Discovery of Biochemical Biomarkers for Aggression: A Role for Metabolomics in Psychiatry

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Human aggression encompasses a wide range of behaviors and is related to many psychiatric disorders. We introduce the different classification systems of aggression and related disorders as a basis for discussing biochemical biomarkers and then present an overview of studies in humans (published between 1990 and 2015) that reported statistically significant associations of biochemical biomarkers with aggression, DSM-IV disorders involving aggression, and their subtypes. The markers are of different types, including inflammation markers, neurotransmitters, lipoproteins, and hormones from various classes. Most studies focused on only a limited portfolio of biomarkers, frequently a specific class only. When integrating the data, it is clear that compounds from several biological pathways have been found to be associated with aggressive behavior, indicating complexity and the need for a broad approach. In the second part of the paper, using examples from the aggression literature and psychiatric metabolomics studies, we argue that a better understanding of aggression would benefit from a more holistic approach such as provided by metabolomics.

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Biochemical markers are intermediate to the outward phenotype and the underlying biology of aggression. They can aid in elucidating the causes and (patho)physiology of aggressive behavior [James et al., 2006; Scoriels et al., 2015]. One of the promises of

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biochemical studies of central nervous system diseases is the development of biochemically based biomarkers [Kaddurah-Daouk and Krishnan, 2009]. Biomarkers can be applied in diagnosis, in assessment of disease stages, as indicators of disease prognosis, and for prediction or monitoring of interventions and treatment responses. Some biomarkers are “surrogate endpoints” which substitute clinical endpoints that reflect “how a patient feels, functions, or survives” [Atkinson et al., 2001]. There are several comprehensive overviews of biochemical compounds as potential biomarkers for aggression [e.g., Siever, 2008; Yanowitch and Coccaro, 2011], including reviews of neurotransmitters [e.g., de Almeida et al., 2005; Seo et al., 2008; Siever, 2008; Wallner and Machatschke, 2009; Chichinadze et al., 2011; Yanowitch and Coccaro, 2011; Haller, 2013; Umukoro et al., 2013; Morrison and Melloni, 2014; Narvaes and Almeida, 2014; Willner, 2015], hormonal networks [Simpson and Hons, 2001; Wingfield et al., 2006; Siever, 2008; Soma et al., 2008; Chichinadze et al., 2011; Eisenegger et al., 2011; Haller, 2014a,c; Soma et al., 2015], and cytokines [e.g., Zalcman and Siegel, 2006]. With a few exceptions [e.g., Siever, 2008], these reviews focus on a single biochemical class, studying biomarkers belonging to various biochemical pathways in isolation. This makes it more difficult to draw substantive conclusions about aggression on a biochemical network or “systems biology” level. A systems biological description of aggression would be particularly useful, although “metabolic snapshots” (as provided for instance by metabolic profiling of a single sample from each individual participating in a molecular epidemiological study) are insufficient to reconstruct models of the metabolic network underlying this phenotype [Kell, 2004]. Further progress in aggression research might benefit from a more “holistic” approach, where biochemical markers from a wide variety of biochemical classes are studied simultaneously. One such a holistic approach is by metabolomics, the comprehensive study of all metabolites in a given sample. A metabolomics approach might allow for the discovery of novel biomarkers of aggression and its subtypes for increasing insight into the biochemical mechanisms of aggression, and consequently to identify new drug targets [Kaddurah-Daouk and Krishnan, 2009].

This article consists of two parts. In the first part, we present a review of studies that aimed at the evaluation and discovery of biochemical biomarkers for aggression, aggression-related disorders, and for distinct aggression subtypes. We start with a short overview of classification systems for aggression and then present an overview of research into biochemical biomarkers for aggression for disorders of which aggression is a component, and for its subtypes. In the second part of the article, we discuss the role metabolomics can play in the quest for biochemical biomarkers of aggression.

AGGRESSION CLASSIFICATIONS

Aggression is a heterogeneous concept that is commonly defined as “hostile, injurious, or destructive behavior” [Siever, 2008], but for which many alternative definitions, subtypes and classification systems have been suggested [Weinshenker and Siegel, 2002; Ramírez and Andreu, 2003; Vitaro et al., 2006; Ramírez, 2009, 2011]. Human aggressive behavior encompasses a wide range of behaviors and occurs in multiple contexts. Subtypes of aggression have been classified by the mode of aggression, target (self, other, objects), and cause/function. Supplementary Table SI lists the different subtypes as they apply to each of the different forms of classification systems. Based on the target of aggression, a distinction is made between direct and indirect aggression. Direct aggression can encompass both physical and verbal aggression as long as there is a direct confrontation between an aggressor and a target. Indirect aggression is characterized by a lack of direct confrontation; examples of indirect aggression include social manipulations and secretly damaging property [Collett et al., 2003]. Direct or indirect aggression can be further distinguished by the modes of aggression, that is, both can be physical or verbal, which is sometimes denoted as relational aggression. Sometimes an argument is made for adding a third subtype referring to nonverbal behavior or body language, such as facial expressions [Ramirez and Andreu, 2003].

Next, all types of aggression can be classified following the functional aspect of aggressive behavior (see Supplementary Table SI). It has been argued that the differences among the functional classification systems are semantic in nature and that different fields of study refer to the same kind of aggression under different names [Weinshenker and Siegel, 2002]. Therefore, Ramírez and Andreu [2006] and Ramírez [2009, 2011] proposed a single dichotomous system including a “social-cognitive subtype” and an “emotional subtype” of aggression. The first subtype is goal-oriented and is associated with a positive evaluation of aggression. It encompasses the instrumental [purposeful and goal-oriented; McEllistrem, 2004], proactive [aimed at securing resources or dominion; Vitaro et al., 2006], and premeditated [planned goal-oriented; Ramírez, 2009] forms of aggression, including behaviors such as “controlling others” and “habitual lying”. The second subtype is uncontrolled aggressive behavior and includes the hostile [“intent to harm another person”; Ramírez and Andreu, 2003], reactive [response to a perceived threat or provocation; Vitaro et al., 2006], and impulsive [aimed at gratification without concern for consequences; Ramírez, 2009] subtypes of aggression, which includes behaviors such as “getting angry” and “starting fights” [Ramirez and Andreu, 2006; Ramírez, 2009, 2011].

The Diagnostic and Statistical Manual for mental disorders [DSM-IV; American Psychiatric Association, 2000] recognizes psychiatric disorders in children and adults in which aggression is one of the characterizing symptoms. In children, oppositional defiant disorder (ODD) is characterized by negative, defiant, disobedient, and hostile behavior toward authority figures, while conduct disorder (CD) encompasses a behavior pattern marked by violations of the basic rights of others and of societal norms and rules [Loeber et al., 2000]. CD is mainly characterized by physical and relational aggression, and ODD by anger [Haller, 2014b]. Proactive aggression during childhood is a risk factor for the development of delinquent behavior, ODD, and CD during adolescence [Vitaro et al., 1998]. In adults intermittent explosive disorder (IED), borderline personality disorder (BPD) and antisocial personality disorder (APD) each have aggression or an aggression-related phenotype (e.g., impulsivity, hostility, anger [Ramirez and Andreu, 2006]) as potential symptoms. Patients meeting criteria for IED suffer from multiple episodes of
disproportionate impulsive aggression [Coccaro, 2012], whereas patients diagnosed with one of the other disorders are not necessarily aggressive; individuals can be diagnosed with one of these disorders without presenting aggressive tendencies [Haller, 2014b]. APD is characterized by a pattern of persistent antisocial behavior and is preceded by conduct problems evident before age 15 [Hare et al., 1991]. APD is associated with physical aggression, irritability, and impulsivity. BPD is characterized by emotional dysregulation, which often manifests as impulsive, self-destructive, or (predominantly self-directed) aggressive behavior [Siever, 2008; Scott et al., 2014]. Other psychiatric disorders, such as autism [Farmer and Aman, 2011; Farmer et al., 2015] or schizophrenia [Weiss, 2012; Bo et al., 2014], are also associated with aggression, but aggression is not one of the criteria required for the DSM-IV diagnosis.

AGGRESSION BIOCHEMISTRY

An overview of studies (published between 1990 and 2015) focusing on putative biochemical biomarkers for aggression subtypes, disorders of which aggression is a (potential) symptom, or overall aggression in humans is given in a set of (supplementary) tables. Table I summarizes the biomarkers reported for total aggression scores, which often are based on assessments by questionnaires. Table II gives an overview of the biomarkers reported for DSM-IV disorders as discussed above, and Table III summarizes the biomarkers reported for the aggression classification systems per subtype. More details are provided in Supplementary Tables SII–IV, indicating for each study in which a significant association of aggression with at least one biochemical biomarker was found in which population the study was conducted; the assayed biofluid; whether the association(s) of the metabolite(s) or enzyme(s) with aggression was (were) positive or negative; and the main conclusions reached by the authors based on the study findings. All tables contain measures of aggression assessed in population and clinical samples, and report significant associations only. Reviews of potential biochemical biomarkers of aggression often focus on a particular class of metabolites. In contrast, in Table I, we list all studies focusing on potential biomarkers for aggression across all classes of biochemicals, ordering the studies by type of aggression measure rather than by biochemical class. As can be seen in this table, markers from various classes have been observed to associate with each of the different aggression measures. Consistent with a review on the role of cytokines in aggression [Zalcman and Siegel, 2006], we observed several studies indicating an increase in inflammation marker (IL-6, CRP) levels in relation to aggression, in both adolescents and adults using different aggression measures [Coccaro, 2006, 2014; Holtmann et al., 2013]. Increased levels of C-reactive protein and interleukin-6 have also been observed in patients with IED [Table II; Coccaro et al., 2014], which seems to suggest that inflammation markers mainly play a role in impulsive aggression.

Investigations into neuroendocrine mechanisms underlying aggressive behavior have focused mainly on gonadal sex steroid hormones (e.g., testosterone, which is regulated by the hypothalamic–pituitary–gonadal axis [HPG]). As can be seen in Table I, increased levels of the HPG hormone testosterone are observed in aggressive adolescents [Yu and Shi, 2009] and adult males convicted for crimes [Horn et al., 2014]. In contrast, a decreased free testosterone index has been associated with CD in girls [Table II; Pajer et al., 2006]. Overall, meta-analyses revealed a weak positive relationship between testosterone and aggression [Archer, 1991; Book et al., 2001; Archer et al., 2005]. Conduct disorder and ODD have also been linked to other androgens (Table II). The testosterone precursor androstenedione and its precursor, dehydroepiandrosterone sulfate (DHEA-S), were elevated in boys with CD [van Goozen et al., 1998a]. Increases in DHEA-S levels have also been observed in a mixed sample of CD patients [Dmitrieva et al., 2001] and in children with ODD [van Goozen et al., 2000]. Another approach has been to investigate the role of the endocrine structures underlying the hypothalamic–pituitary–adrenal (HPA) axis, in order to evaluate the role of the stress response system in aggression [Barzman et al., 2010]. In humans, the most important stress hormone is cortisol as produced by the adrenal glands [Crowley and Girdler, 2014], and as can be seen throughout the tables this hormone was significantly associated with aggression in many studies. Low cortisol levels have been associated with high total aggression measures [Table I; van de Wiel et al., 2004; Yu and Shi, 2009; Poustka et al., 2010; Platje et al., 2013a,b; Horn et al., 2014; McBurnett et al., 2014], CD [Table II; Pajer et al., 2001; Oosterlaan et al., 2005], ODD [Table II; van Goozen et al., 1998b], and with reactive and proactive aggression [Table III; Poustka et al., 2010; Stoppelbein et al., 2014]. In contrast, increased levels of cortisol have also been observed for higher total aggression measures [Table I; Barzman et al., 2013], CD [Table II; van Bokhoven et al., 2005], and reactive aggression [Table III; van Bokhoven et al., 2005; Lopez-Duran et al., 2009]. While the results of studies that investigate the cortisol-aggression relationship are not conclusive, overall, decreased cortisol levels have been observed with higher aggression levels. Further evidence for a role of the stress response system in aggressive behavior comes from studies investigating salivary levels of the adrenocortical stress response marker alpha-amylase [Nater et al., 2005]. An interaction effect for alpha-amylase and cortisol was observed, in which low cortisol level was only predictive for aggressive behavior for low alpha-amylase levels [Table I; Gordis et al., 2006].

The HPA and HPG axes are not isolated systems: DHEA is co-released from the adrenal glands together with cortisol and has anti-glucocorticoid effects [Crowley and Girdler, 2014]. This co-release of DHEA and cortisol occurs through stimulation of adrenocorticotropic hormone (ACTH; cortisol precursor) to the adrenal glands, stimulating DHEA synthesis [Crowley and Girdler, 2014]. In CD patients, an increase in ACTH has been observed [Table II; Dmitrieva et al., 2001], which would also explain the observed increase in DHEA(-S) levels in CD patients [van Goozen et al., 1998a, 2000; Dmitrieva et al., 2001]. Furthermore, a decrease in the ratio of cortisol to DHEA-S has been observed in girls with CD with respect to non-CD controls [Table II; Pajer et al., 2006], which might indicate that the majority of ACTH is used to stimulate DHEA synthesis and not used as a precursor for cortisol. The hormonal influences on aggressive behavior are complex and vastly interconnected, with other hormone classes than androgens and glucocorticoids also playing a role in aggression. The thyroid hormones triiodothyronine and thyroxin have both been associated with aggression. Low levels of free thyroxin have been...
<table>
<thead>
<tr>
<th>Population</th>
<th>Questionnaire</th>
<th>Biomarker(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>69 IED patients</td>
<td>Composite Aggression Score (from multiple questionnaires)</td>
<td>Increased</td>
<td>Coccaro et al. [2014]</td>
</tr>
<tr>
<td>61 non-aggressive patients axis I/II disorder</td>
<td></td>
<td>IL-6</td>
<td></td>
</tr>
<tr>
<td>67 controls</td>
<td></td>
<td>CRP</td>
<td></td>
</tr>
<tr>
<td>38 boys with disruptive behavior</td>
<td>Composite Aggression Score (from multiple questionnaires)</td>
<td>Decreased</td>
<td>McBurnett et al. [2014]</td>
</tr>
<tr>
<td>20 healthy volunteers</td>
<td>Composite Aggression Score (from multiple questionnaires)</td>
<td>Increased</td>
<td>Coccaro and Lee [2010]</td>
</tr>
<tr>
<td>40 personality disorder subjects</td>
<td></td>
<td>IL-6</td>
<td></td>
</tr>
<tr>
<td>10 healthy volunteers</td>
<td>Composite Aggression Score (from multiple questionnaires)</td>
<td>Increased</td>
<td>Coccaro et al. [2013]</td>
</tr>
<tr>
<td>28 personality disorder subjects</td>
<td></td>
<td>Glutamate</td>
<td></td>
</tr>
<tr>
<td>99 subjects with personality disorders</td>
<td>Buss–Durkee Hostility Inventory</td>
<td>Increased</td>
<td>Coccaro [2006]</td>
</tr>
<tr>
<td>38 subjects with personality disorders</td>
<td>Buss–Durkee Hostility Inventory</td>
<td>Increased</td>
<td>Coccaro et al. [2012]</td>
</tr>
<tr>
<td>77 psychiatrically healthy adults</td>
<td>Buss–Durkee Hostility Inventory</td>
<td>Decreased</td>
<td>Bjork et al. [2001]</td>
</tr>
<tr>
<td>60 male subjects committed for serious criminal acts</td>
<td>Karolinska Scale of Personality</td>
<td>Increased</td>
<td>Stalenheim [2004]</td>
</tr>
<tr>
<td>66 male controls</td>
<td></td>
<td>Free triiodothyronine</td>
<td></td>
</tr>
<tr>
<td>67 adolescents from longitudinal study to effects of maltreatment</td>
<td>Reactive–Proactive Aggression Questionnaire</td>
<td>Decreased</td>
<td>Gordis et al. [2006]</td>
</tr>
<tr>
<td>20 aggressive adolescents</td>
<td>Child Behaviour Checklist/Youth Self-Report</td>
<td>Decreased</td>
<td>Yu and Shi [2009]</td>
</tr>
<tr>
<td>20 non-aggressive adolescents</td>
<td></td>
<td>Cortisol</td>
<td></td>
</tr>
<tr>
<td>425 adolescents</td>
<td>Child Behaviour Checklist/Youth Self-Report</td>
<td>Decreased</td>
<td>Platje et al. [2013b]</td>
</tr>
<tr>
<td>390 adolescents</td>
<td>Child Behaviour Checklist/Youth Self-Report</td>
<td>Decreased</td>
<td>Platje et al. [2013a]</td>
</tr>
<tr>
<td>22 children with disruptive behavior</td>
<td>Child Behaviour Checklist/Youth Self-Report</td>
<td>Decreased</td>
<td>van de Wiel et al. [2004]</td>
</tr>
<tr>
<td>51 children and adolescents with a CBCL-DP</td>
<td>Child Behaviour Checklist/Youth Self-Report</td>
<td>Increased</td>
<td>Holtmann et al. [2013]</td>
</tr>
<tr>
<td>82 CBCL-DP control children and adolescents</td>
<td></td>
<td>CRP</td>
<td></td>
</tr>
<tr>
<td>Study 1</td>
<td>Child Behaviour Checklist/Youth Self-Report</td>
<td>Decreased</td>
<td>van Goozen et al. [1999]</td>
</tr>
<tr>
<td>15 ODD boys</td>
<td></td>
<td>Albumin</td>
<td></td>
</tr>
<tr>
<td>25 controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 2</td>
<td>Child Behaviour Checklist/Youth Self-Report</td>
<td>Decreased</td>
<td></td>
</tr>
<tr>
<td>22 ODD</td>
<td></td>
<td>5-HIAA</td>
<td></td>
</tr>
<tr>
<td>25 controls</td>
<td></td>
<td>HVA</td>
<td></td>
</tr>
</tbody>
</table>
associated with total aggression in male convicts [Table I; Stalenheim, 2004], while increased levels of free triiodothyronine have been found in male convicts [Table I; Stalenheim, 2004] and CD patients [Table II; Dmitrieva et al., 2001]. However, only a small percentage of thyroid hormones travels unbound through the bloodstream; one of the carriers of thyroid hormones is albumin, for which low levels have been associated with total aggression [Table I; Holtmann et al., 2013], which might suggest that when albumin levels are low the levels of circulating unbound triiodothyronine increase (associated with increased aggression).

Most major neurotransmitters are believed to play a role in aggressive behavior, with the serotonergic system responsible for the inhibition of aggressive behavior; the dopaminergic system responsible for the initiation of aggressive behavior; and the gamma-Aminobutyric acid-ergic (GABAergic) system responsible for appraising aggression-related cues [Willner, 2015]. Monoamine oxidase (MAO) catabolizes serotonin (5HT) into 5-hydroxyindolealdehyde (5-HIAL), which is then converted into 5-hydroxyindoleacetic acid (5-HIAA) by aldehyde dehydrogenase [Wong et al., 2005]. Both MAO levels and 5-HIAA are associated with total aggression (Table I). Decreases in the MAO levels have been observed in aggressive male criminals [Stalenheim, 2004] and low levels of 5-HIAA are associated with total aggression [Kruesi et al., 1990, 1992; van Goozen et al., 1999; Placidi et al., 2001], ODD [Table II; van Goozen et al., 1999], and physical aggression [Table III; Kruesi et al., 1992]. However, increased levels of 5-HIAA have also been observed for total aggression [Table I; Coccaro and Lee, 2010], and impulsive aggression [Table III; Coccaro and Lee, 2010]. The association of low 5-HIAA levels and aggression might be explained by reduced 5HT transport activity as observed in aggression [Siever, 2008] or by activation of the pre-synaptic 5HT \textsubscript{1B} receptor which inhibits 5HT release and thereby 5HT concentration [Sari, 2004]. Also, altered activation of 5HT receptors plays a role in aggression, including increased 5HT \textsubscript{1A} activation [de Almeida et al., 2005] and reduced 5HT \textsubscript{2C} activation [Siever, 2008].

Overall, disinhibition of (impulsive) aggressive behavior seems related to hypo-activity of the serotonergic system in the prefrontal cortex and anterior cingulate [de Almeida et al., 2005]. This hypo-activity also results in reduced control by serotonin of the dopaminergic system, leading to dopamine hyperactivity, thereby increasing aggressive behavior even further [Soderstrom et al., 2003; Seo

### Table I. (Continued)

<table>
<thead>
<tr>
<th>Populationa</th>
<th>Questionnaire</th>
<th>Biomarker(s)b</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 healthy volunteers</td>
<td>Buss–Perry Aggression Questionnaire</td>
<td>Decreased</td>
<td>Troisi and D’Argenio [2006]</td>
</tr>
<tr>
<td>20 aggressive subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>245 adolescents at risk for psychopathy</td>
<td>Buss–Perry Aggression Questionnaire</td>
<td>Decreased</td>
<td>Poustka et al. [2010]</td>
</tr>
<tr>
<td>17 psychiatrically hospitalized boys</td>
<td>Brief Ratings of Aggression by Children and Adolescents</td>
<td>Increased</td>
<td>Barzman et al. [2013]</td>
</tr>
<tr>
<td>29 children with disruptive behavior disorders</td>
<td>Modified Overt Aggression Scale</td>
<td>Decreased</td>
<td>Kuersi et al. [1990, 1992]</td>
</tr>
<tr>
<td>43 children with OCD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995 participants from the HSCORE consortia</td>
<td>Cook–Medley Hostility Scale</td>
<td>Decreased</td>
<td>Sahebzamani et al. [2013]</td>
</tr>
<tr>
<td>545 newly convicted males</td>
<td>MacArthur Community Violence Instrument</td>
<td>Decreased</td>
<td>Horn et al. [2014]</td>
</tr>
<tr>
<td>93 MDD patients</td>
<td>Life History Aggression</td>
<td>Decreased</td>
<td>Placidi et al. [2001]</td>
</tr>
<tr>
<td>26 subjects with personality disorder</td>
<td>Life History Aggression</td>
<td>Increased</td>
<td>Coccaro et al. [1998]</td>
</tr>
</tbody>
</table>

\*Abbreviations as mentioned in the population column: IED, intermittent explosive disorder; CBCL-DP, child behavior checklist dysregulation profile; ODD, oppositional defiant disorder; OCD, obsessive compulsive disorder; MDD, major depressive disorder.

\*Abbreviations as mentioned in the biomarker(s) column: IL–6, interleukin-6; CRP, C-reactive protein; 5-HIAA, 5-hydroxindolacetic acid, HVA, homovanillic acid; GABA, gamma-aminobutyric acid; MAO, monoamine oxidase; Apo-AI, apo lipoprotein AI; Apo-B, apo lipoprotein B; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; MHPG, 3-methoxy-4-hydroxyphenylglycol.
Interconnections between 5HT and dopamine are mediated through receptors which act both as autoreceptors (controlling activity of “own” neurotransmitter, for example, receptor from the serotonergic system controlling activity of serotonin) and heteroreceptors [controlling activity of “other” neurotransmitters, e.g., receptor from the serotonergic system controlling activity of dopamine; Hamon and Blier, 2013]. Serotonin influences the activity of the dopaminergic system through the 5HT 2C receptor: activation of this receptor reduces dopamine release [de Deurwaerdere et al., 2004]. Increased levels of the dopamine breakdown product 3-methoxy-4-hydroxyphenylglycol (MHPG) have been associated with total aggression [Table 1; et al., 2008].

### TABLE II. Significant Associations of Potential Biomarkers for the DSM-IV Disorders With Aggression as a (Potential) Characterizing Symptom

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Populationa</th>
<th>Biomarker(s)b</th>
<th>References</th>
</tr>
</thead>
</table>
| Intermittent explosive disorder | 69 IED patients  
61 non-aggressive patients axis I/II disorder  
67 controls                      | Increased IL-6  
Increased CRP                      | Coccaro et al. [2014] |
| Conduct disorder              | 194 boys from longitudinal population study       | Increased Cortisol     | van Bokhoven et al. [2005] |
| Conduct disorder              | 28 CD patients  
13 controls                          | Increased ACTH  
Decreased DHEA-S  
Decreased Free triiodothyronine  
Decreased IGF-I  
Decreased IGFBP-3        | Dmitrieva et al. [2001] |
| Conduct disorder              | 15 CD boys  
25 control boys                       | Increased DHEA-S  
Increased Androstenedione          | van Goozen et al. [1998a] |
| Conduct disorder              | 250 male criminal offenders with APD             | Decreased Total cholesterol | Repo-Tiihonen et al. [2002] |
| Conduct disorder              | 47 girls with CD  
36 control girls                      | Decreased Cortisol to DHEA-S ratio  
Decreased SHBG  
Decreased Free testosterone index | Pajer et al. [2006] |
| Conduct disorder              | 47 girls with CD  
37 control girls                      | Decreased Cortisol     | Pajer et al. [2001] |
| Oppositional defiant disorder | 18 ODD/CD children  
7 antisocial children without ODD/CD | Decreased Cortisol     | Dosterlaan et al. [2005] |
| Oppositional defiant disorder | 21 ODD boys  
31 control boys                       | Decreased Cortisol     | van Goozen et al. [1998b] |
| Oppositional defiant disorder | 24 ODD children  
42 psychiatric controls  
30 normal controls                | Decreased DHEA-S       | van Goozen et al. [2000] |
| Oppositional defiant disorder | Study 1  
15 ODD boys  
25 controls                          | Decreased 5-HIAA  
Increased HVA                     | van Goozen et al. [1999] |
| Study 2                        | 22 ODD  
25 controls                          |                        |                             |
| Antisocial personality disorder | 250 male criminal offenders with APD            | Decreased Total cholesterol | Repo-Tiihonen et al. [2002] |

aAbbreviations as mentioned in the population column: IED, intermittent explosive disorder; CD, conduct disorder; ODD, oppositional defiant disorder; APD, antisocial personality disorder.
bAbbreviations as mentioned in the biomarker(s) column: IL–6, interleukin-6; CRP, C-reactive protein; ACTH, adrenocorticotropic hormone; DHEA-S, dehydroepiandrosterone sulphate; IGF-I, insulin-like growth factor I; IGFBP–3, insulin-like growth factor binding protein 3; SHBG, sex hormone binding globulin; 5-HIAA, 5-hydroxindoleacetic acid; HVA, homovanillic acid.
MAO converts MHPG into homovanillic acid (HVA), which is most frequently measured when investigating dopamine function. In apparent contradiction with the theory that hyperactivity of the dopaminergic systems leads to increased aggression, low levels of HVA have been associated with total aggression [Table I; van Goozen et al., 1999; Coccaro and Lee, 2010], ODD [Table II; van Goozen et al., 1999], and impulsive aggression [Table III; Coccaro and Lee, 2010].

The main GABA precursor is alpha-ketoglutarate (α-KG), a tricarboxylic acid (TCA) cycle intermediate. Glutamate dehydroxylation of α-KG synthesizes glutamate, which is then converted into GABA by glutamic acid decarboxylase (GAD) [Soghomonian and Martin, 1998; Bak et al., 2006]. In aggressive animals, low levels of neuronal GABA and GAD have been observed [de Almeida et al., 2005], and inhibition of GABA re-uptake (leading to higher postsynaptic GABA levels) inhibits aggressive behavior [Siever, 2008]. Congruently, low plasma levels of GABA are observed in human individuals with high total aggression scores [Table I; Bjork et al., 2001]. Increased levels of CSF glutamate have been associated with increased total and impulsive aggression in humans [Table I and III; Coccaro et al., 2013]. Neuronal GABA levels are influenced by 5HT (through unknown mechanisms) and positively modulate GABAA receptor activity which increases alcohol-induced aggressive behavior [de Almeida et al., 2005]. In contrast, positive modulation of GABAA receptors with benzodiazepines decreases violent outbursts in psychiatric patients [de Almeida et al., 2005].

The different neurotransmitter systems work in tight concert with one another and with other systems to initiate or inhibit the complex behavior aggression. For example, impaired synaptic 5HT transport (associated with increased aggression) is potentially explained by reduced lipid micro-viscosity due to low central nervous system cholesterol levels [Engelberg, 1992; Wallner and Machatschke, 2009]. Indeed, low serum cholesterol levels have been associated with CD, APD [Table II; Repo-Tiihonen et al., 2002], and premeditated aggression [Table III; Conklin and Stanford, 2008], and a decreased ratio of serum high density lipoprotein cholesterol (HDL-C) to total serum cholesterol has

### TABLE III. Significant Associations of Potential Biomarkers for the Aggression Subtypes From the Different Bimodal Classification Systems

<table>
<thead>
<tr>
<th>Aggression subtype</th>
<th>Population</th>
<th>Biomarker(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal aggression</td>
<td>40 healthy volunteers 20 aggressive subjects</td>
<td>Decreased HDL-C: total cholesterol ratio</td>
<td>Troisi and D’Argenio [2006]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased HVA</td>
<td></td>
</tr>
<tr>
<td>Physical aggression</td>
<td>29 children with disruptive behavior disorders 43 children with OCD</td>
<td>Decreased 5-HIAA</td>
<td>Kruesi et al. [1992]</td>
</tr>
<tr>
<td>Impulsive aggression</td>
<td>10 healthy volunteers 28 personality disorder subjects</td>
<td>Increased Glutamate</td>
<td>Coccaro et al. [2013]</td>
</tr>
<tr>
<td>Impulsive aggression</td>
<td>20 healthy volunteers 40 personality disorder subjects</td>
<td>Increased 5-HIAA</td>
<td>Coccaro and Lee [2010]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased HVA</td>
<td></td>
</tr>
<tr>
<td>Premeditated aggression</td>
<td>18 males with substance dependence</td>
<td>Increased Total cholesterol</td>
<td>Conklin and Stanford [2008]</td>
</tr>
<tr>
<td>Reactive aggression</td>
<td>158 girls with psychiatric problems</td>
<td>Decreased Cortisol</td>
<td>Stoppelbein et al. [2014]</td>
</tr>
<tr>
<td>Reactive aggression</td>
<td>245 adolescents at risk for psychopathy</td>
<td>Decreased Cortisol</td>
<td>Poustka et al. [2010]</td>
</tr>
<tr>
<td>Reactive aggression</td>
<td>194 boys from longitudinal population study</td>
<td>Increased Cortisol</td>
<td>van Bokhoven et al. [2005]</td>
</tr>
<tr>
<td>Reactive aggression</td>
<td>40 boys at risk for CD</td>
<td>Increased Cortisol</td>
<td>Lopez-Duran et al. [2009]</td>
</tr>
<tr>
<td>Proactive aggression</td>
<td>158 girls with psychiatric problems</td>
<td>Decreased Cortisol</td>
<td>Poustka et al. [2010]</td>
</tr>
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<td>Proactive aggression</td>
<td>245 adolescents at risk for psychopathy</td>
<td>Decreased Cortisol</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations as mentioned in the population column: OCD, obsessive compulsive disorder; CD, conduct disorder.**

**Abbreviations as mentioned in the biomarker(s) column: 5-HIAA, 5-hydroxindoleacetic acid; HVA, homovanillic acid, LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.**
been associated with verbal aggression [Table III; Troisi and D’Argenio, 2006]. Similarly, for other lipoproteins, such as HDL-C and LDL-C, low levels have also been associated with both total aggression [Table I; Troisi and D’Argenio, 2006; Sahebzamani et al., 2013] and verbal aggression [Table III; Troisi and D’Argenio, 2006]. All these findings support the hypothesis that the association of low CNS cholesterol/lipoprotein levels with increased aggression is mediated through impaired synaptic 5HT transport. Furthermore, glucose levels could influence GABA synthesis as glycolysis synthesizes pyruvate, a necessary metabolite for TCA cycle initiation [Soghomonian and Martin, 1998]. Indeed, low blood glucose levels have been associated with intra-spousal aggression [Bushman et al., 2014]. Finally, influences of the HPA and HPG axes on the neurotransmitter systems have been observed [Summers and Winberg, 2006], including a trifecta effect in which high testosterone, low cortisol and low serotonin lead to impulsive aggression [Montoya et al., 2012] and the modulating effect of DHEA-S on GABA A receptors [Crowley and Girdler, 2014].

We arrive at indications for the involvement of inflammation markers (e.g., IL-6 and CRP), neurotransmitters (e.g., 5-HIAA, HVA, glutamate, GABA, MAO, and MHPG), lipoprotein markers (e.g., HDL-C, LDL-C, Apo-AI, and Apo-B), and hormones from several classes, including thyroid hormone markers (e.g., triiodothyronine and thyroxin), and steroid hormone markers (e.g., cortisol, testosterone, DHEA-S, and ACTH) in aggression. These systems and pathways usually have been studied by focusing on one known compound. It is often not known whether this compound is the best one to represent deviation in the pathway or system. Approaches that evaluate a whole metabolic pathway or system may identify the most informative markers, but we can use the current information to guide the selection of pathways.

**METABOLOMICS APPROACHES TO BIOCHEMICAL BIOMARKER DISCOVERY**

With the complex and heterogeneous nature of aggression, it is unlikely that a single biochemical biomarker can fully represent the aggression phenotype, whereas the combination of multiple biomarkers may reflect the etiology of psychiatric disorders more comprehensively, and hence provide improved insight in the underlying biological processes [Glenn, 2009; Boksa, 2013]. This is even more evident, when we consider the possibility of multiple subgroups with different pathways of pathology, requiring different biomarkers. Most biomarker studies have focused on the quantification and assessment of only a few biomarkers at a time [Zhao and Lin, 2014]. With the advances in techniques for massively parallel analysis [i.e., “omics”-type analyses, that allow for the acquisition of data for many variables in a single analysis of a single biological sample; Dunn et al., 2011a], it now is possible to aid in diagnosis and subclassification based on a large range of biochemicals [Alawieh et al., 2012; Kobeissy et al., 2013]. One of these “omics” approaches, that is, metabolomics, aims at providing a holistic overview of the metabolome of a biofluid (e.g., blood, saliva, cerebrospinal fluid, urine) or tissue sample [Wishart, 2007]. The metabolome compromises all low molecular weight (<1 kDa) molecules that are involved in metabolic reactions (i.e., metabolites) and that are present in the biofluid or tissue under consideration [Dunn and Ellis, 2005].

In metabolomics, typically, two types of strategies are considered: “targeted” and “untargeted.” In targeted analyses, the researcher targets the analysis to a relatively small number of metabolites or a selected metabolic pathway, based on some prior hypothesis of the involvement of these metabolites in the biological process of interest. Untargeted approaches, on the other hand, do not depart from a particular biological hypothesis but attempt to obtain an overview of the metabolites in the metabolome that is as broad (“global”) as possible [Dunn et al., 2011b]. The untargeted approach is narrowed somewhat in practice by the selection of the platform for analysis focusing on for instance amines, oxidation products or steroids, and more importantly by the sensitivity of the analytical technology chosen. An overview of a typical metabolomics workflow, regardless of the choice for targeted or untargeted approach, for a biomarker discovery study is depicted in Figure 1. The many considerations that apply to the experimental design and sample collection and storage are beyond the scope of this paper and are not discussed, but we will include an outline of the data
acquisition. The two main analytical technologies routinely applied in metabolomics are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Both NMR and MS can be preceded by a separation step (leading to so-called “hyphenated” methods), such as liquid chromatography (LC) or capillary electrophoresis. Such separation steps allow for the targeting of the analysis to specific groups of compounds in targeted metabolomics approaches, but hyphenated methods are also being applied in global metabolomics [Patti et al., 2012]. The main difference between NMR and MS is the analytical sensitivity (i.e., the detection limit ranges). The analytical sensitivity of NMR ranges between NMR and MS is the analytical sensitivity (i.e., the detection limit ranges). The analytical sensitivity of NMR ranges between \( \mu \text{M} \) and mM. Therefore, for quantification below \( \mu \text{M} \) a more sensitive technique needs to be used. MS sensitivity allows detection of small molecules reaching concentrations in the nM range. NMR and MS have their own relative advantages and disadvantages [Griffin, 2006]. Blow [2008] gave an accessible “technological feature” overview of metabolomics, including a list of commercial metabolomics providers. Many of the commercial options discussed by Blow [2008] are routinely and widely used in modern metabolomics studies. However, additional suppliers (e.g., the Finnish NMR BrainShake platform [http://www.brainshake.fi]) have since been introduced.

In the analysis of metabolomics data, univariate methods fail to differentiate among groups if there is little variance on the single molecule level and the use of multivariate statistical methods is needed to capture not only modification of levels of single metabolites, but also the correlation among the molecules. Such correlations among the molecules are often present, as many metabolites measured on a particular metabolomic platform are biochemically related. A typical problem in the analysis of metabolomics data is the so-called “small N, large p” problem (relatively small number of samples, relatively large number of variables.) This may make the application of multivariate statistical approaches such as MANOVA impossible and multivariate techniques tend to include principal component analysis (PCA), an unsupervised method, used to give an overview of the data and to identify clusters, trends and outliers; and supervised methods for pattern recognition such as partial least squares regression, and discriminant analysis. From a supervised analysis the set of VIPs (important variables on the projection [Wold et al., 2001]), the main metabolites responsible for the separation, can be identified [Barker and Rayens, 2003; Trygg et al., 2007]. These VIPs are important for diagnostic development, but not enough to understand mechanisms. One way to overcome this limitation is using tools that correlate VIPs with the chain of reactions of their metabolic pathways. The challenge is to identify proper routes through the metabolic network that connect metabolites from a large number of routes that can exist between two compounds [Feist et al., 2009]. Here the multiple other metabolites tested, showing a change, but not reaching a status of VIP are of importance.

**Potential Advantages of Metabolomics for Aggression Research**

Metabolomics techniques have the ability for providing increased insight in compounds associating with the traditional biochemical biomarkers and provide the promise of finding better discriminat-
mine which of the fatty acids included in the supplements have a positive influence on the reduction of aggressive behavior.

**Biomarker Discovery With Metabolomics in Psychiatry**

Metabolomics is gaining popularity in the study of psychiatric disorders [Kaddurah-Daouk and Krishnan, 2009]. From February 2004 till November 2015, 58 hits for human “metabolomics” AND “psychiatry” are listed in PubMed. Phenotypes include autism [e.g., West et al., 2014; Wang et al., 2015], bipolar disorder [e.g., Howells et al., 2013; Kurita et al., 2015], addictive disorders [Patkar et al., 2009], major depressive disorder [e.g., Paige et al., 2007; Liu et al., 2015], and schizophrenia [e.g., Liu et al., 2014; Wood and Holderman, 2015]. Two reviews by Sethi and colleagues summarize different “-omics” strategies for biomarker discovery in neuropsychiatric disorders [Sethi and Brietzke, 2015; Sethi et al., 2015]. Here, we focus on examples from adult (schizophrenia) and childhood (autism) psychiatric disorders to illustrate how metabolomics can contribute to the study of (pathological) aggression.

In a metabolomics study of autism, metabolic changes in serum between cases and controls were detected. In a discovery phase of 73 patients and 63 controls, 17 metabolites were identified as potential autism biomarkers. In an independent cohort of 100 cases and 100 controls, 11 of the metabolites were replicated. Multiple logistic regression modeling indicated that sphingosine 1-phosphate and docosahexaenoic acid were potential autism biomarkers [Wang et al., 2015]. Metabolic differences in peripheral blood mononuclear cells from schizophrenia cases and controls were investigated in a training set of 45 patients and 50 controls and 18 metabolites discriminated between cases and controls. Differences in pyroglutamic acid, sorbitol, and tocopherol-α replicated in 24 patients and 35 controls [Liu et al., 2014]. These studies indicate the promise of metabolomics in distinguishing between cases and controls, in both children and adults. This is especially impressive when considering the relatively small sample sizes, as compared with for example genome-wide association studies, which have proven to be far less successful for many psychiatric disorders.

Our literature survey and those by Sethi and colleagues [Sethi et al., 2015; Sethi and Brietzke, 2015] did not identify applications of metabolomics aggression in humans. However, we might learn from studies in other species since in general metabolite profiles are highly similar between species [van der Greef et al., 2006]. A study of honey bees used exposure to isopentyl acetate, which is the active compound in the honey bee “alarm pheromone” to induce aggressive behavior. The aqueous metabolites were extracted from the honey bee brains at 5 or 60 min after exposure. Targeted metabolomics detected 122 metabolites. Honey bees showed an increase in glucose, fructose, and alanine levels after induced aggression and increased aggression was associated with increased glycolysis and decreased oxidative phosphorylation in the brain, that is, aerobic glycolysis (increased glycolysis relative to oxidative phosphorylation despite adequate availability of oxygen; Warburg effect). Neurotransmitter levels were altered with decreases in GABA, taurine, alpha-ketoglutarate, and increases in glutamate and glycine. Also, oxidative stress was observed in aggressive bees, with an increase in nitrotyrosine and a decrease in taurine and glutathione. This study by Chandrasekaran et al. so far is the only metabolomics study of aggression in model organisms, and points to pathways previously observed in human aggression via “classic” techniques (Tables I–III).

**DISCUSSION**

Our review included studies with heterogeneous definitions of aggression, varying sample sizes, and heterogeneous methods and biomarkers are considered. Such diversity across studies limited the conclusions. However, based on examples from autism and schizophrenia metabolomics research, well-identified subgroups for training and validation purposes of around 50–100 cases can provide new leads into biomarkers for psychiatric disorders. The validation of new biomarkers needs to be demonstrated with strong consistent associations between the new marker and the condition of interest, and should include high sensitivity and specificity [Xia et al., 2013]. In the case of aggression, the definition of subgroups requires more attention and this approach may have an iterative character, also defining subgroups definitions based on outcomes of biomarker studies. Our review did not include studies and results in which non-significant associations of biomarker levels with aggression were found. This is a limitation that reduces the possibility to exclude biomarkers a priori, however, when implementing untargeted metabolomics one is not dependent on such a priori assumptions. By collecting the data from multiple studies across several biochemical pathways, we have presented an overview of several biochemical biomarkers in aggression. This provides a basis for several platforms of targeted metabolomics for more in-depth analyses of the exact metabolites involved in each system. This would lead to improved diagnostic approaches, hopefully for well-distinguishable subgroups. Using the observed relation between metabolites and genetic loci, this translation of biochemical biomarkers into metabolites provides a possibility to identify mechanisms and target genes. This can be the basis for leaving a “one fits all” treatment heading to individualized treatment and development of medication for aggression.

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