Genetic variants associated with aggressive behaviour in humans: the serotonergic system

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Orientadora

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

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Resumo

O Comportamento Agressivo nos seres humanos não é uma característica simples de estudar. Os mecanismos moleculares e bioquímicos que regulam o comportamento humana estão sob a influência de múltiplos fatores, quer genéticos quer do meio envolvente. Múltiplos estudos recentes se têm dedicado ao estudo da componente genética do comportamento agressivo, e têm sido compilados conjuntos de genes, polimorfismos e vias de sinalização que são considerados candidatos fortes a esta componente. O objetivo principal deste trabalho foi analisar alguns destes polimorfismos, para perceber qual o seu papel na etiologia do comportamento humano. O estudo focou-se em variantes de genes do sistema da serotonina e na sua interação, que tem sido consistentemente associado com comportamento agressivo.

Palavras Chave: polimorfismos, comportamento agressivo, serotonina, MAOA, SLC6A4, TPH1, TPH2, HTR1A, HTR1B, HTR2A, HTR2B

Abstract

Human Aggressive Behaviour is not a simple characteristic to study and analyse. The molecular and biochemical mechanisms that regulate one's behaviour are under the influence of multiple components, both genetic and environmental. Several recent association studies have compiled lists of candidate genes, polymorphisms and molecular pathways considered to be strong candidates for genetic factors partially responsible for some form of aggressive behaviour. The main goal of the project was to study some of these polymorphisms, in order to understand their evolution and current role in the human system. This study focused on variants in genes from the serotonergic system, that has consistently been associated with aggression.

Keywords: polymorphisms, aggressive behaviour, serotonin, MAOA, SLC6A4, TPH1, TPH2, HTR1A, HTR1B, HTR2A, HTR2B

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Abbreviations

5-HTT	5-HT transporter
5-HTTLPR	(5-HTT linked polymorphic region)
AB	Aggressive Behaviour
ACC	Anterior Cingulate Cortex (ACC)
ASPD	Antisocial Personality Disorder
bp	Base Pairs
BPAQ	Buss Perry Aggression Questionnaire
CADD	Combined Annotation–Dependent Depletion
CD	Conduct Disorder
CDS	Coding Sequence
CGAS	Candidate Gene Association Studies
C-score	CADD Raw Score
CU	Callous-Unemotional (traits)
dVNTR	Distal VNTR
GERP	Genomic Evolutionary Rate Profiling
GWAS	Genome-Wide Association Studies
HMM	hidden Markel model
HTR1A	5-HT Receptor 1A
HTR1B	5-HT Receptor 1B
HTR2A	5-HT Receptor 2A
HTR2B	5-HT Receptor 2B
InDel	Insertion/Deletion
LD	Linkage Disequilibrium
MAF	Minor Allele Frequency
MAOA	Monoamine Oxidase A
MAOA-H	MAOA High Activity Variants
MAOA-L	MAOA Low Activity Variants
NMD	Nonsense Mediated Decay
ODD	Oppositional Defiant Disorder
OFC	Orbitofrontal Cortex
PFC	Prefrontal Cortex

PSC	Premature STOP Codon
RefSeq	Reference Sequence
RS	Rejected Substitutions
SERT	Serotonin Transporter
SNP	Single Nucleotide Polymorphisms
S-score	CADD Scaled Score
STin2	Serotonin Transporter intron 2 polymorphism
TF	Transcription Tactor
TFBS	TF Binding Site
TPH	Tryptophan Hydroxylase
UTR	Untranslated Region
uVNTR	Upstream VNTR
VEP	(Ensembl) Variant Effect Predictor
vmPFC	Ventromedial Prefrontal Cortex
VNTR	Variable Number of Tandem Repeats

1. Introduction

In 1993, a team led by the geneticist Han Brunner published a study describing, for the first time, an association between a specific genetic mutation and abnormal behaviour (Brunner et al., 1993). In their work, Brunner and colleagues studied a Dutch family in which several male members exhibited "borderline mental retardation and a tendency toward aggressive outbursts", but the women of the family were "normal". They discovered that the affected members of the family had a genetic mutation in the X-linked *MAOA* gene. This mutation, a non-conservative C > T substitution, creates a premature stop codon in place of a glutamine, in the 8th exon, which causes loss of function. This drastic reduction in MAOA expression and activity was hypothesized by the authors to alter neurotransmitter metabolism in the brain. Male carriers of this mutation, having only one copy of the X chromosome, were virtually MAOA knock outs, but female carriers maintained a near normal level of MAOA activity (Brunner et al., 1993).

About 25 years later, a comprehensive analysis of genes and biological pathways that had been consistently associated with aggression was conducted (Zhang-James et al., 2019). The authors compared evidence from different sources, including human association studies and animal models, and identified several enriched common pathways, as well as over 1700 genes that had been identified as possible candidates for causing aggressive phenotypes. They created a ranked list of the 40 most relevant genes – and *MAOA* was at the top of that list.

Aggression and aggressive behaviours (ABs) are evolutionary natural traits, present in most animal species (Waltes et al., 2016). They have been thought to play important roles in the survival of a species, such as providing a competitive advantage in securing resources or sexual partners, achieving social dominance, and in protection against predators or attackers (Odintsova et al., 2019). During the early stages of human evolution, aggression was probably an adaptive trait, with a display of aggression hypothetically leading to better chances for survival and reproduction.

While some level of aggression may be a competitive advantage in nature, inadequate aggression (which can escalate into violence) can lead to severe consequences for the individual and society (Pavlov et al., 2012). Therefore, there has been a growing interest in understanding the biological mechanisms that regulate aggression in humans.

1.1 What is aggressive behaviour?

Aggression is not a uniform behavioural construct. Broadly, it can be defined as a behaviour (or set of behaviours) that intends to cause physical or emotional harm to others or to the self (American Psychological Association's Dictionary of Psychology). AB may express itself through different phenotypes. However, there have been different types of AB described, that are suggested to be regulated by different neural paths.

Two types of aggression are frequently distinguished, one proposed to result from a lack of emotional sensitivity (offensive/proactive aggression) and the other from an excess of it (impulsive/reactive aggression) (Waltes et al., 2016). Proactive aggression is pre-meditated and frequently goal directed; it is characteristically associated with psychopathy, lacking both empathy and remorse (Dodge et al., 1997). Reactive aggression is typically triggered by negative experiences and emotions including anger and/or anxiety. It appears to result from exaggerated threat perception and response to it, together with an inability to control the resultant enhanced emotional state (Card & Little, 2006). Furthermore, proactive (but not reactive) aggression is correlated with positive expectation of outcomes that would result from the behaviour (Zhu et al., 2019).

While proactive and reactive aggression are the most often used categories, there are several other classifications for AB. A frustrative/non-reward form of aggression can sometimes be studied with reactive and proactive forms (Veroude et al., 2016). There can be physical, verbal or social forms of aggression, which differ in the type of action exerted (Andraszczyk & Gierczyk, 2020; Ettekal & Ladd, 2017). Overt and covert forms of aggression refer to the difference between obvious, openly aggressive behaviours or more deceptive actions (Olson et al., 2013; Waltes et al., 2016). These different types of AB aren't mutually exclusive: for example, physical aggression can be both reactive or proactive in nature, while offensive aggression can be both covert or overt.

Aggression can be considered a behaviour problem in its own, or it may present as a symptom for an underlying behavioural disorder (Hendriks et al., 2020). AB has been studied in the context of multiple mood or behavioural disorders (Veroude et al., 2016). Oppositional Defiant Disorder (ODD) is a condition characterised by an ongoing pattern of angry/irritable mood, argumentative/defiant behaviour or vindictiveness, mostly diagnosed in children (American Psychiatric Association, 2013; Ghosh et al., 2017). Conduct Disorder (CD) presents itself through a pattern of aggressive, destructive and deceitful behaviours, as well as persistent violation of rules (American Psychiatric Association, 2013; Fisher et al., 2013). A specifier for CD involves the presence or absence of Callous-Unemotional (CU) traits, characterized by low empathy, interpersonal callousness, lack of affect, remorse or guilt, and no concern for performance (American Psychiatric Association, 2013; Viding et al., 2013). Antisocial Personality Disorder (ASPD), or simply Antisocial Behaviour (ASB), is characterized by a long-term pattern of disregard for and violation of the rights of others,

deceitfulness, aggressiveness and lack of remorse (American Psychiatric Association, 2013), and seems to be highly associated with criminal behaviour (Ling et al., 2019). Aggression and AB are also often studied in association with other mental disorders that have been found to have high levels of aggressive traits (de Almeida et al., 2015), and have also been assessed based on certain personality traits or dimensions (García-Sancho et al., 2017; Ramírez & Andreu, 2006).

The complexity of constructs associated with Aggression makes it a difficult trait to study. AB presents the same challenges as complex traits, possibly caused by multiple genetic variants, each of small penetrance, in interaction with environmental experiences. Current hypotheses suggest that many environmental effects, such as early life stress or other chronic psychosocial risk factors, and genetic variants in genes related to neuroendocrine, dopaminergic and serotonergic systems increase the risk to develop AB (Davydova et al., 2018).

1.2 Neurobiology of aggressive behaviour

Neuroimaging and *post-mortem* studies have helped identify specific brain areas involved in the regulation of AB. Neural regions of interest are identified by their associations with behavioural phenotypes related to aggression, including abnormal responses to fear and threat (e.g. amygdala), disinhibition (e.g. prefrontal regions), and increased reward sensitivity (e.g. striatum) (Raine, 2019; Shiina, 2015).

The amygdala is the brain region that has more consistently shown association with aggression and violence (Enticott et al., 2020). It is located in the medial parts of the temporal lobe, and its typically thought to be critical for emotional processing, in particular the fear circuitry. Smaller amygdala size is consistently linked to increased aggression (Coccaro et al., 2011; Siever, 2008). Furthermore, there are reports of abnormal patterns of activation in clinically impulsive/aggression groups (Blair, 2016; Rosell & Siever, 2015). Reactive aggression has been associated with enhanced amygdala activation (Blair, 2016; Pardini et al., 2014) together with reduced prefrontal activity. This would cause an individual to be easily aroused emotionally, but have reduced ability to regulate this arousal (Raine, 2019). On the other hand, proactive aggression with CU traits and instrumental aggression in psychopathy have been associated with reduced amygdala activation during emotion-processing (Glenn & Raine, 2009; Lozier et al., 2014).

Aggression has also been associated with dysregulation in a corticolimbic network (Dorfman et al., 2014; Siever, 2008), specifically, a deficient regulation of the amygdala via *prefrontal cortex* (PFC) areas (Coccaro et al., 2011). The PFC is critical for controlling one's emotional states and behavioural output (Enticott et al., 2020), and exerts cortical control over lower level brain circuits involved in emotion and reward (Gillespie et al., 2018). Both the lateral and medial PFC play a role in emotion regulation; the lateral PFC mediates the deliberate aspects of emotional regulation (Gillespie et al., 2018) whilst the medial PFC subserves automatic processes. Furthermore, the *orbitofrontal cortex* (OFC)/*ventromedial prefrontal cortex* (vmPFC) and the *anterior cingulate cortex* (ACC) are particularly relevant, as they reveal extensive structural connectivity with the amygdala (Rosell & Siever, 2015); the ACC projects extensively to the amygdala, while the posterior OFC receives projections from the amygdala.

One meta-analysis of 29 structural and functional brain imaging studies of violent offenders documented reduced structure/function of the prefrontal cortex in violent offenders (Yang & Raine, 2009). Reduced grey matter and volume in the ACC have been associated with aggression, including in children. Aggression is also linked to the reduced functional activation of the OFC/vmPFC, which participates in the brain's reward system (Enticott et al., 2020).

The striatum is a subcortical region, which plays a critical role in selection and inhibition of various competing response sequences, reward-based decision-making and is implicated in voluntary

movement (Raine, 2019; Rosell & Siever, 2015). Neurochemically, the striatum is modulated by glutamatergic, dopaminergic, and serotonergic systems (Waltes et al., 2016). Specifically, serotonin (5-HT) appears to be critical for determining how time delay will affect the value of a particular reward (Rosell & Siever, 2015). The *nucleus accumbens*, which forms part of the striatum, is also implicated in the brain's reward system, and is connected with critical PFC regions involved in regulating behavioural output (Blair, 2016). Increased striatum size and activity have been associated with AB (da Cunha-Bang et al., 2017; Gan et al., 2016; Y. Yang et al., 2017).

1.3 Genetics of aggressive behaviour

Aggression and AB are complex traits: instead of one single causal gene, AB is influenced by a genetic component and an environmental component. The genetic component does not refer to a single gene, instead, it is the result of multiple genetic factors, and their epistatic interaction (Odintsova et al., 2019). The environmental component involves an individual's experiences and relationships, family interactions and social environment (Beaver et al., 2018). Furthermore, there has also been described a Gene x Environment interaction component, so that an individual's genetic architecture can change the way he or she reacts to environmental queues, and environment factors can modulate expression of certain genes, for example through epigenetic modifications (Palumbo et al., 2018).

Twin and adoption studies have estimated the heritability (the proportion of phenotypic variance accounted for by genetic variation) of AB to be about 50% (Craig & Halton, 2009; Tuvblad & Baker, 2011). However, this fraction changes according to the specific characteristics of the sample, the dimension or disorder being studied, and the instruments used to evaluate aggressive behaviours (Fairchild et al., 2019; Ghosh et al., 2017; Tremblay et al., 2018). Physical aggression shows larger heritability estimates (65%) than both reactive (20–43%) and proactive aggression (32–48%) (Waltes et al., 2016). Seeing as the variance left unexplained by heritability is accounted for by environmental influences (and error), these are also estimated to influence phenotypic variation in about 50%, in a manner inverse to that of the genetic influence. Once again, these measures vary a lot depending on the subjects' characteristics, the phenotype being measured and the instruments for evaluation (Gard et al., 2019).

There has been a growing interest in studying the genetic component of AB, and advances in the genomic sciences are making it possible to investigate these influences at a molecular and genetic level. Many association studies have been performed to identify genetic factors underlying ABs in humans, through either candidate gene (CGAS) or genome-wide association studies (GWAS) (Veroude et al., 2016).

Candidate gene studies use neurobiological information and animal studies to focus on genes that are part of functional pathways known to be associated with AB. Therefore, they tend to centre around the same set of genes, mostly in pathways regulating neurotransmitter signalling (mainly in the serotonergic and dopaminergic pathways) and hormonal functions (Waltes et al., 2016). Some of these genes include serotonin and dopamine receptors such as HTR2A, DRD2 and DRD4 (Banlaki et al., 2015; Buchmann et al., 2014; Chester et al., 2016), the serotonin and dopamine transporters SLC6A3 and SLC6A4 (Tielbeek et al., 2016; Vassos et al., 2014), androgen and estrogen receptors AR and ESR1 (Vaillancourt et al., 2012; Vermeersch et al., 2010), and the genes that code for Nitric Oxide Synthase 1 and 3, NOS1 and NOS3 (Rujescu et al., 2008).

On the other hand, GWAS have a hypothesis-free approach, which may lead to results that had not previously been considered. GWAS studies require a very large number of subjects, which is not always available, and therefore current results may exhibit a lot of false positives, while skipping over variants of small effect (Fernàndez-Castillo & Cormand, 2016). There have been hundreds of polymorphisms identified in genome-wide studies (Odintsova et al., 2019); however, only a small number of variants have reached the threshold for genome-wide significance (p < 5.0E-08). Furthermore, there has been little overlap between results from GWAS and candidate genes, although some signals have been identified in candidate genes, such as *HTR1B* (Viding et al., 2010), *SLC6A3* and *TPH2* (Sonuga-Barke et al., 2008). Interestingly, a few genes have been identified as possibly relevant in different Genome-Wide studies, such as *CSMD1* (Brevik et al., 2016; Tielbeek et al., 2012), *PRDM2* (Mick et al., 2014; Rautiainen et al., 2016; Tiihonen et al., 2015) and *SOX5* (Aebi et al., 2016; Mick et al., 2011; Tiihonen et al., 2015), none of which had been previously considered in candidate gene studies.

CGAS and GWAS have allowed the identification of potential susceptibility genes for ABs, despite their respective limitations. A recent study was published, comparing several association studies in both Humans and Rodent models (mice and rats) (Zhang-James et al., 2018). The authors compared eight different sets of genes, including genes identified in GWAS of AB and related constructs, genes associated with aggression in mice and rats, and genes responsible for mendelian diseases with aggressive behaviours (Zhang-James & Faraone, 2016), and found some overlap in multiple genes and several biological pathways, indicating a few that were considered candidates associated to some form of AB.

1.4 Project goals

In a broad sense, the main goal of the project was to analyse some of the polymorphisms that have been reported in the literature as associated with aggressive behaviour (and its related constructs), in order to gain some insight onto the genetic mechanisms underlying aggression. This study focused on variants in genes from the serotonergic system, due to the known role of serotonin in the circuitry of aggression, and because several genes in this pathway are systematically identified as strong candidates for causing abnormal behaviour.

2. Methods

2.1 Polymorphism selection

Eight genes from the serotonergic system were selected based on 35 literature reviews on the genetics of human aggression. All these genes code for proteins with central roles in the serotonin pathway, and have been associated with aggression and/or related endophenotypes by multiple independent sources (Table 1).

Table 1 - Genes from the serotonergic system chosen for this project

GENE ID	Location	Direction	Gene Lenght	Exon Count (Coding)	Protein Expressed	Protein Lenght
HTR1A	Chr 5; 5q12.3	Reverse	9245 bp	1	Serotonin Receptor 1A	422 aa
HTR1B	Chr 6; 6q14.1	Reverse	-	1	Serotonin Receptor 1B	390 aa
HTR2A	Chr 13; 13q14.2	Reverse	70657 bp	4 (3)	Serotonin Receptor 2A	471 aa
HTR2B	Chr 2; 2q37.1	Reverse	-	4 (3)	Serotonin Receptor 2B	481 aa
MAOA	Chr X; Xp11.3	Forward	97661 bp	15	Monoamine Oxidase A	527 aa
SLC6A4	Chr 17; 17q11.2	Reverse	48618 bp	15 (13)	Serotonin Transporter	630 aa
TPH1	Chr 11; 11p15.1	Reverse	27252 bp	11 (10)	Tryptophan Hydroxilase 1	444 aa
TPH2	Chr 12; 12q21.1	Forward	100596 bp	11	Tryptophan Hydroxilase 2	490 aa

Genes *TPH1* and *TPH2* encode for the two isoenzymes of Tryptophan Hydroxylase. This protein is responsible for the first, rate-limiting step in serotonin synthesis, catalysing the aminoacid L-Tryptophan into 5-hydroxytryptophan. While TPH1 is mostly found in peripheral tissues, TPH2 is the brain-specific form of this protein (Runions et al., 2019). Genes coding for four different serotonin receptors were also selected for analysis. Serotonin receptors 1A and 1B, coded for by *HTR1A* and *HTR1B*, are inhibitory receptors localized presynaptically on all serotonergic neurons and postsynaptically on many non-serotonergic neurons (Olivier, 2004). Presynaptic 1A and 1B autoreceptors help regulate serotonergic activity by inhibiting 5-HT release. The type 2 excitatory receptors 2A and 2B, encoded for by genes *HTR2A* and *HTR2B* are positive mediators of serotonin transmission (Jager, 2019). The gene *SLC6A4* (or *SERT*) codes for the serotonin transporter protein, which is responsible for serotonin reuptake from the synaptic cleft back to the presynaptic neuron. The serotonin molecules can then be reused in a different signal transmission or degraded (Toshchakova et al., 2018). Finally, the *MAOA* gene codes for the Monoamine Oxidase A protein, which catalyses the oxidative deamination of serotonin following reuptake into the presynaptic neuron (Taylor, 2014), the first step in serotonin destruction.

The PubMed database was used to retrieve relevant studies on each of the chosen genes. A keyword-based search was used, screening for the words “aggression OR aggressive behavio* OR violence OR violent behavio* OR anger OR hostility OR conduct OR antisocial AND [gene name]” for each specific gene in the fields “Title/Abstract”. The abstracts were screened for inclusion, and the selected studies were manually analysed in order to identify specific polymorphisms that had been associated with aggression or its associated constructs.

Once a preliminary list was constructed, the LitVar database (Allot et al., 2018) was used to retrieve relevant studies on each of the chosen polymorphisms. While some of the literature would have already been returned in the PubMed search, a variant-base screening was expected to yield more specific results, including studies not directly about aggressive behaviour, but with information on the possible functional effects of each variant. Some additional variants, not previously considered, were added from this literature search, and then ran through the LitVar database again.

2.2 Variant analysis

The databases *Ensembl* and dbSNP were used to collect general information on each of the polymorphisms, particularly the chromosomal and transcript position, the location within the gene, the type of variant, change, and known molecular consequences.

The Combined Annotation–Dependent Depletion (CADD) framework was used to retrieve further information on the variants. CADD integrates multiple available annotations to calculate a score that measures the deleteriousness of a variant, a property that correlates with molecular functionality and pathogenicity (Kircher et al., 2014).

CADD returns two scores for each variant. The "raw" scores summarize the extent to which the variant is likely to have derived from a set of "fixed" variants or a set of *de novo* variants. Negative values indicate a larger likelihood of that variant belonging to the "fixed" set; positive values indicate a larger likelihood of the variant belonging to the "simulated" *de novo* set (Rentzsch et al., 2019). The second score returned by CADD is a scaled score, derived from the relative ranking of the raw C scores of all 8.6 billion possible SNPs. Substitutions with the highest 10% (10^{-1}) of all C-scores – considered the most deleterious - were assigned values of 10 or greater, whereas variants in the highest 1% (10^{-2}), 0.1% (10^{-3}), etc., were assigned scores of '≥20', '≥30', etc (Rentzsch et al., 2019).

Furthermore, the full annotation files from CADD were retrieved to analyse the possible effect of each variant in gene or protein function. Specifically, annotations regarding sequence conservation and regulatory elements were selected for analysis. Additional information on these annotations, plus additional annotations, were retrieved from the *Ensembl* Variant Effect Predictor (VEP; McLaren et al., 2016), and from the UCSC genome browser (Haeussler et al., 2019).

CADD integrates annotations regarding the conservation score of each site from several different tools; the conservation scores from GERP, PhastCons and PhyloP were analysed and compared.

Genomic Evolutionary Rate Profiling (GERP) is a framework for the identification of constrained elements (Cooper et al., 2005). GERP estimates evolutionary rates for individual alignment columns and identifies regions that exhibit nucleotide substitution deficits. These elements are scored according to the magnitude of this substitution deficit, measured as "rejected substitutions" (RS), which reflect the intensity of past purifying selection, and are used to rank and characterize constrained elements (Davydov et al., 2010). Positive scores represent a substitution deficit (fewer substitutions than the average neutral site) and indicate that a site may be under evolutionary constraint. Negative scores indicate that a site is probably evolving neutrally. Positive scores scale with the level of constraint, such that the greater the score, the greater the level of evolutionary constraint inferred to be acting on that site. The same does not happen with negative scores, due to confounders such as alignment uncertainty or rate variance (Goode et al., 2010).

PhastCons is a program based on a two-state phylo-HMM (hidden Markel model), which considers both the process by which nucleotide substitutions occur at each site in a genome and how this process changes from one site to the next (Siepel & Haussler, 2005). The program aligns the genomes from multiple species and takes into account the phylogeny by which these species are related. PhastCons scores, ranging from 0 to 1, translate the posterior probability that the corresponding alignment column was "generated" by the conserved state (rather than the non-conserved state) of the phylo-HMM (Siepel et al., 2005). A PhastCons score of 1 predicts the highest possible conservation, while a score of 0 indicates the lowest.

PhyloP (phylogenetic *P*-values) is a program that integrates four different conservation scoring methods, including a GERP-like test, a number-of-substitutions test, a likelihood ratio test and a score test (Pollard et al., 2010). Positive scores indicate a conserved site, with slower-than-expected evolution; negative scores indicate sites predicted to be fast-evolving.

Only SNPs were ran through CADD, due to the limitations of the framework regarding the size of the variants, and the unreliable data regarding the rs ID and chromosomal locations of size polymorphisms, necessary for CADD analysis.

There has been consistent evidence of differences in aggression levels between different populations. Furthermore, genetic differences between populations have been considered an important contributor to health disparities around the globe (Haberstick et al., 2015). Therefore, allele frequencies of the selected SNPs were retrieved from the gnomAD database (v3 - Karczewski et al., 2020). Six different populations were selected for analysis: European (Finnish and Non-Finnish populations), East and South Asian, African (including African American) and Latino (Admixed-American). These populations were selected due to the large samples available on gnomAD v3. In addition, the total frequency of the allele, across all available genomes and populations on gnomAD was also retrieved.

Only SNP frequencies were retrieved from gnomAD. For other polymorphisms, allele frequencies were retrieved from the available literature.

3. Results

3.1 Selected polymorphisms

Table 2 shows the polymorphisms selected for analysis based on the literature search, and summarizes the basic information for each variant. A total of 31 polymorphisms across the 8 genes were selected for analysis. Most of them (n=27; 87.1%) were single-nucleotide variants (SNPs), while four were size polymorphisms consisting in a Variable Number of Tandem Repeats (VNTR). Most of the polymorphisms identified were located in non-coding regions. Only 6 SNPs were located within the CDS of their respective gene (18.75%); four were silent mutations (rs6296 in *HTR1B*, rs6313 in *HTR2A*, and rs6323 and rs1137070 in *MAOA*), one was a missense SNP (rs6314 in *HTR2A*) and one was a nonsense mutation (rs79874540, *HTR2B*).

Table 2 - Polymorphisms associated with AB selected for analysis

Gene	Variant ID	Type	Chromosome: Position	Location	HGVS Position	Change
HTR1A	rs6295	SNP	Chr5: 63 962 738	Promoter Region	-1019	G > C
HTR1B	rs11568817	SNP	Chr6: 77 463 665	Promoter Region	-262	T > G
HTR1B	rs130058	SNP	Chr6: 77 463 564	Promoter Region	-162	A > T
HTR1B	rs6296	SNP	Chr6: 77 462 543	CDS (silent)	861	G > C V[GT**G**] > V[GT**C**]
HTR1B	rs6297	SNP	Chr6: 77 462 224	3' UTR	1180 (*7)	G > A
HTR1B	rs13212041	SNP	Chr6: 77 461 407	3' UTR	1997 (*824)	G > A
HTR2A	rs6311	SNP	Chr13: 46 897 343	5' UTR	-1438	G > A
HTR2A	rs6313	SNP	Chr13: 46 895 805	CDS (silent) Exon 2	102	C > T S[TC**C**] > S[TC**T**]
HTR2A	rs1923886	SNP	Chr13: 46 849 156	Intron 3	614 -13 517	G > A
HTR2A	rs6561333	SNP	Chr13: 46 846 177	Intron 3	614 -10 538	A > G
HTR2A	rs7322347	SNP	Chr13: 46 835 968	Intron 3	614 -329	A > T
HTR2A	rs6314	SNP	Chr13: 46 834 899	CDS (missense) Exon 4	1354	C > T H[**C**AT] > Y[**T**AT]
HTR2B	rs79874540 (Q20*)	SNP	Chr2: 231 123 707	CDS (nonsense) Exon 2	58	C > T Q[**C**AG] > *[**T**AG]
HTR2B	rs17440378	SNP	Chr2: 231 114 641	Intron 2	353 -712	G > C

Gene	Variant ID	Type	Chromosome: Position	Location	HGVS Position	Change
MAOA	10VNTR (dVNTR)	VNTR	ChrX: 43 654 464	Promoter Region		varying number of a 10bp repeat motif; 8R, 9R, 10R, 11R
MAOA	30VNTR (uVNTR)	VNTR	ChrX: 43 655 101	Promoter Region		varying number of a 30bp repeat motif; 2R, 3R, 3.5R, 4R, 5R
MAOA	rs5906957	SNP	ChrX: 43 688 062	Intron 2	168 +4455	A > G
MAOA	rs909525	SNP	ChrX: 43 693 955	Intron 3	306 +527	C > T
MAOA	rs6323 (*Fnu4HI*)	SNP	ChrX: 43 731 789	CDS (silent) Exon 8	891	G > T R[CG**G**] > R[CG**T**]
MAOA	rs1137070 (*EcoRV*)	SNP	ChrX: 43 744 144	CDS (silent) Exon 14	1453	T > C D[GA**T**] > D[GA**C**]
MAOA	rs2064070	SNP	ChrX: 43 749 435	Downstream Variant	(*4922)	A > T
SLC6A4	5-HTTLPR	VNTR	Chr17: 30 237 455	Promoter Region		varying number of a 20-23bp repeat motif; 14R, 16R
SLC6A4	rs25531	SNP	Chr17: 30 237 328	Promoter Region		A > G; changes the L allele of 5-HTTLPR
SLC6A4	STin2	VNTR	Chr17: 30 221 590	Intron 2		varying number of a 16-17bp repeat motif; 9R, 10R, 12R
TPH1	rs1800532	SNP	Chr11: 18 026 269	Intron 7	803 +221	C > A
TPH1	rs1799913	SNP	Chr11: 18 025 708	Intron 7	804 -7	C > A
TPH2	rs6582071	SNP	Chr12: 71 936 332	Upstream Variant	-2655	G > A
TPH2	rs4570625	SNP	Chr12: 71 938 143	5' UTR	-844	G > T
TPH2	rs1352250	SNP	Chr12: 72 004 004	Intron 8	1068 +943	A > G
TPH2	rs10879352	SNP	Chr12: 72 013 178	Intron 8	1069 -9221	T > C
TPH2	rs1487275	SNP	Chr12: 72 016 512	Intron 8	1069 -5887	C > G

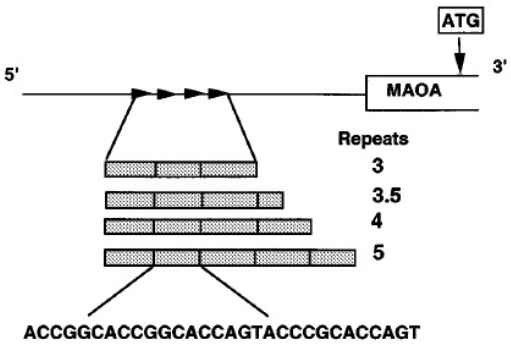
A large portion of the polymorphisms identified were located in intronic regions (n=12; 38.7%), and rs1799913 in *TPH1* was located near a splice site. The VNTR STin2 of *SLC6A4* has been shown to influence transcription activity, but it has so far not been described to affect the splicing mechanism.

Six variants (19.4%) within the upstream or downstream regions of relevant genes have been described in association with ABs. Two of them (rs6311 in *HTR2A* and rs4570625 in *TPH2*) are located in the 5' UTR regions, within 1kb of the translation start site. Two SNPs (rs6297 and rs13212041 in *HTR1B*) are located in the 3' UTR regions, one (rs6582071 in *TPH2*) is located 2.6kb upstream of the translation start site, and one (rs2064070 in *MAOA*) is located ~5kb downstream of the STOP codon.

Seven variants (22.6%), including three of the four VNTRs, were located in the promoter regions of their respective genes. Some of these polymorphisms have been shown to modulate transcriptional activity in the gene (Duan et al., 2003; Lemonde et al., 2003; Manca et al., 2018; Veroude et al., 2016).

The two VNTRs in the *MAOA* gene are located in the promotor region, close to each other: an upstream (u) and a distal (d) VNTR. The uVNTR is located approximately 1kbp upstream of the transcription start site; the dVNTR is located approximately 1700bp upstream from the transcription start site. These polymorphisms have been shown to be in strong LD, and work together to modulate transcriptional activity of the *MAOA* gene (Manca et al., 2018; Philibert et al., 2011).

Sabol et al. (1998) first described the promoter uVNTR as a repetition of a 30-bp sequence, followed by half a sequence (Figure 1). The *MAOA* gene reference sequence in NCBI has 3 full sequences, followed by the half sequence described by Sabol et al. (1998); this is a 3-repeat (3R) allele. There have been identified alleles with 2R, 3.5R, 4R, 5R and 6R in different populations (Guo et al., 2008; Sabol et al., 1998).



Repeat Motif: ACC GGC ACC GGC ACC AGT ACC CGC ACC AGT

RefSeq: $(ACCGGCACCGGCACCAGTACCCGCACCAGT)_3$ ACCGGCACCGGCACC => 3R

2R => $(ACCGGCACCGGCACCAGTACCCGCACCAGT)_2$ ACCGGCACCGGCACC
3.5R => $(ACCGGCACCGGCACCAGTACCCGCACCAGT)_3$ $(ACCGGCACCGGCACC)_2$
4R => $(ACCGGCACCGGCACCAGTACCCGCACCAGT)_4$ ACCGGCACCGGCACC
5R => $(ACCGGCACCGGCACCAGTACCCGCACCAGT)_5$ ACCGGCACCGGCACC

Figure 1 - *MAOA* uVNTR polymorphism structure and sequence.
(a) uVNTR structure; black arrows indicate transcription direction (Sabol et al., 1998).
(b) uVNTR alleles sequence.

Different uVNTR alleles have been associated with different transcriptional activities of the *MAOA* promoter, which in turn result in different levels of *MAOA* expression. Most authors indicate alleles with 3.5 or 4 repeats as the high transcription (H) variants, and alleles with 2, 3 or 5 repeats as having lower expression (L) (Guo et al., 2008; Kim-Cohen et al., 2006; Sabol et al., 1998). The low activity alleles have been considered the risk alleles for aggression and violence (Beaver et al., 2013; Buckholtz & Meyer-Lindenberg, 2008; Ficks & Waldman, 2014; Tharshini, 2019; Tiihonen et al., 2015).

Philibert et al. (2011) noted another CpG rich region near the promoter uVNTR. Once studied, this region revealed to be a VNTR, consisting of two decamer repeat units which differed only in the 7th position (Figure 2). The *MAOA* RefSeq in NCBI has 10 repeat units, consisting of a 10R allele. Other alleles discovered have 8R, 9R, and 11R (Philibert et al., 2011; Manca et al., 2018).

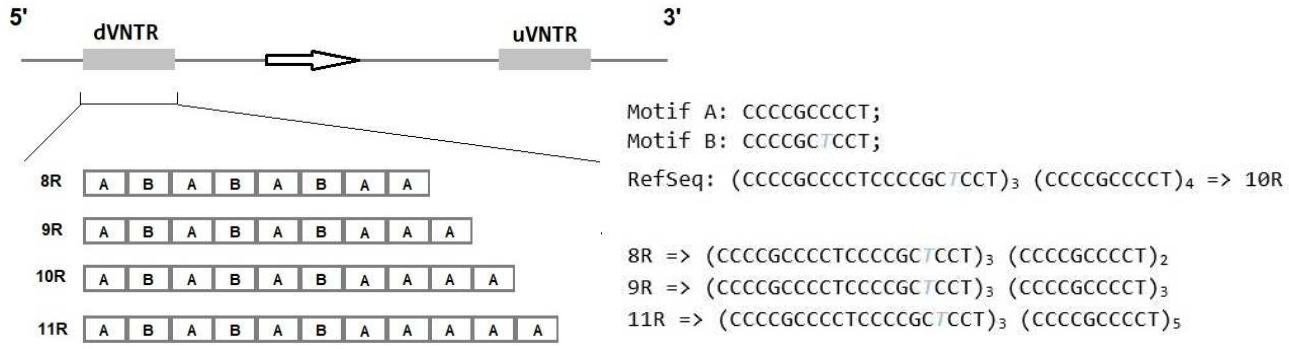


Figure 2 - MAOA dVNTR polymorphism structure and sequence.
(a) dVNTR structure as described by Manca et al. (2018); the black arrow indicates transcription direction.
(b) dVNTR alleles sequence.

This polymorphism has also been shown to alter the transcriptional activity of the *MAOA* gene. In a cell line model, the 9R variant was the most active, 8R and 11R had intermediate activity, and the 10R version had the lowest transcriptional activity (Philibert et al., 2011). In fact, Manca et al. (2018) suggested that the dVNTR alleles may exert an even greater role than the uVNTR on *MAOA* expression.

One of the most studied polymorphisms in association with AB is a VNTR located in the promoter region of *SLC6A4*, the *5-HTT*-linked polymorphic region (5-HTTLPR). This VNTR consists of varying numbers of copies of a 20–23bp degenerate repeat sequence (Figure 3); the presence or absence of a 43bp sequence in this region defines the two most common alleles, a short (S) and a long (L) allele, with 14 and 16 repeats respectively (Heils et al., 1996). Still, there are variants with 15, 19, 20 or 22 repeats described (Nakamura et al., 2000).

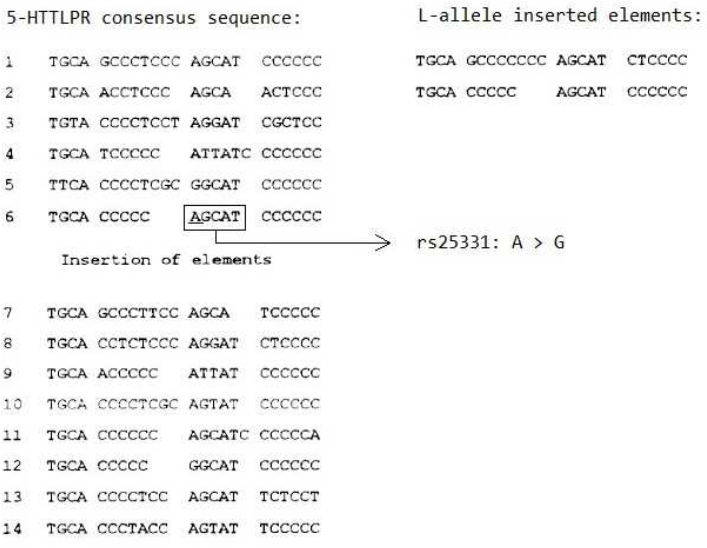


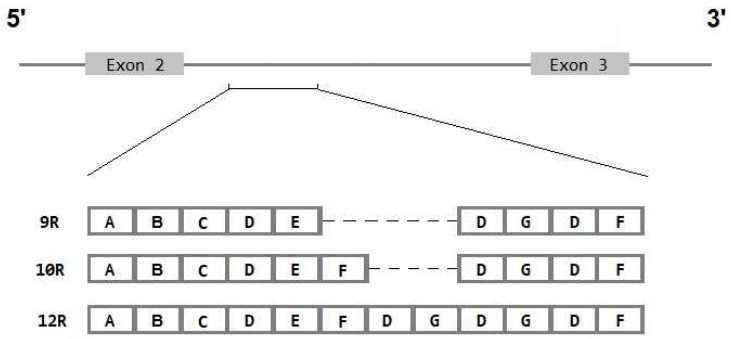
Figure 3 - 5-HTTLPR sequence, as described by Kunugi et al. (1997).

The different alleles of 5-HTTLPR have been shown to result in different transcription levels. Heils et al. (1996) found that the basal activity of the L variant was more than two times that of the S variant. The S allele, which produces less serotonin transporter protein, was associated with lower serotonin uptake

(Lesch et al., 1996). Furthermore, an SNP within this region, rs25531, has been shown to change the transcriptional levels of 5-HTTLPR. This polymorphism is an A > G SNP, which mainly occurs in the long version of 5-HTTLPR. An L allele with a G (L_G) has a low transcriptional activity, similar to that of the S allele (with either A or G), while the L_A version has the highest activity of the four possible haplotypes. While some authors have proposed that 5-HTTLPR together with rs25531 could be considered a single tri-allelic locus (Haberstick et al., 2015; Hu et al., 2006), with alleles L_A, L_G and S, others have suggested otherwise (Wendland et al., 2006).

Most studies on the influence of this polymorphism in aggressive traits have indicated the shorter, low activity variant as the risk allele. The S allele has been found associated with antisocial behaviours, increased anger and hostility, higher neuroticism scores, CD, and criminality (Caspi et al., 2002; Hill et al., 2002; Manco et al., 2018; Nilsson et al., 2015).

The last VNTR that has been studied in association with AB and related phenotypes is a VNTR in the second intron of *SLC6A4*, denominated STin2 (Serotonin Transporter intron 2). This variant comprises various repeats of a 16-17 bp imperfect motif (Kaiser et al., 2001). The most common alleles have 9, 10, and 12 repeats (Figure 4). Studies have found that the allele with 12 repeats enhances transcription of *SLC6A4*, while the 10R variant has been associated with less efficient serotonin turnover. This polymorphism has been associated with increased aggression in children and adults, and with cognitive impulsivity (Beitchman et al., 2003; Hemmings et al., 2018; Oades et al., 2008).



Motif Sequence:

A => GGCTGT GACC CAGGG TG
B => GGCTGT GACC C**GGA**G TG
C => GGCTGT GACC C**GG**GG TG
D => GGCTGT GACC C**G_**GG TG
E => GGCTG**C** GACC **TG**GGG TG
F => GGCTGT GACC **TG**GG**A** TG
G => GGCTGT GACC **TG**GGG TG

Figure 4 - STin2 polymorphism structure and sequence.
(a) STin2 structure, as described by Kaiser et al. (2001).
(b) Repeat Motifs' sequences.

Furthermore, there have been four distinct *MAOA* polymorphisms associated with Brunner Syndrome by different authors, three SNPs and a 1bp insertion (Table 3). All four of them are located within the coding sequence and cause significant changes in the transcription levels of the gene. Two of these variants (rs796065311 and rs7255463) create premature stop codons, which result in truncated versions of *MAOA*, which are then signalled for destruction via Nonsense Mediated Decay (NMD) (Brunner et al., 1993; Palmer et al., 2016). The two missense polymorphisms (rs796065312 and rs587777457) have both been described to cause significant changes in the chemical proprieties of MAOA (Palmer et al., 2016; Piton et al., 2014).

Since Brunner's Syndrome is a Mendelian disease, with known causal variants and, and is characterized by an aggressive phenotype, variants identified in Brunner's Syndrome were also ran through CADD, to compare the results with those of the variants chosen in this project.

Table 3 - *MAOA* polymorphisms identified in Brunner's Syndrome.

Variant ID	Type	Location	Position (HGVS)	Change	Reference
rs796065312	SNP	CDS (missense) Exon 2	43 683 572; 133	C > T R[**C**GG] > W[**T**GG]	Palmer et al. (2016)
rs796065311	Insertion	CDS (frameshift, PSC) Exon 7	43 731 345; 750	A > TA SSD[TCA AGT GAC] > SK*[TC**T** **A**AG TGA]	Palmer et al. (2016)
rs587777457	SNP	CDS (missense) Exon 8	43 731 695; 797	G > T C[T**G**C] > F[T**T**C]	Piton et al. (2014)
rs72554632	SNP	CDS (nonsense) Exon 8	43 731 784; 886	C > T Q[**C**AG] > *[**T**AG]	Brunner et al. (1993)

3.2 Population frequencies

3.2.1 SNPs

Table 4 shows the allele frequencies of the SNPs selected for analysis. It is important to consider that the allele frequencies considered as "Total" may be skewed towards the ones found in populations with the largest sample in gnomAD, and may not be a faithful representation of the real worldwide frequency of the variants.

Table 4 - Minor Allele Frequencies (MAF) for the selected SNPs, except for rs25531

Gene	Variant ID	Alleles* (RefSeq > Alt)	Minor Allele Frequency[1]						
			Total[2]	NFE	Fin	Afr	EAs	SAs	Amr
HTR1A	rs6295	C > G	0,4860 (G)	0,4997 (C)	0,4271	0,4406	0,2438 (C)	0,3983 (C)	0,4880
HTR1B	rs11568817	A > C	0,3673 (C)	0,4485	0,4822	0,2015	0,1331	0,4508	0,3927
HTR1B	rs130058	T > A	0,2307 (A)	0,2975	0,3770	0,0719	0,1012	0,2434	0,2834
HTR1B	rs13212041	C > T	0,296 (C)	0,2046	0,2003	0,4668 (T)	0,2271	0,1363	0,1772
HTR1B	rs6296	C > G	0,2694 (G)	0,2601	0,2434	0,2318	0,4994	0,3412	0,3794
HTR1B	rs6297	C > T	0,0996 (C)	0,1522	0,1315	0,0283	0,1110	0,0745	0,0651
HTR2A	rs1923886	C > T	0,355 (C)	0,4561	0,4258 (T)	0,0925	0,4987	0,4858 (T)	0,4430
HTR2A	rs6311	C > T	0,4025 (T)	0,4094	0,3373	0,3896	0,3895 (C)	0,4034	0,3978
HTR2A	rs6313	G > A	0,3984 (A)	0,4095	0,3357	0,3762	0,3914 (G)	0,4216	0,3921
HTR2A	rs6314	G > A	0,0949 (A)	0,0900	0,0523	0,1255	0,0026	0,0887	0,0737
HTR2A	rs6561333	T > C	0,3969 (T)	0,4770	0,4211 (C)	0,1977	0,4422	0,4724	0,4687
HTR2A	rs7322347	T > A	0,4859 (A)	0,4402	0,2971	0,3707 (T)	0,2255	0,3680	0,4692
HTR2B	rs17440378	C > T	0,1521 (T)	0,1655	0,2905	0,1086	0,0712	0,1321	0,1378
HTR2B	rs79874540	G > A	0,0012 (A)	0,0002	0,0151	0	0	0	0
MAOA	rs1137070	T > C	0,3467 (T)	0,3002	0,3909	0,3802	0,4148 (C)	0,3587 (C)	0,3705
MAOA	rs2064070	A > T	0,3538 (A)	0,3080	0,4440	0,3795	0,4145 (T)	0,3696 (T)	0,3706
MAOA	rs5906957	A > G	0,2853 (A)	0,2455	0,3821	0,2951	0,4156 (G)	0,4152 (G)	0,2806
MAOA	rs6323	G > T	0,2699 (G)	0,2989	0,3923	0,1487	0,4181 (T)	0,3804 (T)	0,2927
MAOA	rs909525	C > T	0,3337 (C)	0,3326	0,4492	0,2765	0,4187 (T)	0,3591 (T)	0,3345
TPH1	rs1799913	G > T	0,3345 (T)	0,3905	0,4369	0,1817	0,4475	0,3399	0,3841
TPH1	rs1800532	G > T	0,3346 (T)	0,3908	0,4368	0,1814	0,4477	0,3399	0,3834
TPH2	rs10879352	T > C	0,4403 (T)	0,4107	0,4763	0,4754 (C)	0,3784	0,3183	0,3677
TPH2	rs1352250	A > G	0,4879 (A)	0,4349	0,4812 (G)	0,4164 (G)	0,4585 (G)	0,4768	0,4507
TPH2	rs1487275	C > A	0,2928 (C)	0,2837	0,3796	0,2746	0,3479	0,3493	0,2983
TPH2	rs4570625	G > T	0,2738 (T)	0,2171	0,1884	0,3636	0,4476 (G)	0,2846	0,2770
TPH2	rs6582071[3]	G > A	0,3142 (A)	0,2189	0,1912	0,4930	0,4441 (G)	-	0,3726

NFE= Non-Finnish European; Fin= Finnish; Afr= African; Eas= East Asian; Sas= South Asian; Amr= Latino/Admixed American;
*Data retrieved from dbSNP build 154
[1]Data retrieved from gnomAD v3
[2]Minor Allele of Total is considered the default; populations that differ are indicated as so
[3]Data retrieved from gnomAD v2.1.1 as it was unavailable on v3

With the exception of rs79874540 in the gene *HTR2B*, all polymorphisms were found in all populations analysed. Bevilacqua et al. (2010), who first described this polymorphism, had proposed this variant to be almost exclusive of Finnish populations. In fact, on gnomAD, only 17 alleles were found outside of Finnish populations, 14 of those in other European individuals, and only one individual (a Finnish male) was found to be homozygous for this variant. Regardless, and in spite of its molecular consequence, Bevilacqua et al. (2010) found that Q20* carriers were not uncommon across Finland.

Only two other polymorphisms revealed a MAF≤0.1, rs6297 in *HTR1B*, and rs6314 in *HTR2A*. No functional role has yet been described for rs6297; however, Conner et al. (2010) found that the A allele predicted higher hostility levels in males when compared with G allele carriers. On the other hand, rs6314 is a missense SNP that has been described to affect gene function (Banlaki et al., 2015). This C > T variant causes a histidine to be substituted by a tyrosine, and thus a change between a basic amino acid residue and an uncharged one. This change has been predicted to affect protein structure and pre-mRNA splicing, via destruction of a binding site for SRp40, a protein that binds exonic splicing enhancer elements (Blasi et al., 2013). It has also been associated with slower receptor response (Hazelwood & Sanders-Bush, 2004).

The MAOA polymorphisms showed a completely different distribution pattern in Asian populations compared to the remaining groups. While the minor allele is the reference allele in almost all populations, in Asian groups the minor allele is the alternative allele. In the East Asian population, the different variants had very similar allele frequencies between each other.

The two variants of *TPH1*, rs1799913 and rs1800532, showed very similar allele frequencies in all populations. These variants are located in intron 7 of the gene, within 500bp, and have been described to be in strong LD (Keltikangas-Järvinen et al., 2007).

3.2.2 VNTRs

The *MAOA* gene has been one of the most studied in association with AB. The bulk of the work has been dedicated to the uVNTR (Waltes et al., 2016). The most common alleles in humans are the variants with 3 and 4 repeats (3R and 4R, respectively). Other less common but still frequent variants include alleles with 2, 3.5 and 5 repeats (Deckert et al., 1999; Sabol et al., 1998), and there have been two rare alleles described: a 1R allele in an Iraqi population (Al-Tayie & Ali, 2018), and a single 6R allele in a sample from Taiwan (Lu et al., 2002). Table 5 shows allele frequencies retrieved from the literature.

Table 5 - *MAOA* uVNTR allele frequencies in different populations, separated by continent.

MAOA uVNTR Allele Frequencies					
Population (Reference)	**2R**	**3R**	**3.5R**	**4R**	**5R**
Germany/Italy (Deckert et al., 1999)	0,0061	0,3262	0,0061	0,6478	0,0138
Sweden (Åslund et al., 2011)	0	0,3662	0,0137	0,6148	0,0053
Germany (Kuepper et al., 2013)	0,0077	0,3590	0,0077	0,6103	0,0154
Spain (Voltas et al., 2015)	0,0054	0,3666	0,0189	0,5957	0,0135
Portugal (Dias et al., 2016)	0,0038	0,3329	0,0038	0,6507	0,0088
UK mixed (Manca et al., 2018)	0	0,3505	0,0155	0,6134	0,0206
Iran (Saify & Saadat, 2015, 2016)	0,008	0,3932	0	0,5674	0,0314
Iraq (Al-Tayie & Ali, 2018)	0	0,3568	0	0,5205	0,1114
Taiwan (Lu et al., 2002)	0,0069	0,5636	0	0,4261	0
Taiwan (Lung et al., 2011)	0,0065	0,5823	0	0,4080	0,0032
Japan (Matsusue et al., 2019)	0,0127	0,5924	0	0,3949	0
South Africa/Black (Malan, 2014)	0,0576	0,3864	0	0,5186	0,0373
South Africa/White (Malan, 2014)	0	0,2328	0	0,7241	0,0431
South Africa/White (Erasmus et al., 2015)	0	0,2750	0	0,6700	0,0350
USA/White (Sabol et al., 1998)	0	0,3309	0,0049	0,6483	0,0160
USA/White (Widom & Brzustowicz, 2006)	0,0105	0,3864	0,0210	0,5734	0,0087
USA/White (Lavigne et al., 2013)	0	0,3680	0,0160	0,6080	0
Asian American (Sabol et al., 1998)	0	0,6098	0,0122	0,3780	0
African American (Sabol et al., 1998)	0,0000	0,5909	0,0227	0,3636	0,0277
African American (Beaver et al., 2013)	0,0517	0,5230	0,0057	0,4195	0
Hispanic (Sabol et al., 1998)	0	0,2935	0	0,7065	0
USA mixed (Cooke et al., 2018)	0	0,2890	0,0051	0,7008	0,0051
USA mixed (Hamilton et al., 2000)	0	0,3621	0,0288	0,6049	0,0041
New Zealand (Caspi et al., 2002)	0,0015	0,3392	0,0046	0,6381	0,0165

Overall, the 3R and 4R alleles showed similar frequencies across different populations, with the 4R allele being the most frequent in almost all populations, followed by the 3R allele. The exception, once again, was in Asian populations, for which the 3R allele was more frequent than the 4R allele; this was also observed in an Asian American subsample. This follows the same deviation pattern as observed with the SNPs. Interestingly, the two African American populations analysed also had a higher frequency for the 3R allele than the 4R allele, much like in Asian populations. It should be noted that both African, European American and Hispanic populations showed the inverse pattern.

The other *MAOA* VNTR that has been associated with aggressive behaviour is also located in the promoter region, ~1500 bp upstream of the transcriptional start site (Philibert et al., 2011). This distal polymorphism (dVNTR) consists of a 10 bp repeat motif that can be present in 8, 9, 10 or 11 copies. Only two studies that have investigated the association of this polymorphism with aggressive traits have reported allele frequencies (Philibert et al., 2011; Manca et al., 2018). These studies showed similar allele frequencies in mixed samples, one from the US, one from the UK, of ~74% for the 9R-allele, ~20% for 10R, ~5% for 11R and less than 1% for 8R.

The serotonin transporter gene (*SLC6A4*, *5-HTT* or *SERT*) is located in the long arm of Chromosome 17 (q11.1–q12). Together with *MAOA*, it is one of the genes that has been more consistently associated with aggressive traits (Waltes et al., 2016). One of the most studied variants of this gene is a VNTR located in the promoter region, usually referred to as 5HTTLPR (for *5HTT*-Linked Polymorphic Region). This polymorphism is located in a repetitive region, consisting of a varying number of a 20–23bp degenerate repeat sequence. The two most common alleles differ in the presence or absence of a 43-bp sequence (two repeats). The short (S) allele has 14 repeats, while the long (L) allele has 16. Other rare alleles have been described, with 19, 20 or 22 repeats, and are usually grouped as XL (extra-large) variants (Nakamura et al., 2000).

Table 6 – 5-HTTLPR/rs25531 allele frequencies in different populations, separated by continent.

5-HTTLPR/rs25531 Allele Frequencies			
Population (Reference)	**S**	**L_G**	**L_A**
Finland (Hu et al., 2006)	0,4001	0,0901	0,5097
Portugal (Pereira, 2015)	0,4757	0,0510	0,4733
Portugal (Manco et al., 2018)	0,4419	0,0505	0,5076
Russia (Butovskaya et al., 2018)	0,4610	0,0460	0,4930
Croatia (Ravić et al., 2018)	0,3711	0,0602	0,5688
Iran (Safarinejad, 2010)	0,4714	0,0947	0,4339
Cameroon (Haberstick et al., 2015; CLGS)	0,2070	0,1862	0,6068
Tanzania (Butovskaya et al., 2018)	0,1889	0,1687	0,6424
South Africa (Hemmings et al., 2018)	0,3034	0,1914	0,5052
Singapore/Chinese (Haberstick et al., 2015; SCCS)	0,6799	0,1597	0,1597
Singapore/Malay (Haberstick et al., 2015; SCCS)	0,6197	0,1300	0,2503
Singapore/Indian (Haberstick et al., 2015; SCCS)	0,5694	0,1697	0,2595
USA/White (Hu et al., 2006)	0,3602	0,1449	0,4949
USA/White (Odgerel et al., 2013)	0,4350	0,0700	0,4940
USA/White (Haberstick et al., 2015; FTP)	0,4117	0,0712	0,5170
USA/White (Haberstick et al., 2015; AddH)	0,4290	0,0696	0,5013
African American (Hu et al., 2006)	0,2500	0,2404	0,5096
African American (Odgerel et al., 2013)	0,2510	0,2100	0,5270
African American (Haberstick et al., 2015; AddH)	0,2634	0,2067	0,5299
Asian American (Haberstick et al., 2015; AddH)	0,6668	0,1224	0,2108
Native American (Hu et al., 2006)	0,6490	0,0098	0,3412
Native American (Haberstick et al., 2015; AddH)	0,5050	0,0414	0,4536
Native American (Williams et al., 2017)	0,4706	0,0452	0,4819
USA/mixed (Haberstick et al., 2015; AddH)	0,5112	0,0620	0,4268
Hispanic (Haberstick et al., 2015; GIC)	0,5938	0,0313	0,3750
Hispanic (Haberstick et al., 2015; RYDS)	0,4310	0,1552	0,4138

The different forms of 5-HTTLPR have been shown to have different transcription levels. Heils et al. (1996) found that the basal activity of the L variant was more than two times that of the S variant. The S allele, which produces less serotonin transporter protein, was associated with lower serotonin uptake (Goldman et al., 2010). Furthermore, an SNP (rs25531, A > G) modifies its transcription: the L allele with a G (L_G) at rs25531 drives low transcription levels, similar to the short allele (S), whereas the L_A allele determines high transcription levels. Table 6 shows the allele frequencies for the tri-allelic locus of 5-HTTLPR with rs25531.

In most populations, the L_A allele was the most frequent, followed by the S allele. However, in Asian populations (including in an Asian American subsample) the S allele had a much higher frequency than the other two alleles. In some Hispanic and Native American populations, the S allele was also the most frequent, but the L_A allele had a higher frequency than in Asian populations. The L_G allele was the less frequent allele in all populations. While in European (and white American) populations it is present at very low frequencies (f <0.1), in Asian and African populations the frequency was much similar to that of the second most frequent allele (L_A in Asian populations and S in African populations). The highest L_G frequencies were found in African American populations (f >0.2).

Lastly, a VNTR in the second intron of *SLC6A4*, denominated STin2 (Serotonin Transporter intron 2) has been investigated. This polymorphism comprises various repeats of a 16-17 bp imperfect motif. The most common alleles have 9, 10, and 12 repeats. Studies have found that the allele with 12 repeats enhances transcription of *SLC6A4*, while the 10R variant has been associated with less efficient serotonin turnover.

Table 7 - STin2 allele frequencies in different populations, separated by continent.

STin2 Allele Frequencies			
Population (Reference)	**9R**	**10R**	**12R**
Scotland (Ogilvie et al., 1996)	0,0199	0,4022	0,5779
Denmark (Ogilvie et al., 1998)	0,0203	0,3517	0,6280
Germany (Kaiser et al., 2001)	0,0236	0,3847	0,5913
Italy (Racchi et al., 2004)	0,0110	0,4231	0,5659
Hungary (Szilagyi et al., 2006)	0,0145	0,3584	0,6270
Portugal (Dias et al., 2016)	0,0057	0,3901	0,6042
Croatia (Ravić et al., 2018)	0,0158	0,3883	0,5960
Turkey (Yilmaz et al., 2001)	0	0,3922	0,6078
Iran (Safarinejad, 2010)	0	0,3348	0,6652
China (Yang et al., 2012)	0	0,1061	0,8286
China (Huang et al., 2016)	0,0116	0,1977	0,7907
South Africa (Hemmings et al., 2018)	0	0,2224	0,7776
African American (Gelernter et al., 1998)	0,0099	0,2624	0,7277
European American (Gelernter et al., 1998)	0,0068	0,3507	0,6425
USA/mixed (Assal et al., 2004)	0,0141	0,4085	0,5775

Table 7 shows the allele frequencies retrieved from the literature. The 12R allele showed the highest frequencies (f >0.56) in all populations, while the 9R allele was the less frequent (f <0.1). In Asian populations, the 10R had its lowest frequencies (f <0.2) and the 12R allele had its highest frequencies (f >0.79).

3.3 CADD scores

Table 8 - CADD scores for the SNPs chosen for analysis; variants are ranked by their raw score, from highest to lowest

Gene	Variant ID	Raw Score	Scaled Score
HTR2B	rs79874540	7,011294	37
HTR1B	rs13212041	1,555693	16,09
HTR1B	rs130058	1,163623	13,34
TPH1	rs1799913	1,01592	11,82
HTR1B	rs6296	1,013072	11,79
TPH2	rs10879352	0,88847	10,31
HTR1B	rs11568817	0,88824	10,31
SLC6A4	rs25531	0,562928	7,148
HTR2B	rs17440378	0,554793	7,066
HTR2A	rs6313	0,482417	6,32
HTR2A	rs6314	0,46026	6,084
MAOA	rs909525	0,427756	5,732
MAOA	rs6323	0,390788	5,324
HTR1B	rs6297	0,353075	4,899
MAOA	rs5906957	0,295366	4,235
MAOA	rs2064070	0,283088	4,092
HTR2A	rs6561333	0,25825	3,801
HTR2A	rs7322347	0,253785	3,749
MAOA	rs1137070	0,131074	2,384
HTR1A	rs6295	0,068833	1,826
HTR2A	rs1923886	0,037522	1,588
TPH2	rs6582071	0,028936	1,527
TPH2	rs1352250	-0,074166	0,938
TPH2	rs1487275	-0,175504	0,576
HTR2A	rs6311	-0,395345	0,197
TPH2	rs4570625	-0,607929	0,07
TPH1	rs1800532	-0,623341	0,065

Table 8 shows the selected variants, ranked by their Raw Score (from now on simply C-score). Overall, the variants had relatively low scores, indicating that they are not predicted to be functionally relevant. As previously discussed, the genetic component of aggression is thought to be composed by multiple genes/variants, each of small effect, and their interactions. Therefore, even if the variants described are not predicted to be deleterious individually, the synergistic effect may result in an aggressive phenotype.

The polymorphism that scored highest was rs79874540 in the *HTR2B* gene (C=7.011; S=37), also known in the literature as Q20*. This variant creates a premature STOP codon (TAG) in the place of a Glutamine (CAG), producing a blunt protein of only 19 amino acids, instead of the native 481. This loss of function leads to variable NMD and blocked expression (Bevilacqua et al., 2010). It is not surprising that this variant has the highest score in our set; an S-score > 30 indicates that this polymorphism belongs to the 0.1% of the most deleterious variants in all of the genome.

Seven of the selected variants had S-scores of >10. According to Rentzsch et al. (2019), they are predicted to belong to the top 10% most “deleterious” polymorphisms in the genome. However, only one polymorphism other than rs79874540 was scored at the somewhat significant threshold of S > 15: the functional polymorphism rs13212041 in the gene *HTR1B* (C=1.556; S=16.09). This polymorphism disrupts a binding site for the microRNA miR-96 (Conner et al., 2010). In a Luciferase Reporter-Gene Assay conducted by Jensen et al. (2009), mRNA containing the A-allele was found to be repressed by miR-

96, while the G-allele attenuated this regulatory function. While not necessarily deleterious, this functional polymorphism has a potentially phenotypically influential effect, since it may affect gene expression.

Interestingly, 4 of the 7 variants that scored above 10 were in the *HTR1B* gene, which has been consistently identified as a strong candidate for aggression (Davydova et al., 2018; Odintsova et al., 2019). The lowest scoring *HTR1B* variant, rs6297 (C=0.3531; S=4.899) is the only one in this gene for which no functional role has been described (Conner et al., 2010).

Five of the polymorphisms analysed had a negative C-score. These are more likely to belong to the *proxy-neutral* set (Rentzsch et al., 2019), and are likely benign or neutral.

In contrast, the four variants that have been identified in Brunner's Syndrome scored much higher than all of the variants from the set chosen for analysis, apart from Q20* (Table 9). All variants presented S-scores > 20, indicating that they are predicted to be the in the top 1% most deleterious substitutions that you can do to the human genome, and rs72554632, with an S-score of 36, is on the top 0.1%.

Table 9 - CADD scores for SNPs identified in Brunner's Syndrome; variants are ranked by their raw score, from highest to lowest.

Gene	Variant ID	Type	RefAA	AltAA	Raw Score	Scaled Score
MAOA	rs72554632	SNV	Q	*	6,83833	36
MAOA	rs587777457	SNV	C	F	4,258497	29,2
MAOA	rs796065312	SNV	R	W	2,93232	23,3
MAOA	rs796065311	INS	S	SK	2,612359	22,7

These results are not surprising, considering the known effects of these polymorphisms in the *MAOA* gene. Both rs72554632 and rs796065311 create premature STOP codons, (Brunner et al., 1993; Palmer et al., 2016). These truncated mRNA sequences are signalled for destruction via NMD, leading to a drastic reduction in *MAOA* expression and activity. Male carriers are particularly affected, since they only have one copy of the *MAOA* gene. On the other hand, both rs796065312 and rs587777457 are missense variants, predicted by SIFT and PolyPhen to be deleterious (Supplementary Table 1). rs796065312 changes an amino acid in the FAD binding domain of the MAOA protein; this change was predicted to impact on substrate binding and protein folding (Palmer et al., 2016). The amino acidic change introduced by rs587777457 drastically changed the chemical characteristics of a side chain from a highly conserved residue (Piton et al., 2014). Furthermore, this variant may also affect the splicing mechanism. It is, thus, not surprising that these variants have received higher CADD scores.

3.4 Conservation scores

Tables 10, 11 and 12 show the 10 variants with highest conservation scores from each of the CADD programs. The full tables, with conservation scores for each variant, are included in the supplementary material.

Table 10 – Variants with the highest conservation scores in GERP++; data for the remaining variants can be found in Supplementary Table 2

Gene	Variant ID	Location	GERP++	
			RS	Neutral
HTR1B	rs13212041	downstream	5,39	6,54
HTR2B	rs79874540	CDS (nonsense)	4,74	6,52
HTR1B	rs6296	CDS (silent)	3,7	6,45
HTR2A	rs6314	CDS (missense)	2,88	6,53
HTR2B	rs17440378	intron	2,48	4,26
HTR1B	rs11568817	5' UTR	1,93	5,59
MAOA	rs909525	intron	1,56	5,22
MAOA	rs2064070	downstream	1,24	3,63
SLC6A4	rs25531	upstream	0,561	0,561
HTR2A	rs6561333	intron	0,56	5,04

Interestingly, the variants with the highest scores for each program were not the same. This is likely due to the different approaches that each tool uses. Of the 28 polymorphisms selected for analysis, only 3 had high conservation scores in all three tools. The *HTR2B* polymorphism rs79874540 (Q20*) was one of the three SNPs that received high scores in all three programs. The others were rs6296 and rs13212041, both in *HTR1B*. Furthermore, the top variants in PhastCons were the most similar with the highest C-scoring variants.

Table 11 - Variants with the highest conservation scores in PhastCons; data for the remaining variants can be found in Supplementary Table 3. Vert. = Vertebrates

Gene	Variant ID	Location	PhastCons		
			Primates	Mammals	Vert.
HTR2A	rs6313	CDS (silent)	0,972	0	0
HTR2B	rs79874540	CDS (nonsense)	0,965	0,985	0,93
HTR1B	rs6296	CDS (silent)	0,881	1	0,986
TPH2	rs10879352	intron	0,826	0,402	0,345
TPH1	rs1799913	splice, intron	0,69	0,982	0,996
HTR1B	rs13212041	downstream	0,689	1	1
HTR1B	rs130058	5' UTR	0,55	0,944	0,199
MAOA	rs6323	CDS (silent)	0,462	0,964	0,988
HTR1B	rs6297	3' UTR	0,44	0,014	0
MAOA	rs5906957	intron	0,413	0	0

Table 12 – Variants with the highest conservation scores in PhyloP; data for the remaining variants can be found in Supplementary Table 4. Vert. = Vertebrates

Gene	Variant ID	Location	PhyloP Scores		
			Primates	Mammals	Vert.
TPH1	rs1800532	intron	0,595	-1,039	-0,91
HTR2A	rs6313	CDS (silent)	0,595	-1,167	-1,257
HTR2A	rs6314	CDS (missense)	0,588	0,931	1,331
HTR2B	rs79874540	CDS (nonsense)	0,576	2,107	4,921
TPH2	rs6582071	upstream	0,475	0,002	0,009
HTR1B	rs13212041	downstream	0,469	3,352	3,48
HTR1B	rs6297	3' UTR	0,469	-0,045	-0,073
HTR2A	rs7322347	intron	0,453	0,253	-0,001
HTR1B	rs6296	CDS (silent)	0,418	0,435	0,227
HTR2A	rs6561333	intron	0,418	0,018	0,02

It is important to remember that, while these were the variants that scored higher in our set, that does not necessarily mean that the scores they obtained were high. For example, only five variants had an RS score above 2 on GERP++, a cut-off suggested in the Track Description for GERP on the UCSC Genome Browser. Furthermore, only 7 variants had a PhastCons score > 0.5, meaning only 7 variants are more likely to be explained by the "conserved" state; of these, only 4 of these scored above 0.8.

Overall, the *MAOA* variants identified in Brunner's Syndrome did not score much higher than the variants selected for the project. The rs587777457 SNP obtained the highest score in all three programmes. However, the remaining variants scored differently in the three programs. GERP++ and PhastCons ranked the variants in the same order.

Table 13 – Conservation Scores for the *MAOA* SNPs identified in Brunner's Syndrome. pri = Primates; mam= Mammals; ver = Vertebrates

Variant ID	Consequence	GERP++		PhastCons			PhyloP		
		RS	Neutral	pri	mam	ver	pri	mam	ver
rs587777457	missense	5,22	5,22	0,604	0,997	1	0,528	3,729	5,872
rs72554632	nonsense	5,08	5,08	0,501	0,999	1	0,382	2,5	3,421
rs796065312	missense	3,2	5,01	0,115	0,194	0,993	0,382	0,618	2,88
rs796065311	frameshift	1,17	5,22	0,141	0,002	1	0,404	0,415	5,008

4. Discussion

4.1 Serotonin synthesis

4.1.1 *TPH1*

TPH1 is coded for by the *TPH1* gene, located on chromosome 11p15.3–p14. Two polymorphisms in intron 7, (A218C and A779C) have been described to be in strong linkage disequilibrium (Keltikangas-Järvinen et al., 2007), and have been frequently studied in association with AB and its related constructs in humans.

The rs1800532 (A218C) polymorphism is associated with reduced gene expression level due to the change in a GATA type TFBS (Davydova et al., 2018). The A allele, however, has been considered the risk allele for AB, having been associated with increased aggression, anger and impulsivity (Baud et al., 2009; Beden et al., 2016; Manuck et al., 1999; Rujescu et al., 2002). However, some associations with C-allele carriers have also been found (Koh et al., 2012; Staner et al., 2002).

Interestingly, this polymorphism was considered the most conserved on the set by PhyloP (table 11) but the least conserved variant by GERP on the CADD analysis (Supplementary Table 2).

The rs1799913 (A779C) polymorphism is located at the polypyrimidine stretch immediately upstream of the 3′ acceptor splice site, a somewhat conserved sequence in mammals. A purine base (A allele) in this consensus sequence has been shown to decrease the fidelity of splicing (Nielsen et al., 1997).

The A allele of rs1799913 has been associated with increased anger (Rujescu et al., 2002), higher aggressive hostility (Hennig et al., 2005) aggression in cases with psychiatric history (Vassos et al., 2014). Furthermore, it was also associated with reduced activity in the medial PFC under conditions of response inhibition (Ruocco et al., 2016).

There was also a GxE effect described for both these variants. (Keltikangas-Järvinen et al., 2007) found that individuals with an A/A haplotype who had experienced hostile childhood environments had higher levels of adulthood harm-avoidance.

In the CADD analysis, these two polymorphisms obtained very different scores; while rs1799913 was one of the highest scoring SNPs (C= 1.0159; S= 11.82), rs1800532 had the lowest scores of all selected variants (C= -0.6233; S= 0.065). Both the literature and the population analysis in this project suggest that these two polymorphisms are in strong LD.

4.1.2 *TPH2*

Tryptophan hydroxylase-2 (TPH2) is a recently identified TPH isoform responsible for serotonin synthesis in the brain (Walther, 2003). The *TPH2* gene is located on chromosome 12q21. Five polymorphisms from this gene were selected for analysis.

Most of the TPH2 polymorphisms selected for this project were amongst the lowest scoring in the CADD analysis, with 3 polymorphisms retrieving a Raw score below 0 (rs1352250, C= -0.0742; rs1487275, C= -0.1755; rs4570625, C= -0.6079), which would indicate these polymorphisms are more likely to be fixed.

The intronic polymorphism rs1352250 was associated with increased aggression (Perez-Rodriguez et al., 2010) and cognitive impulsivity (Oades et al., 2008). Individuals who were homozygous for the A allele of rs1487275 tended to have larger amygdala size (Li et al., 2015),. The upstream polymorphism rs4570625 has been one of the most studied TPH2 variants in AB. Studies indicated that individuals homozygous for the G allele had higher scores in several anger subscales (J. Yang et al., 2010; Yoon et al., 2012), while individuals with TT genotype had lower scores of aggression, antisocial behaviour, neuroticism and maladaptive impulsivity (Laas et al., 2017; Lehto et al., 2015). Furthermore, the T allele was associated with greater amygdala activation when processing facial expressions (Brown et al., 2005; Canli et al., 2005).

The highest scoring TPH2 polymorphism in the CADD analysis was also one of the overall highest scoring in the set, rs10879352 (C= 0.8885, S= 10.31). No functional role has so far been described for this upstream polymorphism; however, it was reported to be associated with cognitive impulsivity (Oades et al., 2008).Likewise, Oades et al. (2008) found that an under-transmission of the A allele of rs6582071 was associated with behavioural impulsivity. This polymorphism was also associated with differential serotonin production in the OFC (Booij et al., 2012). This polymorphism also had a rather low CADD score (C= 0.0289; S= 1.527).

4.2 Serotonin receptors

4.2.1 *HTR1A*

The rs6295 C(-1019)G polymorphism has been reported to regulate gene expression of the HTR1A receptor (Lemonde et al., 2003). This variant integrates recognition sites for two different transcription factors, NUDR (a homolog of the Drosophila DEAF-1) and Hes5 (a mouse homolog). Both NUDR and Hes5 function as transcriptional repressors. The G allele prevents binding of NUDR, and partially decreases binding of Hes5, resulting in enhanced HTR1A receptor expression in raphe neurons (Lemonde et al., 2003). An increase in the number of HTR1A receptors leads to reduced serotonin release and thus reduced serotonergic neurotransmission, which in turn has been suggested to increase aggression (Albert et al., 2011). The G allele has been associated with greater amygdala reactivity (Fakra et al., 2009; Savitz & Drevets, 2009) and aggression measures (Benko et al., 2010; Strobel et al., 2003).

This polymorphism had low scores on CADD (C= 0.0688; S= 1.826), and was not predicted to be in a conserved site by any of the conservation tools (Supplementary Material).

4.2.2 *HTR1B*

Four of the five HTR1B polymorphisms selected for analysis scored high on the CADD analysis (S > 10). The lowest scoring variant was rs6297 (C= 0.3531; S= 4.899), which was also one of the rarest variants analysed (Table 4; MAF (total) = 0.0996). No functional role has so far been described for this polymorphism; however, Conner et al. (2010) reported that male carriers of the G allele showed significantly lower hostility scores when compared with individuals homozygous for the A allele..

The polymorphism rs6296 (c.G861C) is a synonymous mutation in the CDS of *HTR1B*, and it was one of the highest scoring variants in the CADD analysis (C= 1.0131; S= 11.79). It was also one of the only three polymorphisms to be scored highly in all three CADD conservation tools (Tables 10-12). The C allele has been associated with higher HTR1B receptor expression (Huang et al., 1999), but also with lower binding of serotonin to receptors in the brain, which leads to lower serotonergic activity (Moul et al., 2013). The C allele has been generally considered a risk allele for AB (Clark & Neumaier, 2001; Hakulinen et al., 2013; Krischer & Sevecke, 2008; Soyka et al., 2004).

The two promoter polymorphisms rs11568817 and rs130058 have been shown to work together to modulate gene expression (Duan et al., 2003). The G allele of rs11568817, paired with the A allele of rs130058, lead to a 2.3-fold increase in gene transcription, which leads to increased HTR1B expression and higher serotonergic activity. Furthermore, rs130058 has also been shown to overlap

the binding site for AP1; while it does not change the motif sequence, it does alter the matrix similarity (Sun et al., 2002). The T allele reduced specificity and binding of this transcription factor to the promoter, which was proposed to partly account for the different transcriptional activities of the alleles in this polymorphism (Zouk et al., 2007).

Zouk et al. (2007) found that individuals homozygous for the T allele showed increased reactive AB. Conner et al. (2010) reported that male carriers of the T allele of rs130058 showed increased hostility scores, although this association did not reach significance (p= 0.057). The rs11568817 polymorphism was associated with increased levels of Callous-Unemotional traits in antisocial boys (Moul et al., 2015).

The rs13212041 polymorphism (A1997G) was the second highest scoring polymorphisms in the CADD analysis (C= 1.5557; S= 16.09), and one of the only to be in the top 10 highest scoring variants in all three CADD conservation tools (Tables 10-12). Recently, this polymorphism was shown to modulate gene expression through a change in the recognition sequence for a microRNA (Jensen et al., 2009). This polymorphism corresponds to the nucleotide that would anneal to the third nucleotide of miR-96, and changes an A:U pairing to a weaker G:U pair. This miRNA was shown to repress gene activity; this variant markedly attenuated the repressive effect of miR-96 over HTR1B expression (p= 0.001; Jensen et al., 2009), which would predict increased serotonergic activity.

Jensen et al. (2009) reported that A-allele homozygotes reported a history of more CD-related behaviours (p= 0.005). Conner et al. (2010) found a significant association of this variant with hostility in men, with AA homozygotes showing higher levels of hostility than G-allele carriers (p= 0.001).

4.2.3 *HTR2A*

The HTR2A gene has been consistently associated with AB, but none of the five variants selected for analyses had high CADD scores.

The silent polymorphism rs6313 was the *HTR2A* variant that scored higher on CADD (C= 0.4824; S= 6.32). The T allele has been associated with a higher number of HTR2A receptors in the central nervous system. Both alleles (C>T) of rs6313 have been associated with AB or related endophenotypes. The C/C genotype has been associated with increased agitation and aggression in Alzheimer's disease (AD) (Lam et al., 2004). An association between high levels of behavioural impulsivity and the C/C genotype in alcohol-dependent patients was also observed. On the other hand, the T/T genotype was associated with increased hostility in adulthood, and in a Caucasian cohort, the T-allele was associated with aggression (NPI) in AD (Assal et al., 2004).

The promoter polymorphism rs6311 has been described to be in nearly complete linkage disequilibrium with rs6313 (ref). Allele frequencies for these two polymorphisms were relatively similar, particularly in European and American populations. A-allele homozygotes were found to express 1.6-fold lower extended 5′UTR mRNA. Tomson et al. (2016) reported that subjects with the A/A genotype had higher scores of maladaptive impulsivity, but not adaptive impulsivity. The A allele has been linked to a genetic susceptibility to bipolar disorder, as well as a general tendency towards impulsivity (Bonnier et al., 2002; Nomura et al., 2006). Giegling et al. (2006) observed a significant association between G/G homozygotes and anger- and aggression-related behaviours. The rs6311 was one of the lowest scoring variants in the CADD analysis (C= -0.3953; S= 0.197).

The missense variant rs6314 (C>T) leads to the substitution of a basic amino acid (Histidine) to an uncharged one (Tyrosine), thought to result in changes to the secondary structure of the receptor (Heiser et al., 2007). This causes slower receptor response and has been suggested to interfere with splicing of pre-mRNA, leading to lower expression both on RNA and protein level. In the CADD analysis, both SIFT and PolyPhen considered the variant tolerated (Supplementary Table 1). This was the second rarest polymorphism in our set (MAF= 0.0949).

Both alleles (C/T) of rs6314 have been associated with aggression. Adolescents homozygous for the C allele had significantly higher levels of antisocial behaviour and rule breaking, and individuals homozygous for the C allele showed higher CU traits (Burt & Mikolajewski, 2008).

The variant rs7322347 has been suggested to disrupt a potential miRNA binding site (Banlaki et al., 2015). The T-allele showed significant association with BPAQ subscales for hostility, anger, and physical (proactive) aggression in a nonclinical adult population (Banlaki et al., 2015). Finally, two polymorphisms in the 3rd intron have been associated with cognitive impulsivity (Oades et al., 2008).

4.2.4 *HTR2B*

As previously described, the population-specific variant, rs7987454, leads to blocked expression of the HTR2B receptor. Carriers of this polymorphism will have a much lower level of HTR2B, with only one functional allele. This was the highest scoring polymorphism in the set selected for analysis, and also the rarest. It was previously associated with severe impulsivity (Bevilacqua et al., 2010). Tikkanen et al. (2016) found that Q20* predicted alcohol-related risk behaviours, and that carriers of this polymorphism showed high prevalence of mood disorder symptoms and emotional dysregulation.

The intronic polymorphism rs17440378 was the only one from the set that was reported in a genome-wide study on aggression (Montalvo-Ortiz et al., 2018). This polymorphism did not score very high

in the CADD analysis (C= 0.5548; S= 7.066). No other study has so far reported an association of this variant with aggression.

4.3 Serotonin reuptake: *SLC6A4*

As previously stated, one of the most studied genes in the context of AB is the gene that codes for the serotonin transporter, *SLC6A4* (or *5-HTT*). 5-HTTLPR is a polymorphic region in the promoter consisting of variable number of a 20-23bp degenerate repeat. While several different alleles have been described (Nakamura et al., 2000), most studies have focused on only two, which are the most common: a long (L) allele, with 16 motifs, and a short allele with 14. The short (S) allele, lacking a 43-bp sequence, leads to lower expression of the transporter, which results in reduced serotonin reuptake from the synaptic cleft (Hanna et al., 1998; Lesch et al., 1996).

Furthermore, an SNP in this region (rs25331, A>G) changes the transcriptional activity of these alleles, bringing the expression levels of the L allele down to that of the S allele (Hu et al., 2006). Thus, there have been three different alleles considered when studying 5-HTTLPR: the S allele, regardless of rs25531 genotype, an L_A allele with high transcriptional activity, and an L_G allele with low activity. Some researchers have considered these three alleles as part of a single, multi-allelic locus (Wendland et al., 2006). The low activity variants are generally considered the risk alleles for AB (Cicchetti et al., 2012; Conway et al., 2012; Lopez-Castroman et al., 2014; Manco et al., 2018; Sadeh et al., 2013). Furthermore, several Gene x Environment interactions have been described for 5-HTTLPR in association with AB (Åslund et al., 2013; Caspi et al., 2003; Hankin et al., 2011; Vaughn et al., 2009).

Some epistatic interactions have also been described between 5-HTTLPR with variants in *HTR1A* (David et al., 2005) and the dopamine receptor D4 gene, *DRD4* (Hohmann et al., 2009; Holmboe et al., 2011; Szekely et al., 2004) in the modulation of aggressive behaviour and temperament.

Another VNTR, in the second intron of *SLC6A4*, has been studied in association with aggression, STin2 (Serotonin Transporter intron 2 polymorphism). This variant comprises various repeats of a 16-17 bp imperfect motif. The most common alleles have 9, 10, and 12 repeats, but a 7-repeats allele has been described (Kaiser et al., 2001). Studies have found that STin2.12 enhances transcription of *SLC6A4*, while STin2.10 has been associated with less efficient serotonin turnover (Heils et al., 1996; MacKenzie & Quinn, 1999). Overall, the STin2.12 allele has been considered the risk allele for AB and impulsivity (Aluja et al., 2009; Beitchman et al., 2003; Davidge et al., 2004; Hemmings et al., 2018; M. Yang et al., 2012b)

5-HTTLPR and STin2 have been associated with aggression, both independently and together. Interestingly, their risk alleles seem to point in opposite directions, with the low activity variants of 5-HTTLPR and the high activity allele of STin2 both being associated with increased aggression. Overall, the serotonin transporter gene shows strong evidence for association with AB.

4.4 Serotonin destruction: *MAOA*

MAOA has been one of the most studied genes in relation with aggressive and criminal behaviours. The bulk of the work has been dedicated to a VNTR in the promoter region. This upstream polymorphism (uVNTR) consists of a repeated 30 bp motif that can be present in a variable number of copies. The most common alleles in humans are the variants with 3 and 4 repeats (3R and 4R, respectively). Other less common but still frequent variants include alleles with 2, 3.5 and 5 repeats (Deckert et al., 1999; Sabol et al., 1998).

These different uVNTR variants are associated with different transcriptional activities of the MAOA promoter, which in turn result in different levels of expression of the MAOA gene. Most authors indicate alleles with 3.5 or 4 repeats as the high transcription (H) variants, and alleles with 2, 3 or 5 repeats as having low expression (L).

The low activity monoamine oxidase (*MAOA*-L) variants are less efficient and therefore result in a higher concentration of serotonin in the brain than the more efficient high activity variants (*MAOA*-H) (Eme, 2013). As MAOA degrades catecholamines such as serotonin, reduced *MAOA* expression is associated with elevated levels of serotonin, which could then shape activation and connectivity within corticolimbic regions involved in social evaluation and emotion regulation, though these effects may happen neurodevelopmentally, early in life (Fowler et al., 2007). Most studies indicate that low activity alleles (2R, 3R and 5R) of the *MAOA*-uVNTR are the risk alleles associated with aggressive traits (Armstrong et al., 2014; Beaver et al., 2014; Cooke et al., 2018; Pickles et al., 2013; Tiihonen et al., 2015; Veroude et al., 2016).

This polymorphism has also shown effects of Gene x Environment interaction. *MAOA*-L genotype was predicted as an increased risk for aggressive and antisocial behaviour in Caucasian males, but only when participants had experienced severe childhood maltreatment (Caspi et al., 2002).

Five *MAOA* SNPs were also selected for analysis for this project: two intronic (rs5906957 and rs909525), two silent mutations (rs6323 and rs1137070) and a downstream polymorphism (rs2064070). None of these polymorphisms scored high on the CADD analysis.

Pingault et al. (2013) found a significant main effect of rs5906957 on physical aggression, since levels of aggression for T carriers decreased less than for C carriers. T carriers also had a trend toward lower initial levels of physical aggression than C carriers (p= 0.05).

Antypa et al. (2013) found that homozygous carriers of the G allele of rs909525 had higher levels of anger expressed outwards (STAXI ‘‘anger-out’’ subscale) compared to controls. This was the highest scoring MAOA variant in the CADD analysis (C= 0.4277; S= 5.732).

The silent variant rs6323 has been shown to modulate enzymatic activity for MAOA: studies have associated the A allele with a reduction in MAOA enzymatic activity compared to the G allele, and heterozygotes show intermediate levels of activity. Elevated MAOA enzyme activity, in turn, results in increased amine degradation and decreased availability of neurotransmitters.

While most of the work regarding the role of MAOA in the aetiology of AB has been focused on the promoter uVNTR, there is some evidence that other polymorphisms also affect gene activity, and have some association with aggression. The *MAOA* gene also plays an important role in the degradation of dopamine, not just serotonin (Waltes et al., 2016), which may explain why this particular gene has been shown to influence AB much more than any other.

4.5 Forensic context

In the last 30 years, and ever since the seminal work by Brunner et al. (1993), there has been growing interest in the relationship between genetics and behaviour.. There have been several attempts at introducing behavioural genetics' evidence in court, particularly information regarding the *MAOA* genotype of individuals on trial for murder (McSwiggan et al., 2017).

The first case in which the defendant's genetic information was successfully introduced as mitigating evidence was State v. Waldroup. Prosecutors charged Waldroup with the felony murder of a friend of his wife's, and attempted first-degree murder of his wife. However, after the defence presented evidence for Waldroup's *MAOA*-L genotype, and revealed he had suffered abuse during his childhood, he was instead charged with voluntary manslaughter (Barber, 2010).

In 2009, an Italian court became the first in European to take behavioural genetics into consideration when passing a sentence (Forzano et al., 2010). After a psychiatric report was released claiming that the defendant's genes would make him more prone to behaving violently if provoked, his sentence was reduced from 10 years to 9.

According to McSwiggan et al. (2017), behavioural genetics has been introduced in at least 11 criminal cases (9 in the U.S. and 2 in Italy). The authors found that, of the 11 cases, test results establishing the accused's MAOA genotype were known for eight, and only two of them resulted in sentence reductions. While so far this information has been introduced as mitigating evidence, some authors have argued that the defendant's increased risk for aggression may be viewed as an aggravating factor, in what was described as a "double edged sword" (Denno, 2011).

Selita et al. (2019) conducted a survey to explore people's views on the use of genetic information in the justice system. Most participants agreed to some extent that there is genetic disadvantage, and that genetic information should be taken into account in deciding the form and length of sentence. However, very few participants chose to 'reduce' (4.9% and 5.7%) or 'increase' (0.6% and 2.5%) the sentence based on this genetic risk, when presented with the possibility.

5. Conclusion

The goal of this project was to better understand the genetic mechanisms underlying Aggressive Behaviour. Since there is strong evidence for the role of the serotonergic system in aggression, polymorphisms from serotonergic genes were the focus.

Most of the polymorphisms identified were not in the coding sequence and several were described to affect gene function. The variants that scored highest on CADD (and were thus the ones predicted to be the more deleterious) were in regulatory regions, and not on the CDS. Overall, the variants studied in this project were not found to be highly deleterious, and may play only a small role in the general architecture of AB.

The *MAOA* gene variants showed an interesting distribution pattern across different groups. *MAOA* genotype was significantly different in Asian populations when compared with others.

This study had some limitations. By searching for genes and variants that had already been identified in association with AB, the results were already skewed from the start towards a positive association with AB. However, by analysing polymorphisms that had received less focus together with the ones that had been more investigated, there was an effort into differentiating the results.

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7. Supplementary Materials

Supplementary Table 1 - SIFT and PolyPhen scores for the missense mutations; retrieved from CADD

Gene	Variant ID	AA Change	SIFT	Score	PolyPhen	Score
HTR2A	rs6314	H > Y	tolerated	0,23	benign	0,034
MAOA	rs796065312	R > W	deleterious	0	probably damaging	0.999
MAOA	rs587777457	C > F	deleterious	0	possibly damaging	0.763

Supplementary Table 2 - GERP conservation scores for the SNPs chosen for analysis; variants are ranked by RS score, from highest to lowest.

Gene	Variant ID	Location	GERP++	
			RS	Neutral
HTR1B	rs13212041	downstream	5,39	6,54
HTR2B	rs79874540	CDS (nonsense)	4,74	6,52
HTR1B	rs6296	CDS (silent)	3,7	6,45
HTR2A	rs6314	CDS (missense)	2,88	6,53
HTR2B	rs17440378	intron	2,48	4,26
HTR1B	rs11568817	5' UTR	1,93	5,59
MAOA	rs909525	intron	1,56	5,22
MAOA	rs2064070	downstream	1,24	3,63
SLC6A4	rs25531	upstream	0,561	0,561
HTR2A	rs6561333	intron	0,56	5,04
TPH1	rs1799913	splice, intron	0,456	6,43
HTR2A	rs1923886	intron	0,303	4,03
HTR2A	rs7322347	intron	-0,524	4,39
MAOA	rs6323	CDS (silent)	-0,946	5,08
MAOA	rs1137070	CDS (silent)	-1,65	5,22
TPH2	rs6582071	upstream	-1,76	2,46
TPH2	rs1352250	intron	-1,98	5,95
MAOA	rs5906957	intron	-2,33	3,21
HTR1B	rs130058	5' UTR	-2,45	6,54
TPH2	rs10879352	intron	-2,54	6,31
HTR1B	rs6297	3' UTR	-2,83	6,45
HTR1A	rs6295	upstream	-3,66	6,04
TPH2	rs1487275	intron	-4,04	4,93
TPH2	rs4570625	upstream	-4,94	5,01
HTR2A	rs6313	CDS (silent)	-6,38	6,43
HTR2A	rs6311	upstream	-7,37	5,33
TPH1	rs1800532	intron	-7,75	6,11

Supplementary Table 3 - PhastCons conservation scores for the SNPs chosen for analysis; variants are ranked from highest to lowest. Vert. = Vertebrates

Gene	Variant ID	Location	PhastCons		
			Primates	Mammals	Vert.
HTR2A	rs6313	CDS (silent)	0,972	0	0
HTR2B	rs79874540	CDS (nonsense)	0,965	0,985	0,93
HTR1B	rs6296	CDS (silent)	0,881	1	0,986
TPH2	rs10879352	intron	0,826	0,402	0,345
TPH1	rs1799913	splice, intron	0,69	0,982	0,996
HTR1B	rs13212041	downstream	0,689	1	1
HTR1B	rs130058	5' UTR	0,55	0,944	0,199
MAOA	rs6323	CDS (silent)	0,462	0,964	0,988
HTR1B	rs6297	3' UTR	0,44	0,014	0
MAOA	rs5906957	intron	0,413	0	0
HTR2B	rs17440378	intron	0,318	0,199	0,302
MAOA	rs909525	intron	0,227	0,06	0,01
HTR1A	rs6295	upstream	0,212	0	0
HTR2A	rs6314	CDS (missense)	0,212	0,001	0
TPH2	rs6582071	upstream	0,162	0	0
HTR2A	rs7322347	intron	0,154	0,001	0,015
TPH2	rs1487275	intron	0,133	0	0
TPH1	rs1800532	intron	0,103	0	0
SLC6A4	rs25531	upstream	0,069	0,092	0
MAOA	rs2064070	downstream	0,037	0,006	0,006
MAOA	rs1137070	CDS (silent)	0,029	0,136	0,968
HTR2A	rs6561333	intron	0,017	0	0
HTR1B	rs11568817	5' UTR	0,004	0	0
TPH2	rs1352250	intron	0,001	0	0
HTR2A	rs1923886	intron	0,001	0	0
TPH2	rs4570625	upstream	0	0	0
HTR2A	rs6311	upstream	0	0	0

Supplementary Table 4 - PhyloP conservation scores for the SNPs chosen for analysis; variants are ranked from highest to lowest. Vert. = Vertebrates

Gene	Variant ID	Location	PhyloP Scores		
			Primates	Mammals	Vert.
TPH1	rs1800532	intron	0,595	-1,039	-0,91
HTR2A	rs6313	CDS (silent)	0,595	-1,167	-1,257
HTR2A	rs6314	CDS (missense)	0,588	0,931	1,331
HTR2B	rs79874540	CDS (nonsense)	0,576	2,107	4,921
TPH2	rs6582071	upstream	0,475	0,002	0,009
HTR1B	rs13212041	downstream	0,469	3,352	3,48
HTR1B	rs6297	3' UTR	0,469	-0,045	-0,073
HTR2A	rs7322347	intron	0,453	0,253	-0,001
HTR1B	rs6296	CDS (silent)	0,418	0,435	0,227
HTR2A	rs6561333	intron	0,418	0,018	0,02
MAOA	rs909525	intron	0,391	0,761	0,47
MAOA	rs2064070	downstream	0,391	0,832	0,84
MAOA	rs1137070	CDS (silent)	0,382	-0,408	0,175
TPH2	rs1487275	intron	0,328	-0,299	-0,309
SLC6A4	rs25531	upstream	0,281	0,501	-0,518
MAOA	rs5906957	intron	0,273	-0,391	-0,373
TPH2	rs1352250	intron	-0,199	0,008	0,034
HTR1A	rs6295	upstream	-0,2	-0,175	-0,239
TPH1	rs1799913	splice, intron	-0,346	0,291	0,827
HTR2A	rs1923886	intron	-0,346	0,008	0,019
TPH2	rs4570625	upstream	-0,362	-0,434	-0,393
HTR1B	rs11568817	5' UTR	-0,393	0,282	-0,028
TPH2	rs10879352	intron	-0,439	-0,777	-0,857
HTR1B	rs130058	5' UTR	-0,442	-0,479	-0,76
HTR2B	rs17440378	intron	-0,444	0,355	0,155
MAOA	rs6323	CDS (silent)	-0,694	0,067	0,435
HTR2A	rs6311	upstream	-0,7	-2,844	-2,427