and affect serotonergic gene expression and transmission, suggesting that *HTR1B* may play a role in aggressive behavior. Nonetheless, more research is needed to determine the role of these SNPs in serotonergic neurotransmission and gene expression.

***HTR1B* and Aggression**

Because serotonin has consistently been shown to be associated with aggression and that serotonin release and neurotransmission are regulated by HTR1B receptors,

HTR1B may be particularly implicated in aggression. As discussed above, serotonin has been linked to aggressive behavior, such that the availability of serotonin is inversely related to aggression (see Carver et al., 2008, for review). Lower levels of serotonin were found in individuals exhibiting more aggression (Brown et al., 1982, 1990; Linnoila et al., 1983; Raine, 2008) whereas aggression has been reduced when serotonin agonists were administered (Brown et al., 1982, 1990; Raine, 2008; Van Erp & Miczek, 2000; Virkkunen et al., 1993, 1995). These studies indicate that lower levels of serotonin or less serotonin transmission are linked to increased aggression.

Further, *HTR1B* regulates HTR1B receptors, which influence the amount of synaptic serotonin and neurotransmission by being both production and binding sites of serotonin (Olivier & Van Oorschot, 2005). Individuals who have a history of anger and aggression problems showed blunted metabolic activity in response to serotonergic agonists (Dougherty et al., 2004; New et al., 2002), suggesting that there may be lower efficiency in serotonin binding or less neurotransmission. On the other hand, administration of the anti-aggressive drug, CP-94,253, has shown greater binding affinity for the HTR1B receptors (Koe, Nielsen, Macor, & Heym, 1992; Koe & Lebel, 1995), which provides evidence for how HTR1B receptor binding may influence aggression. These few studies have demonstrated how HTR1B receptors may be related to aggression by regulating the release and expression of serotonin, but more research is needed to determine the effect of genetic polymorphisms on HTR1B receptors and the subsequent serotonin transmission. Given that the level of serotonin is negatively linked with aggression and that serotonin is regulated by HTR1B receptors, there has been some indirect evidence supporting the role of *HTR1B* in aggression.

HTR1B receptors are also expressed in areas implicated in aggression. These receptors regulate the release of serotonin in the OFC (Gaspar, Cases, & Maroteaux, 2003) and amygdala (Sari, 2004), thus influencing functioning in areas important for behavioral inhibition and emotional processing. These areas are likely associated with aggression, as lesions and atrophy in the amygdala have resulted in increased impulsive aggression (Van Elst, Woermann, Lemieux, Thompson, & Trimble, 2000) whereas damage to the OFC has resulted in increased impulsivity, aggression, poorer interpersonal skills (Duffy & Campbell, 1994), and deficits in judgment (Bechara, Tranel & Damasio, 2000). Because HTR1B receptors regulate the amygdala and OFC, brain regions shown to be associated with aggression, *HTR1B* may also be a cause of aggression. More research is needed to determine the direct and indirect relations among *HTR1B*, HTR1B receptors, and aggression.

Further evidence from animal studies supports the association between *HTR1B* and aggression. *HTR1B* knockout mice, which lack functional *HTR1B* gene expression, were found to attack intruder mice more impulsively and severely than wildtype mice (Saudou et al., 1994). Physiologically, this study suggests that *HTR1B* gene expression may play a role in suppressing impulsive behavior. *HTR1B* knockout mice also displayed hyper-arousal in response to an acute stressor (Bouwknecht et al., 2001), indicating a strong reactivity to threats and the urge to react immediately. Pharmacological manipulations that deactivated HTR1B receptors in animals have also supported the role of *HTR1B* in aggression. HTR1B receptor antagonists, which blocked serotonin from binding to HTR1B receptors, were found to increase the amount of impulsivity and aggression in mice (Bjork, Dougherty, Moeller, & Swann, 2000; Chiavegatto et al., 2001;

Marsh, Dougherty, Moeller, Swann, & Spiga, 2002). In contrast, HTR1B receptor agonists that mimicked serotonin at the HTR1B receptors were found to reduce aggression in mice (Bannai, Fish, Faccidomo, & Miczek, 2007; De Almeida et al., 2006; De Boer & Koolhaas, 2005; Oliver et al., 2005; Veiga, Miczek, Lucion, & Almeida, 2007). These studies indicate that HTR1B receptor activation is inversely related to aggressive behavior in animals, and therefore that *HTR1B* may be involved in aggression.

Although neurobiological and animal models of aggression suggest a role of *HTR1B* in aggression, *HTR1B* has not been well-examined in human aggression yet, and extant findings have yielded an inconsistent association with aggression. *HTR1B* polymorphisms have generally been related to disorders with aggressive features, such as conduct disorder (Soyka, Preuss, Koller, Zill, & Bondy, 2004), which characterizes individuals who demonstrate aggressive or nonaggressive antisocial behavior toward other people or property, and antisocial alcoholism, a subtype of alcoholism with impulsive aggressive features (Kranzler, Hernandez-Avila, & Gelernter, 2002; Lappalainen et al., 1998). There was also evidence supporting an association between *HTR1B* and Attention-Deficit/Hyperactivity Disorder (ADHD) (Guimaraes et al., 2009; Hawi et al., 2002; Quist et al., 2003), a disorder characterized by inattention and impulsiveness and has been found to be highly correlated with aggression and conduct disorder (Harty, Miller, Newcorn, & Halperin, 2009; Jensen, Martin, & Cantwell, 1997). Specifically, these association studies have found that the rs6296 SNP was associated with antisocial alcoholism, conduct disorder, and ADHD, but not all studies have replicated this association. The rs6296 SNP was not significantly associated with aggression items on the Child Behavior Checklist (CBCL) (Davidge et al., 2004),

physical aggression (Huang et al., 1999), impulsive aggression (Zouk et al., 2007), impulsive self-injury (Nishiguchi et al., 2001; Rujescu, Giegling, Sato, & Möller, 2003), or ADHD (Ickowicz et al., 2007; Li et al., 2005; Smoller et al., 2006).

HTR1B has also been associated with personality traits related to aggression, including hostility and anger (Conner et al., 2010). Specifically, two functional promoter SNPs (rs6297 and rs13212041) and the two 3'UTR SNPs (rs11568817 and rs130058) were shown to be associated to these traits (Connor et al., 2010). The rs13212041 promoter SNP A minor allele accounted for more of the variance in hostility than other SNPs, and individuals homozygous for the A allele in rs13212041 reported more aggression related to conduct disorder (Jensen et al., 2009). Other than these two studies, it is unclear how these SNPs may be involved in aggression. More research on the association between these SNPs and aggression needs to be conducted as these findings have not been consistently replicated.

Among animal and human studies of *HTR1B* and aggression, the extant literature has not explicitly examined the relations between *HTR1B* and either reactive or proactive form of aggression. Some of the above studies have shown that *HTR1B* is associated to impulsive personality trait and hyper-reactive physiological responses related to reactive aggression in both animals (Bjork, Dougherty, Moeller, Swann, 2000; Bouwknecht et al., 2001; Chiavegatto et al., 2001; Marsh, Dougherty, Moeller, & Swann, 2002; Saudou et al., 1994) and in humans (Kranzler, Hernandez-Avila, & Gelernter, 2002; Lappalainen et al., 1998). Thus, these studies suggest that *HTR1B* may be involved in reactive aggression. On the other hand, there are not any studies that have examined the relation between *HTR1B* and the personality traits or physiological characteristics representative

of proactive aggression. As seen by the lack of genetic studies differentiating the two forms of aggression, further research on the relations between *HTR1B* and the two forms of aggression is warranted.

Gene-Based Association Tests

Although mixed findings between *HTR1B* and aggression in human studies may be attributed to not distinguishing reactive from proactive aggression, these mixed findings may also be attributed to examining only one or a few SNPs when testing for association with aggression. This methodology has been a predominant method in candidate gene association studies, but this method accounts for little of the variation across one particular gene. As a result, the one genotyped SNP may not be the actual SNP that carries the true effect on the phenotype, but may instead be a proxy for nearby loci that truly have an effect due to high linkage disequilibrium. SNPs that carry a true effect may be examined in some studies but not in others, yielding mixed results. An alternative approach using gene-based association tests provides better coverage of the genetic variation across *HTR1B* and captures all potential risk-conferring SNPs, rather than testing only a single or few SNPs in the gene (Neale & Sham, 2004). By utilizing linkage disequilibrium among the various SNPs in a gene, researchers can identify the minimum number of 'tagging' SNPs necessary to represent the majority of variation within a gene (Neale & Sham, 2004). This method reduces the costs of genotyping, and more importantly, reduces the number of statistical tests needed to be conducted (Neale & Sham, 2004). By testing the combination of individual SNPs in one omnibus statistical test, researchers reduce the chances of committing Type I errors and finding spurious associations. The current study utilized a gene-based association testing approach, as this

provides greater confidence regarding how the overall gene may be related to a phenotype.

Goals of the Current Study

In summary, *HTR1B* has not been extensively examined in association with human aggression. In the few studies that have examined the association between *HTR1B* and aggression, studies have yielded mixed findings. These inconsistent findings may be due to a true lack of association between *HTR1B* and aggression, but evidence from neurobiological and animal studies suggests otherwise. Instead, inconsistent findings may be partly explained by the fact that the extant literature has focused on testing the association only with general aggression and has not differentiated reactive from proactive aggression. The current study addressed these gaps by examining a theoretically-plausible serotoninergic gene (*HTR1B*) and by comparing the genetic associations with reactive versus proactive aggression. This should show whether *HTR1B* is more associated with one form of aggression than another, thus explaining why association may not be found when testing against overall, general aggression. Further, the current study utilized gene-based association tests to provide better coverage of the variation within *HTR1B*, improving upon the predominant methodology of testing only one or a few SNPs.

The present study had several aims in understanding the etiology of aggression. First, given the evidence from neurobiological and animal studies on the association between *HTR1B* and aggression, we hypothesized that *HTR1B* is associated with total aggression. Second, we compared the associations between *HTR1B* and reactive and proactive aggression. Because studies have shown some phenotypic and genetic overlap

but also temperamental and neurobiological differences between both forms of aggression, *HTR1B* may have shared or differential effect to each form. Based on the indirect evidence supporting the role of *HTR1B* in reactive aggression, we hypothesized that *HTR1B* may be associated with reactive aggression. Nevertheless, it is unclear whether *HTR1B* may be related to proactive aggression due to the lack of extant studies between *HTR1B* and proactive aggression or related characteristics. By examining the relations between a plausible candidate gene and the two forms of aggression, this study aimed to provide a better understanding of the etiology of aggression and its different forms in children.

Method

Participants

A total of 514 children participated in the study, including 343 clinically-referred children and their siblings recruited through the Center for Learning and Attention Deficit Disorders (CLADD) and the Psychological Center at Emory University, and 171 non-referred twins recruited through the Georgia Twin Registry. Children referred to CLADD and the Emory Psychological Center presented primarily with externalizing behaviors. Any diagnoses assigned by clinic staff were not shared with study personnel. Although the twins were not a clinically-referred sample, they showed variability in their levels of behavior problems, including aggression. The children's age ranged between 6 and 18 years old (M=12, SD = 4). The sample was 58% male and primarily of European descent (87% Caucasian, 10% African-American, and 3% Hispanic). The sample characteristics are described in Table 1.

Procedures

Families were recruited by mailing a letter to their homes explaining the study and requesting them to reply if they were interested in participating in the study. Prior to study participation, we obtained signed consent from the parents and verbal assent from children age of 9 and above. Parents completed questionnaires regarding the family's demographic characteristics as well as their children's DSM-IV symptoms and aggression.

Measures

Parents rated their children's reactive and proactive aggression using a scale described by Dodge and Coie (1987), which was empirically derived from a principle-components factor analysis of 12 items of childhood aggressive behaviors. All items were rated on a 5-point scale (1 = never to 5 = almost always), reflecting how often the aggressive behavior occurred. Items for either the reactive or proactive factor were selected based on the factor loadings for teacher-reported aggression (Dodge & Coie, 1987). The reactive aggression factor included 3 items, "When teased, strikes back," "Overreacts angrily to accidents," and "Blames others in fights," and these three items yielded factor loadings that ranged from .70 – .86. These items were averaged into a single measure with higher scores reflecting greater reactive aggression. The proactive aggression factor comprised the following three items, "Threatens and bullies others," "Uses physical force to dominate," and "Get others to gang up on a peer." Factor loadings for these three items loaded less strongly (ranging from .31 – .45) and were averaged into a single measure. Although only three items represented each factor, each demonstrated high internal consistency in the current study ($\alpha = .81$ for reactive aggression and $\alpha = .78$ for proactive aggression). Lastly, a total aggression measure was

created by summing reactive and proactive aggression scores. Descriptive statistics of aggression scores were reported in Table 2.

Genotyping

Deoxyribonucleic acid (DNA) was collected from buccal cells from parents and their children using buccal brushes or Oragene kits (DNA Genotek, Inc., Ontario, Canada) via a 30-mL solution of 4% sucrose held in their mouths for 1 minute. DNA was extracted by a QIAmp Tissue kit at the Center for Medical Genomics at Emory University according to the protocol developed by the manufacturer (Qiagen, Valencia, California). The buccal cells were pelleted at 2000 *g* for 10 min and samples were preserved in TE (10 mmol/L Tris Hcl, 1 mmol/L ethylenediaminetetraacetic acid [EDTA]).

HTR1B polymorphisms were selected for genotyping using information about individual *HTR1B* SNPs and their linkage disequilibrium (LD) from the International Hapmap Project (available at http://hapmap.ncbi.nlm.nih.gov/), which contains allele frequency data for several ethnic reference populations from around the world. We downloaded from the HapMap database all 292 SNPs in *HTR1B* and 20kb of its 3' and 5' flanking regions. We next characterized the LD among the *HTR1B* SNPs using the Haploview 4.2 program (see Figure 1) (Barrett, Fry, Maller, & Daly, 2005), and selected 'tagging SNPs' - a minimum set of SNPS that capture the majority of the genetic variation across the gene - using the program Tagger as implemented within Haploview 4.2. We identified the minimum necessary set of SNPs required to reach a tagging threshold of $r^2 \geq 0.85$ for all the common SNPs within *HTR1B* (i.e., those with a minor

allele frequency of 0.03 or above). A total of 16 tagging SNPs in *HTR1B* were selected and genotyped, as shown in Table 3.

The 16 tagging SNPs were genotyped on the Sequenom iPlex platform in the Psychiatric and Neurodevelopmental Genetics Unit (PNGU) in the Center for Human Genetic Research at Massachusetts General Hospital (MGH) and at the Broad Institute of Harvard and MIT. After the genotyping was completed at the MGH and at the Broad Institute, our lab received spreadsheets containing the final called genotypes for all samples.

Quality Control Analyses

Departure from Hardy-Weinberg equilibrium (HWE) was used as a measure of genotyping reliability. The 16 tagging SNPs did not significantly deviate from HWE ($p >$ .05 for all SNPs) (see Table 4). Genotyping rates for each SNP were high, as almost all were above 90% ranging from 88% to 100%, see Table 4). Both the HWE and the non-missing genotyping measures indicated adequate genotyping reliability.

Data Analyses

Prior to conducting gene-based association tests, we tested for significant demographic covariates in SPSS 20. Generalized Linear Modeling with Generalized Estimating Equations (GEEs) were used because this method allows for alternative distributions other than the normal distribution, and GEE takes into account the nested family data structure in our sample (Liang & Zeger, 1986). Significant covariates were subsequently controlled for in the gene-based association analyses.

We next conducted gene-based association tests in order to investigate the association between *HTR1B* and aggression using the software package Pedigree-Based

Association Test (PBAT) (Laird, Horvath & Xu, 2000; Lange, DeMeo, Silverman, Weiss, & Laird, 2004). The PBAT software yields a family-based association test (FBAT) statistic, which tests the null hypotheses of no linkage and no association. In our PBAT analyses, FBAT-Generalized Estimating Equation (FBAT-GEE) was used given that we simultaneously tested both reactive and proactive aggression in a multivariate test of association. Because FBAT-GEE does not assume normality of the phenotype distribution (Lange et al., 2003), it was thus appropriate for our skewed aggression data. Aggression scores were transformed to normal Z-scores within the program due to their skewedness (Lange et al., 2002). The additive genetic model of inheritance was further specified because this model has been shown to perform well under a variety of true genetic models (Tu, Balise, & Whittemore, 2000). The 16 selected SNPs within *HTR1B* were all included in one omnibus statistical test against aggression.

Results

Associations between Aggression and Covariates

Generalized linear modeling analyses with generalized estimating equations were used to examine which covariates were significantly associated with reactive and proactive aggression. There were significant effects of covariates on reactive aggression, including sex ($\chi^2 = 9.32$, $p = 2.3\text{x}10^{-3}$, $R^2 = 1.8\%$), age ($\chi^2 = 16.66$, $p = 4.5\text{x}10^{-5}$, $R^2 = 3.2\%$), and age^2 ($\chi^2 = 21.44$, $p = 4.0\text{x}10^{-6}$, $R^2 = 4.2\%$, see Table 4), but ethnicity and the interactions of the age terms with sex (i.e., sex x age, sex x age^2) were not significant ($p > .05$). Similarly, for proactive aggression, we found significant associations for sex ($\chi^2 = 4.27$, $p = .039$, $R^2 = 0.8\%$), age ($\chi^2 = 12.45$, $p = 4.2\text{x}10^{-4}$, $R^2 = 2.4\%$), age^2 ($\chi^2 = 9.65$, $p = .002$, $R^2 = 1.9\%$), as well as for sex x age ($\chi^2 = 7.12$, $p = .008$, $R^2 = 1.4\%$, see Table 5).

Association between *HTR1B* and Total Aggression

We first tested the association between *HTR1B*, represented by the 16 tagging SNPs, and total aggression without controlling for the significant covariates. As hypothesized, there was a significant association between *HTR1B* and total aggression ($p = 4.0x10^{-5}$, see Table 6). There were significant associations for three individual SNPs upstream of the gene, rs12527054, rs9359271, and rs13212041 ($p = .025$, $p = .046$, $p = .017$, see Table 7) and two other SNPs downstream of the gene, rs130058 and rs2798949, were marginally significant ($p = .098$, $p = .061$, see Table 7).

We next conducted additional analyses re-examining the association between *HTR1B* and total aggression after controlling for the significant covariates sex, age, and age^2. Similar to the results without covariates, these analyses revealed that *HTR1B* was significantly associated with total aggression ($p = .018$, see Table 6), although the association was somewhat diminished. Some of the significant SNPs in the analysis without covariates (i.e., rs13212041, rs2798949, and rs12527054 remained significant or marginally significant (see Table 7). Overall, the analyses with and without covariates yielded similar results, suggesting that *HTR1B* was significantly associated with total aggression.

Association between *HTR1B* and Reactive Aggression

We next tested the associations between *HTR1B* and reactive and proactive aggression. In analyses without covariates, as hypothesized, there was a significant association between *HTR1B* and reactive aggression ($p = 0.033$, see Table 6). One SNP upstream of the gene (rs9359271) and another SNP downstream of the gene (rs2798949) were significantly associated with reactive aggression ($p = .039$ and $p = .020$, see Table

7) while two other SNPs downstream of the gene, rs130058 and rs12173930, showed a statistical trend (p = .087 and p = .051, see Table 7). There was no evidence of association between reactive aggression and the other twelve SNPs (p > .05, see Table 7).

In analyses that controlled for sex, age, and age^2 covariates, *HTR1B* also was significantly associated with reactive aggression ($p = 1.9 \times 10^{-3}$, see Table 6). The rs2798949 and rs9359271 SNPs remained significant or marginally significant after covariates were controlled (see Table 7). Consistent with our hypothesis, *HTR1B* was significantly associated with reactive aggression.

Association between *HTR1B* and Proactive Aggression

Associations between *HTR1B* and proactive aggression were also found to be significant ($p = 6.9 \times 10^{-4}$, see Table 6). Three SNPs upstream of the gene were significantly associated with proactive aggression, specifically rs9352481 (p = .047), rs12527054 (p = .017), and rs9359271 (p = .015, see Table 7) while one SNP upstream of the gene (rs13212041) and another SNP downstream (rs2798949) were marginally significant (p = .078 and p = .058, see Table 7). When sex, age, and age^2 were controlled, the results were consistent with analyses that did not include covariates, as the association between *HTR1B* and proactive aggression remained significant ($p = 8.2 \times 10^{-5}$, see Table 6). The SNPs that were significant or marginally significant remained so after controlling for the covariates (see Table 7). The results from analyses with and without covariates suggested that *HTR1B* is associated with proactive aggression. Overall, our findings revealed that *HTR1B* is significantly associated with total, reactive, and proactive aggression.

Sex Differences in Association between *HTR1B* and Aggression

We also conducted analyses investigating gene by sex interactions on aggression. Only two downstream SNPs, rs12173930 and rs9352483, revealed nominally significant sex by SNP interaction effects on reactive aggression ($p = .005$ and $p = .011$, respectively, see Table 8) and there were no significant interactions for the other 14 SNPs (see Table 9). For proactive aggression, there was no evidence of gene by sex interactions for any of the SNPs (see Table 9). Further exploratory analyses divided the full sample by sex, yielding results consistent with the gene by sex interactions (see Table 10).

Discussion

Interpretation of Findings

The current study examined whether *HTR1B*, a serotonergic gene that has not been well studied in human aggression, may be associated with aggression. To resolve inconsistent findings between susceptibility genes and aggression, we differentiated reactive from proactive aggression as well as conducted gene-based analyses of the association between *HTR1B* and aggression. Our results confirmed our hypotheses that *HTR1B* was significantly associated with total, reactive, and proactive aggression, and analyses with and without covariates showed similar findings. The association with total aggression is consistent with previous findings showing that *HTR1B* is associated with disorders and personality traits related to aggression, such as impulsive aggression (Lappalainen et al., 1998; Zouk et al., 2007), conduct disorder symptoms (Jensen et al., 2009), antisocial personality (Soyka et al., 2004), and anger and hostility (Connor et al., 2010). Nevertheless, in a sample of 21 French alcohol-dependent patients (Gorwood et al., 2002) and in a sample of 493 European American alcohol-dependent patients

(Kranzler et al., 2002), there was no association between the *HTR1B* rs6296 SNP and antisocial personality disorder. The association between rs6296 and child aggression reported on the Child Behavior Checklist was also reported to be non-significant in a sample of 50 children (Davidge et al., 2004). The lack of association found in these studies is likely due to their small sample sizes and due to testing only one SNP in *HTR1B*. Our study had a much larger sample size and simultaneously tested multiple SNPs within *HTR1B*, which increased coverage of the gene and statistical power. Nevertheless, these mixed findings suggest that further investigation is needed to replicate our finding of a gene-based association between *HTR1B* and total aggression.

Consistent with our hypothesis, this study also found significant associations between *HTR1B* and reactive and proactive aggression. Although previous molecular genetic studies have not explicitly differentiated reactive from proactive aggression, our findings regarding *HTR1B* and reactive aggression are consistent with the association between *HTR1B* and a personality trait related to reactive aggression—impulsivity (Bjork, Dougherty, Moeller, Swann, 2000; Bouwknecht et al., 2001; Chiavegatto et al., 2001; Kranzler, Hernandez-Avila, & Gelernter, 2002; Lappalainen et al., 1998; Marsh, Dougherty, Moeller, & Swann, 2002; Saudou et al., 1994;). Whereas previous literature suggests the role of *HTR1B* in reactive aggression, extant studies on the genetic influences of proactive aggression are limited. More research is needed to determine the association between *HTR1B* and proactive aggression.

In follow-up analyses of the significant association between *HTR1B* and aggression, we found that five individual SNPs were significantly associated with aggression, but not as significant as the gene-based associations. This finding provides

support for the use of omnibus gene-based analyses, as they provide more statistical power and likely yield stronger effects than the more conventional SNP-based analyses.

For the five significant SNPs, none of these SNPs was within the coding region of the gene, but rather were all in flanking regions that play a regulatory role in gene expression and in the production of the HTR1B receptor protein. Because HTR1B receptors are binding sites for serotonin and serotonin levels and activity have been found to be associated with aggression, changes in the HTR1B receptor protein likely influence aggressive behavior. In the present study, three of the five significant SNPs (rs9352481, rs12527054, rs9359271) were located six to nine kb upstream of the gene in the 5'UTR region. Because 5'UTR's have been found to regulate mRNA translation and the subsequent synthesis of proteins (Barrett, Fletcher, & Wilton, 2012; Chatterjee & Pal, 2009), these SNPs may influence the translation process and the production of the HTR1B receptor protein.

Whereas the 5'UTR SNPs may influence translation, another significant SNP (rs13212041) may influence DNA transcription, given that it is a functional promoter SNP that has been found to be significantly associated with aggression in previous studies (Connor et al., 2010; Jensen et al., 2009). The promoter is in a regulatory region immediately upstream of *HTR1B* and functions by beginning the transcription process (Barrett et al., 2012). The minor allele 'A' has been found to disrupt the transcription binding site and to reduce the overall level of gene expression (Baek et al., 2008; Selbach et al., 2008). The fifth significant SNP, rs2798949, is located 17kb downstream of the gene in the 3'UTR. Similar to the effect of the other SNPs on transcription and translation, the 3'UTR region has been shown to regulate post-transcriptional gene

expression and inhibit translation (Barrett et al., 2012), and changes to the 3'UTR may result in reduced *HTR1B* gene expression. Given the relation between the HTR1B receptor protein and aggression, these significant SNPs likely contribute to aggression by influencing the regulation of gene expression and receptor protein production.

In contrast to these significant SNPs, the widely studied synonymous rs6296 SNP located within the coding region of the gene was not associated with aggression in this study, inconsistent with some prior studies that found an association between rs6296 and aggression (e.g., Jensen et al., 2009; Soyka et al., 2004) but consistent with other studies (Huang et al., 1999; Zouk et al., 2007). It may be possible that previous positive findings for rs6296 may not reflect true effects of this SNP, but the effects of other functional SNPs with which rs6296 is in high LD. Our findings lend support to this possibility because the rs6296 SNP was not significant but the promoter SNP (rs13212041) in high LD with rs6296 was significant instead.

Lastly, exploratory analyses did not find evidence of gene by sex interaction effect on any forms of aggression, except for two SNPs (rs12173930 and rs9352483). Additional analyses that divided the full sample into males and females also did not report significant findings. It is possible that the lack of gene by sex interactions reflects a true lack of effect or the lack of statistical power to detect interactions in the current study. Future studies should aim to increase sample size, as greater sample size is particularly necessary to detect interaction effects (McClelland & Judd, 1993; Wahlsten, 1991).

Implications of Findings

This study has several implications. First, our findings contribute to our understanding of the etiology of aggression by providing robust evidence for the role of *HTR1B* in aggression, which has not been well established in the extant literature. Future research should attempt to replicate these associations and elucidate biological pathways that could explain how *HTR1B* may contribute to aggression. One promising future direction may be the examination of endophenotypes, which are intermediate phenotypes that are hypothesized to underlie disorders or traits and are more directly influenced by the genes than the manifest symptoms or traits (Gottesman & Gould, 2003; Waldman, 2005). From our understanding of the neurobiology of *HTR1B*, the gene regulates HTR1B receptors, which are richly expressed in the orbitofrontal cortex (OFC) and amygdala and involved in behavioral inhibition and emotional processing (Carver, Johnson, & Joormann, 2008; Gaspar et al., 2003). When HTR1B receptor activation was inhibited by administering an HTR1B receptor antagonist or knocking out the gene, there was less behavioral control and increased aggressive behavior in mice (Kruk, 1991; Olivier et al., 1990; Olivier & Van Oorschot, 2005; Olivier and Young, 2002). Behavioral inhibition and emotional processing may thus serve as promising endophenotypes that explain how *HTR1B* may contribute to aggression. Identifying these intermediate pathways may be particularly useful for designing clinical interventions to reduce aggression by targeting these *HTR1B*-mediated mechanisms.

Moreover, our findings that *HTR1B* was associated with both reactive and proactive aggression provide some support for the one-factor model of aggression. Previous studies have debated whether aggression should be viewed as one general factor or as two factors with reactive and proactive aggression (e.g., Bushman & Anderson,

2001). Proponents of the two-factor model have argued that reactive and proactive aggression are distinct based on the vast number of behavioral and physiological differences (Dodge & Coie, 1987; Kempes et al., 2005; Raine et al., 2006). In contrast, the one-factor model of aggression has been based on previous quantitative genetic findings that have estimated highly correlated genetic influences of reactive and proactive aggression (Brendgen et al., 2006). Supporting the one-factor model, this study has identified one particular gene (*HTR1B*) that explains how the genetic influences on reactive and proactive aggression may overlap. Future studies should consider examining other candidate genes, as the genetic influences of these genes on reactive and proactive aggression may be different from the shared effect of *HTR1B*. Understanding more about how other genes are similarly or differentially related to the two forms of aggression would help us understand whether the reactive and proactive distinction is meaningful.

Our study has further implications for the neurobiological mechanisms underlying the associations between *HTR1B* and reactive and proactive aggression. Although *HTR1B* is associated with both forms of aggression, some phenotypic differences have been observed in both forms. One possible explanation may be that there are two different neurobiological pathways in the OFC and in the basal ganglia regulated by *HTR1B* simultaneously, which may result in the differences between reactive and proactive aggression. *HTR1B* has been proposed to be related to aggression due to its receptors' involvement in the OFC and behavioral disinhibition. Because behavioral disinhibition and impulsivity are both representative of the immediate overreactions to provocations found in children exhibiting reactive aggression (Dodge & Coie, 1987), the OFC may be particularly involved in reactive aggression. In contrast, it is unlikely that

the behavioral disinhibition implicated in OFC can explain the planful, premeditated characteristics observed in proactive aggression. Instead, proactive aggression may be associated with *HTR1B* through a more goal-directed mechanism. Studies have found that HTR1B receptors are also expressed in the basal ganglia (Gaspar et al., 2003; Jin et al., 1992; Reif & Lesch, 2003), a region responsible for mediating goal-directed behavior and processing of rewards and punishments (Delgado, 2007). Children exhibiting proactive aggression have been shown to view aggression as rewarding and positive rather than as punishment (Crick & Dodge, 1996; Marsee & Frick, 2007), thus they may be motivated to plan aggression to achieve goals or rewards. Given these characteristics of proactive aggression and the implicated role of basal ganglia in goal-directed behavior, the basal ganglia may represent a different pathway for how *HTR1B* may contribute to proactive aggression. The different possible pathways of OFC and basal ganglia regulated by HTR1B receptors provide an interesting explanation for how *HTR1B* may be associated with two forms of aggression that appear to have vastly different behavioral and biological characteristics. Further research should examine these pathways in relation to the genetic associations of *HTR1B* and the two forms of aggression.

Limitations & Strengths

This study has several limitations to be noted. First, because complex traits, such as aggression, are likely accounted for by multiple variants that have modest effect sizes (Lohmueller, Pearce, Pike, Lander, & Hirschhorn, 2003), larger sample sizes are needed to have adequate statistical power to detect these effect sizes. Furthermore, given that gene by sex interactions have been found in a few previous studies (Baker et al., 2008; Jones & Lucki, 2005), it may be possible that there are true interactions that were not

found in the current study due to the small sample size, despite the fact that there were mean differences in aggression between males and females in the expected direction in the current study. Future studies should include larger samples of both sex, as interactions typically require much greater statistical power to detect than main effects (McClelland & Judd, 1993; Wahlsten, 1991).

Second, the majority of the sample was of European-American background and therefore did not represent the full diversity of ethnic backgrounds in the population. Because different ethnic groups have shown large differences in the allele frequency of polymorphisms in *HTR1B* (Proudnikov et al., 2006), the associations found within this sample may not be generalizable to all ethnic groups. Future studies should examine these associations using a more ethnically diverse sample. Lastly, the current study did not investigate the underlying neurobiological mechanisms that may intervene between *HTR1B* and various forms of aggression. Although *HTR1B* was found to be related to both forms of aggression, it is unclear as yet what neurobiological mechanisms may explain this shared genetic association. More research on endophenotypes is needed in order to elucidate the underlying neurobiological mechanisms between *HTR1B* and the different forms of aggression, which also may inform how clinical treatments can intervene at these neurobiological pathways. Given how few studies have examined the role of *HTR1B* in aggression and compared reactive and proactive aggression, future replications of these associations are needed, and we plan to replicate these associations using other samples.

Despite these limitations, our study has several methodological strengths. First, by distinguishing reactive from proactive aggression, we examined how *HTR1B* may

have shared or unique influences on these two forms of aggression. This is important because the two forms of aggression have demonstrated significant differences in their behavioral, physiological, and neurobiological characteristics. Second, this study used more comprehensive gene-based association tests to represent the majority of the variation across *HTR1B*, thus statistically capturing all potential risk-conferring SNPs that may be related to aggression. One of the possible reasons for equivocal findings between *HTR1B* and aggression in extant literature may be due to testing only one or few markers. Our study found that the gene-based association results provided much greater evidence for association with aggression than the individual SNP-based analyses. Compared to testing only a single or a few markers in previous studies, this gene-based omnibus testing approach not only is more comprehensive in its coverage, but also reduces the likelihood of committing Type I errors by reducing the number of statistical tests conducted. This study design allows us to be more confident about our findings in relation to the association between *HTR1B* and aggression, and future research should conduct gene-based association analyses in an effort to replicate the current findings.

Conclusion

The present study provides evidence that *HTR1B* is associated with total, reactive, and proactive aggression. By distinguishing aggression into reactive and proactive aggression, we found that *HTR1B* was associated with both reactive and proactive aggression, suggesting that there is shared genetic influence on seemingly heterogeneous forms of aggression. Further, by using the gene-based association test to determine the association between *HTR1B* and aggression, the current study was able to better represent the majority of the genetic variation across *HTR1B*. Future studies should also examine

associations between other candidate genes and childhood externalizing disorders using gene-based association tests to effectively capture all risk-conferring SNPs within the gene. Such studies would help increase our understanding about the genetic risk factors that contribute to the etiology of various forms of aggression, as well as whether reactive and proactive aggression is a meaningful distinction genetically. More research is needed to replicate these findings and to identify the intermediate neurobiological mechanisms between *HTR1B* and different forms of aggression using endophenotypes. By identifying susceptibility genes and understanding the underlying mechanisms, we can better understand the development of aggression.

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Appendix

Table 1

Sample Characteristics for the Clinic-Referred and Control Samples

	Clinic-Referred Sample	Control Sample	Total
Sample Size			
# of families	222	87	309
Total children	343	171	514
Demographic characteristics			
Age	10.8 (4.0)	13.7 (2.5)	11.7 (3.8)
Gender	229M, 114F	71M, 100F	300M, 214F
Ethnicity			
Caucasian	271	148	419
African-American	42	4	46
Hispanic	14	0	14

Note: Gender - M = Male, F = Female

Table 2

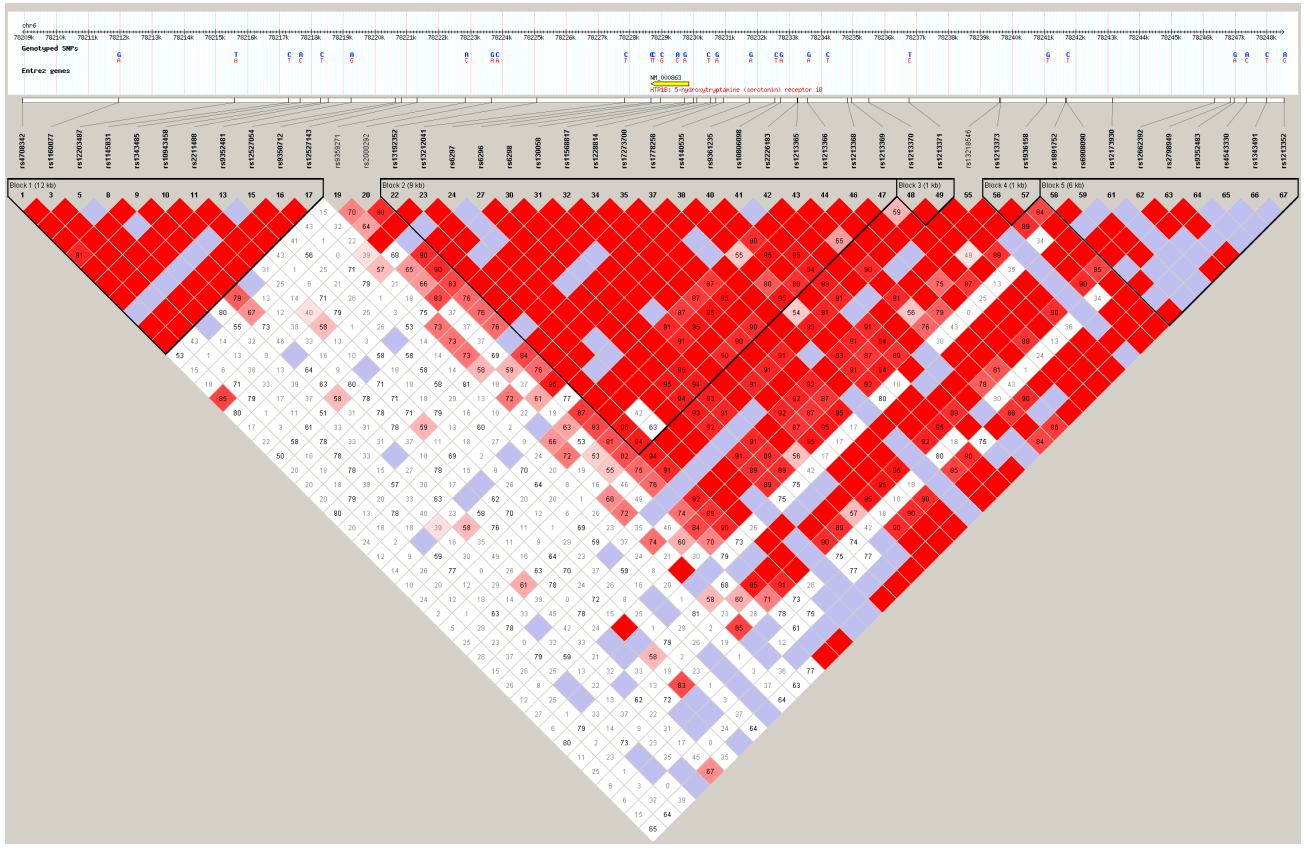
Descriptive Statistics of Aggression Scores

		M (SD)	
Aggression	Full Sample	Males	Females
Reactive	2.90 (2.97)	3.27 (3.05)	2.38 (2.78)
Proactive	.80 (1.57)	.98 (1.75)	.55 (1.24)
Total	3.70 (4.11)	4.24 (4.32)	2.93 (3.68)

Note: Total aggression scores are the sum of reactive and proactive aggression scores.

Figure 1

HTR1B Linkage Disequilibrium Plot



Note: Haploview-generated LD plot illustration. The values shown above are raw D' scores, a measure of linkage disequilibrium between two genetic markers. D' values range from 0 to 1 (blue to red, respectively). Values shown represent 100 x |D'|, empty red boxes represent a value of 100 or complete LD (i.e., D'=1).

Table 3

Allele frequency of Tagging SNPs

SNP	Position	Location	Alleles	MAF
rs9352481	78219255	9kb upstream	G/A	0.46
rs12527054	78220647	8kb upstream	T/C	0.08
rs12527143	78221317	7kb upstream	T/C	0.19
rs9359271	78222839	6kb upstream	A/C	0.32
rs2000292	78223664	5kb upstream	G/A	0.35
rs13212041	78227843	1kb upstream	T/C	0.17
rs6296	78228979	Coding region	C/G	0.34
rs6298	78229711	Coding region	G/A	0.33
rs130058	78230000	<1kb downstream	T/A	0.22
rs2226183	78233257	3kb downstream	G/A	0.18
rs1936158	78241094	11kb downstream	G/T	0.45
rs12173930	78243153	13kb downstream	C/T	0.10
rs2798949	78246853	17kb downstream	G/A	0.34
rs9352483	78246975	17kb downstream	G/A	0.14
rs4543330	78247352	17kb downstream	A/C	0.19
rs1213352	78248530	19kb downstream	G/A	0.13

Notes: MAF = minor allele frequency

Alleles: The first allele listed is the major allele and the second allele is the minor allele.

Table 4

Quality Control Analyses: Hardy Weinberg Equilibrium and Non-Missing Genotypes

SNP	HWE *p*	%Geno
rs9352481	0.10	90
rs12527054	1.00	100
rs12527143	0.15	100
rs9359271	0.60	90
rs2000292	0.57	88
rs13212041	1.00	89
rs6296	0.33	89
rs6298	0.36	90
rs130058	0.35	100
rs2226183	0.87	100
rs1936158	0.80	90
rs12173930	0.91	100
rs2798949	1.00	100
rs9352483	0.49	89
rs4543330	1.00	90
rs1213352	1.00	90

Notes: HWE *p* = probability of departure from Hardy-weinberg equilibrium, an indicator of genotyping error based on the constant allele frequencies assumption.

%Geno is the percentage of participants that were successfully genotyped for this marker.

Table 5

Covariate Analyses for Reactive and Proactive Aggression

	Reactive Aggression			Proactive Aggression		
Predictor	Wald's χ^2	*p*	R^2	Wald's χ^2	*p*	R^2
Sex	9.32	2.3E-03**	0.018	4.27	3.9E-02*	0.008
Age	16.66	4.5E-05***	0.032	12.45	4.2E-04***	0.024
Age^2	21.44	4.0E-06***	0.042	9.65	2.0E-03**	0.019
Sex x Age	1.29	2.6E-01	0.003	7.12	8.0E-03**	0.014

Notes: *<.05. **<.01. ***<.001

Sex x Age is an interaction term of sex and age.

The aggression variables were modeled with a Negative Binomial distribution with a Log Link function.

Table 6

Association between HTR1B and Aggression with and without Covariates

	p	
Aggression	Without covariates	With covariates
Reactive	3.3E-02	1.9E-03
Proactive	6.9E-04	8.2E-05
Total	4.0E-05	1.8E-02

Note: Covariates included: Age, Age^2, sex

Table 7

Associations between HTR1B SNPs and Aggression with and without Covariates

	p					
	Without covariates			With covariates		
SNPs	Total	Reactive	Proactive	Total	Reactive	Proactive
rs9352481	0.139	0.197	0.047	0.200	0.329	0.076
rs12527054	0.025	0.782	0.017	0.081	0.091	0.030
rs12527143	0.716	0.812	0.428	0.616	0.815	0.361
rs9359271	0.046	0.039	0.015	0.133	0.065	0.069
rs2000292	0.173	0.435	0.324	0.580	0.897	0.465
rs13212041	0.017	0.501	0.078	0.003	0.572	0.026
rs6296	0.662	0.426	0.981	0.911	0.825	0.852
rs6298	0.441	0.604	0.212	0.274	0.436	0.111
rs130058	0.098	0.087	0.988	0.312	0.148	0.211
rs2226183	0.324	0.134	0.368	0.424	0.192	0.480
rs1936158	0.337	0.141	0.283	0.324	0.144	0.543
rs12173930	0.148	0.051	0.186	0.497	0.287	0.805
rs2798949	0.061	0.02	0.058	0.026	0.008	0.046
rs9352483	0.607	0.762	0.322	0.625	0.333	0.986
rs4543330	0.625	0.553	0.332	0.467	0.240	0.694
rs1213352	0.712	0.727	0.413	0.534	0.979	0.364

Note: Covariates included: Age, Age^2, sex

Table 8

Sex by HTR1B SNP Interaction Effects on Aggression

	p	
SNPs	Reactive	Proactive
rs9352481	0.953	0.534
rs12527054	0.699	0.828
rs12527143	0.817	0.569
rs9359271	0.744	0.912
rs2000292	0.773	0.084
rs13212041	0.301	0.410
rs6296	0.217	0.667
rs6298	0.520	0.990
rs130058	0.768	0.724
rs2226183	0.177	0.683
rs1936158	0.584	0.892
rs12173930	0.005	0.480
rs2798949	0.256	0.611
rs9352483	0.011	0.120
rs4543330	0.389	0.128
rs1213352	0.312	0.460

Note: All analyses controlled for age and age^2 covariates.

Table 9

Association between HTR1B and Aggression with and without Covariates for Males and Females

	p			
	Males		Females	
Aggression	No covariates	With covariates	No covariates	With covariates
Reactive	0.082	0.001	-	0.804
Proactive	0.075	0.088	-	0.873
Total	0.538	0.088	-	0.932

Notes: Covariates included: Age, Age^2

Analyses without covariates for females did not run

Table 10

Associations between HTR1B SNPs and Aggression for Males and Females

	p					
	Males			Females		
SNPs	Total	Reactive	Proactive	Total	Reactive	Proactive
rs9352481	0.384	0.941	0.284	0.836	0.564	0.564
rs12527054	0.261	0.243	0.119	0.368	0.157	0.190
rs12527143	0.298	0.535	0.402	1.000	1.000	1.000
rs9359271	0.402	0.284	0.203	0.523	0.319	0.257
rs2000292	0.979	0.870	0.839	0.243	0.594	0.414
rs13212041	0.060	0.558	0.187	0.479	0.907	0.259
rs6296	0.503	0.537	0.665	0.395	0.317	0.317
rs6298	0.377	0.308	0.163	0.788	0.576	0.611
rs130058	0.886	0.674	0.923	0.677	0.783	0.378
rs2226183	0.230	0.097	0.459	1.000	1.000	1.000
rs1936158	0.251	0.096	0.348	0.365	0.392	0.159
rs12173930	0.716	0.519	0.430	0.607	0.426	0.564
rs2798949	0.165	0.063	0.201	0.572	0.323	0.933
rs9352483	0.594	0.699	0.344	0.578	0.317	0.317
rs4543330	0.954	0.760	0.823	0.368	0.218	0.551
rs1213352	0.597	0.897	0.321	0.844	0.991	0.740

Note: Covariates included: Age, Age^2