Supplementary Material

Grouping of children participating in the study according to DSM-5 criteria:

Given the degree of deficit, obstacles disorders can be divided into children with autism disorders with a lighter, moderate or severe deficit in social communication and social interaction.

1) Easier deficit in social communication and social interaction. A child has difficulty in making contact. Inadequately responds to other people's social initiatives and may indicate a reduced interest in interacting with others. Inadequately establishing social relationships, there is a lack of reciprocity in communication, poor integrationverbal and non-verbal communication, and difficulties in adapting behavior to different social circumstances.
2) Moderate Deficit in Social Communication and Social Interaction The child has significant shortcomings in verbal and non-verbal social communication. It is noticeable that the establishment of social relations is limited, inadequate response to the social initiatives of others. The child expresses the disability and shares the interests and emotions with others, inadequately establishes an eye contact. His body language is unusual, shows deficits in understanding and using gestures, and difficulties in participating in a symbolic game.
3) The worse deficit in social communication and social interaction The child has very significant deficits in the area of verbal and non-verbal social communication, causing significant disruption to its functioning. It is very limited in establishing social relations and responding to the social initiatives of others to a minimum. He cannot start or respond to the initiative after social interaction, facial mimics and non-verbal communication are absent, they are not interested in peers.
   A) Lighter deficit in the field of behavior, interests and activities. The child's inflexible / rigid behavior causes deviations in the functioning in one or more areas. A child has difficulty in moving between activities. Organizational and planning issues hinder child's independence.
   B) Moderate deficit in the field of behavior, interests and activity The child's inflexible / rigid behavior causes significant deviations in adapting to changes. The child is over-occupied with interests and / or behaviors that occur so often, that they are also obvious to a random observer and influence the child's activity in several different areas. A child shows hardship if he has to change his or her behavior. Adapt to environmental requirements
   C) A more severe deficit in the field of behavior, interests and activities. The inflexible / rigid behavior of a child causes very important deviations in adapting to change. The child is such a preoccupied interest and / or behavior that it prevents this its operation in several different areas. The child shows a lot of hardship if he has to change his / adapt to environmental requirements.

Table S1. Severity levels for autism spectrum disorder.

Severity level	**Social communication**	**Restricted, repetitive behaviors**
Level 3 "Requiring very substantial support"	Level 2 "Requiring substantial support"	Level 1 "Requiring support"
Severe deficits in verbal and nonverbal social Communication skills cause severe impairments in functioning, very limited initiation of social interactions, and minimal response to social overtures from others.	Marked deficits in verbal and nonverbal social Communication skills; social impairments apparent even with supports in place; limited initiation of social interactions; and reduced or abnormal responses to social overtures from others.	Without supports in place, deficits in social communication cause Noticeable impairments. Difficulty initiating social interactions, and clear examples of atypical or unsuccessful response to social overtures of others. May appear to have decreased interest in social interactions.
For example, a person with few words of intelligible speech who rarely initiates interaction and, when he or she does, makes unusual approaches to meet needs only and responds to only very direct social approaches	For example, a person who speaks simple sentences, whose interaction is limited to narrow special interests, and who has markedly odd nonverbal communication.	For example, a person who is able to speak in full sentences and engages in communication but whose to- and-from conversation with others fails, and whose attempts to make friends are odd and typically unsuccessful.
Inflexibility of behavior, extreme difficulty coping with change, or other restricted/repetitive behaviors markedly interfere with functioning in all spheres. Great distress/difficulty changing focus or action.	Inflexibility of behavior, difficulty coping with change, or other restricted/repetitive behaviors appear frequently enough to be obvious to the casual observer and interfere with functioning in a variety of contexts. Distress and/or difficulty changing focus or action.	Inflexibility of behaviour causes significant interference with functioning in one or more contexts. Difficulty switching between activities. Problems of organization and planning hamper independence

The Spearman's Rank Correlation Coefficient was used to verify if the results correlate to the severity of the disorder. No correlations between the severity of the disorder and the levels of metabolite concentrations were observed.

Table S2. The Spearman rank-order correlation coefficient.

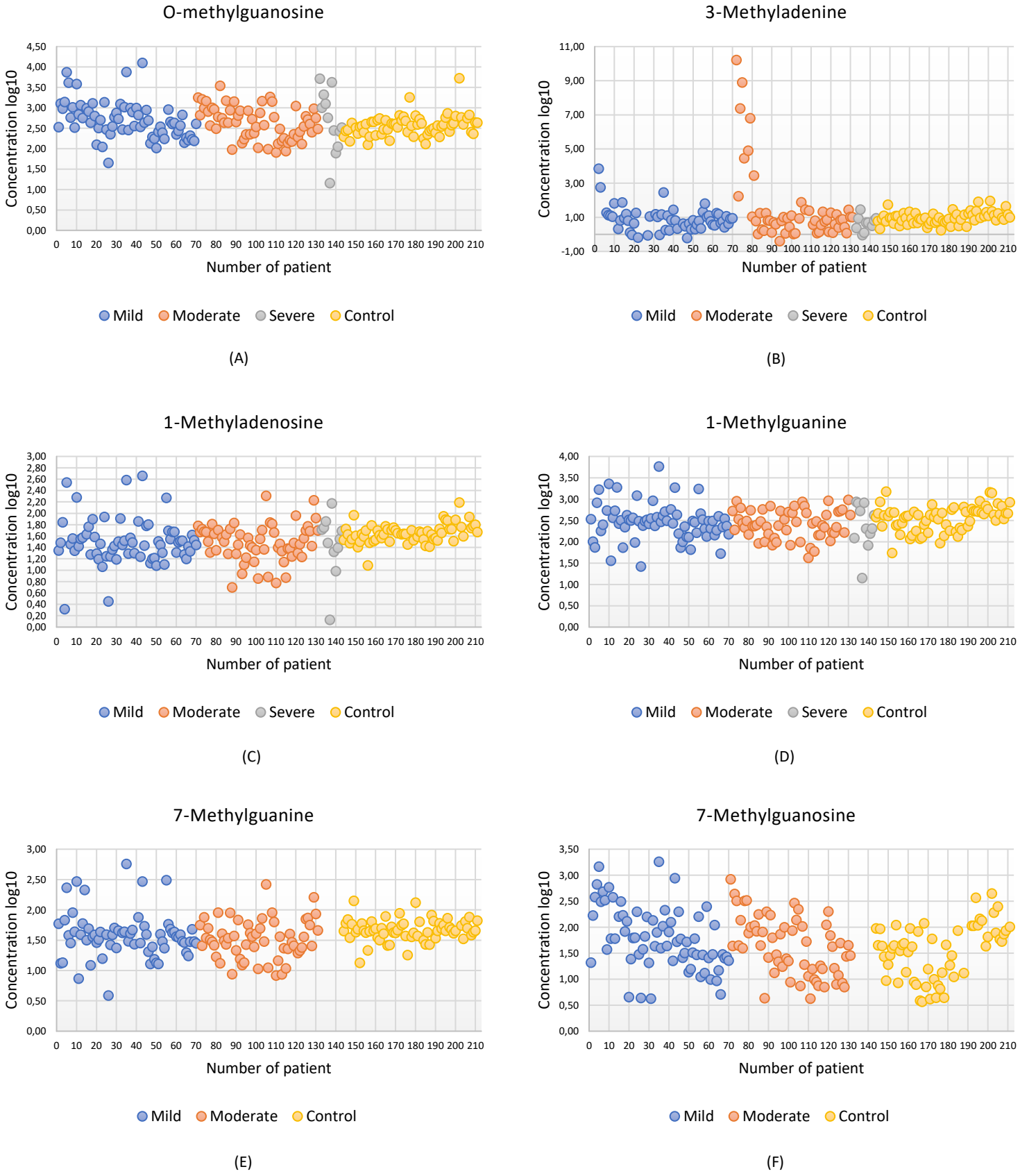
	N	R	t(N-2)	p
Severity of the disorder & O-methylguanosine [ng/mg]	140	-0.03	-0.3377	0.7361
Severity of the disorder & 3-methyladenine [ng/mg]	126	-0.10	-1.0774	0.2834
Severity of the disorder & 1-methylguanine [ng/mg]	136	-0.03	-0.3563	0.7222
Severity of the disorder & 1-methyladenosine [ug/mg]	138	0.06	0.6443	0.5205
Severity of the disorder & 7-methylguanine [ug/mg]	137	-0.02	-0.2763	0.7827
Severity of the disorder & 7-methylguanosine [ng/mg]	140	-0.10	-1.1259	0.2622
Severity of the disorder & 8-hydroxy-2'deoxyguanosine [ng/mg]	118	0.03	0.2945	0.7689

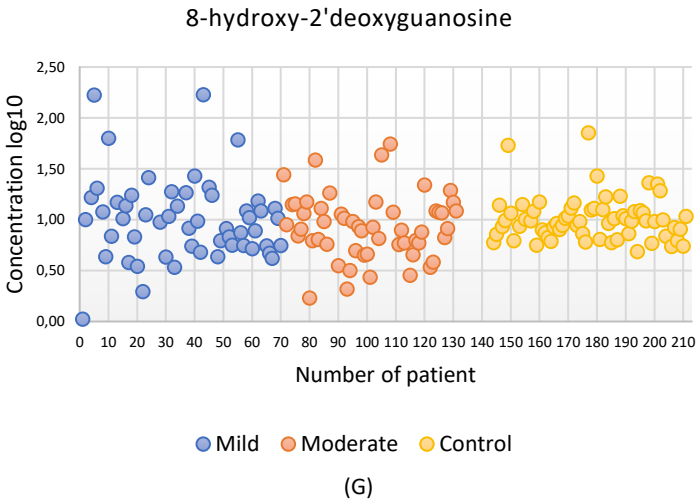
All Spearman rank correlation coefficient values (r), shown in red in the table, the p-value was < 0.001. The p-value ≤ 0.001 was considered to be statistically significant. Nonparametric correlation analysis showed a strong and a moderate positive correlation between the concentration of metabolites determined in ASD urine.

Table S3. The correlation matrix.

	O-methylguanosine [ng/mg]	3-methyladenine [ng/mg]	1-methylguanine [ng/mg]	1-methyladenosine [ug/mg]	7-methylguanine [ug/mg]	7-methylguanosine [ng/mg]	8-hydroxy-2'deoxyguanosine [ng/mg]
O-methylguanosine [ng/mg]	1.00	0.38	0.56	0.79	0.60	0.77	0.73
3-methyladenine [ng/mg]	0.38	1.00	0.46	0.41	0.50	0.23	0.52
1-methylguanine [ng/mg]	0.56	0.46	1.00	0.55	0.89	0.39	0.47
1-methyladenosine [ug/mg]	0.79	0.41	0.55	1.00	0.67	0.57	0.79
7-methylguanine [ug/mg]	0.60	0.50	0.89	0.67	1.00	0.41	0.56
7-methylguanosine [ng/mg]	0.77	0.23	0.39	0.57	0.41	1.00	0.56
8-hydroxy-2'deoxyguanosine [ng/mg]	0.73	0.52	0.47	0.79	0.56	0.56	1.00

Figure S1. 7-panel (A-G), 4-group scatter plot (mild, moderate, severe, and control) for each of the 7 urinary purines measured.




8-hydroxy-2'deoxyguanosine
Concentration log10
2,50
2,00
1,50
1,00
0,50
0,00
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210
Number of patient
Mild
Moderate
Control

(G)

Figure S2. Scatterplots illustrating the relationship between the gender and the concentration of metabolites (A-G) in the urine of ASD patients.

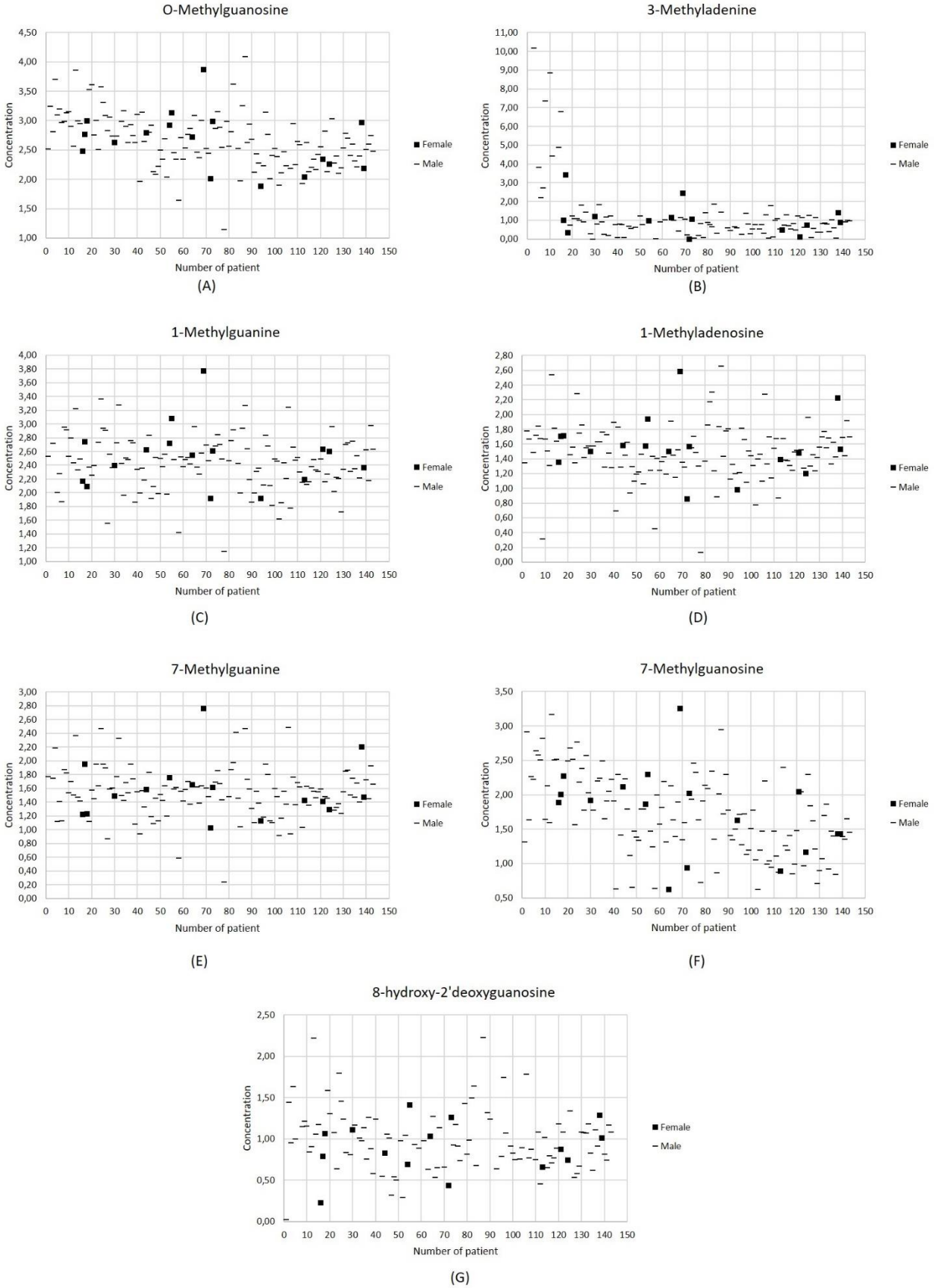


Figure S3. Scatterplots illustrating the relationship between the gender and the concentration of metabolites (A-G) in the urine of control patients.

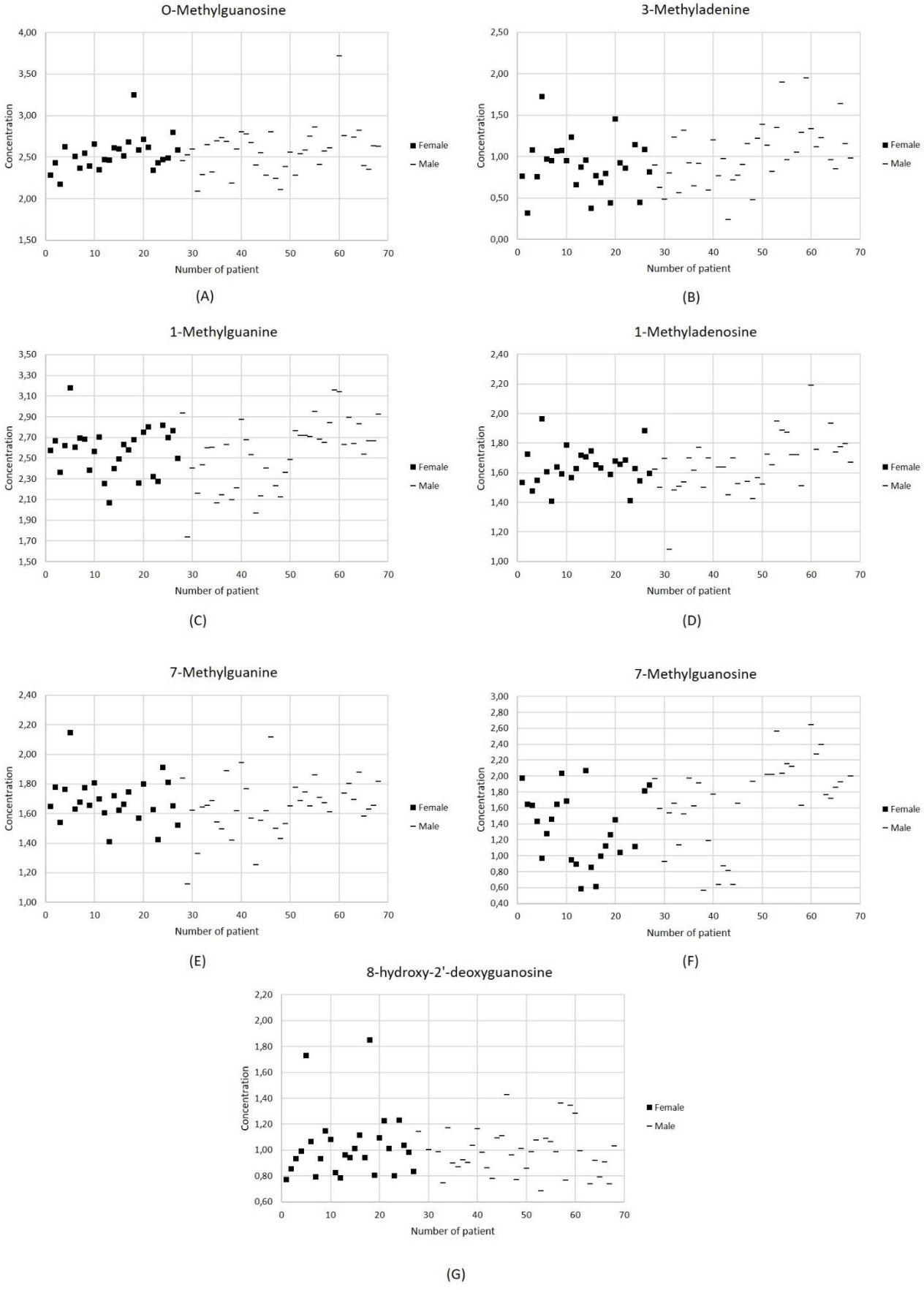


Table S4. Correlation between determined metabolites in urine and age in ASD and control groups.

Compounds	**Group**	**Age**	
		***R* Spearman**	***p*-value**
O-methylguanosine [ng/mg]	ASD	-0.2186	0.0350
	Control	-0.4791	0.0010
3-methyladenine [ng/mg]	ASD	-0.1490	0.1540
	Control	-0.0477	0.7500
1-methylguanine [ng/mg]	ASD	-0.2118	0.0420
	Control	-0.2489	0.0920
1-methyladenosine [ug/mg]	ASD	-0.1717	0.1000
	Control	-0.2282	0.1230
7-methylguanine [ug/mg]	ASD	-0.1893	0.0690
	Control	-0.3047	0.0370
7-methylguanosine [ng/mg]	ASD	-0.1304	0.2130
	Control	0.0338	0.8220
8-hydroxy-2'deoxyguanosine [ng/mg]	ASD	-0.1276	0.2230
	Control	0.1871	0.2080

Figure S4. Scatterplots illustrating the relationship between the age and the concentration of metabolites (A-G) in the urine of ASD patients.

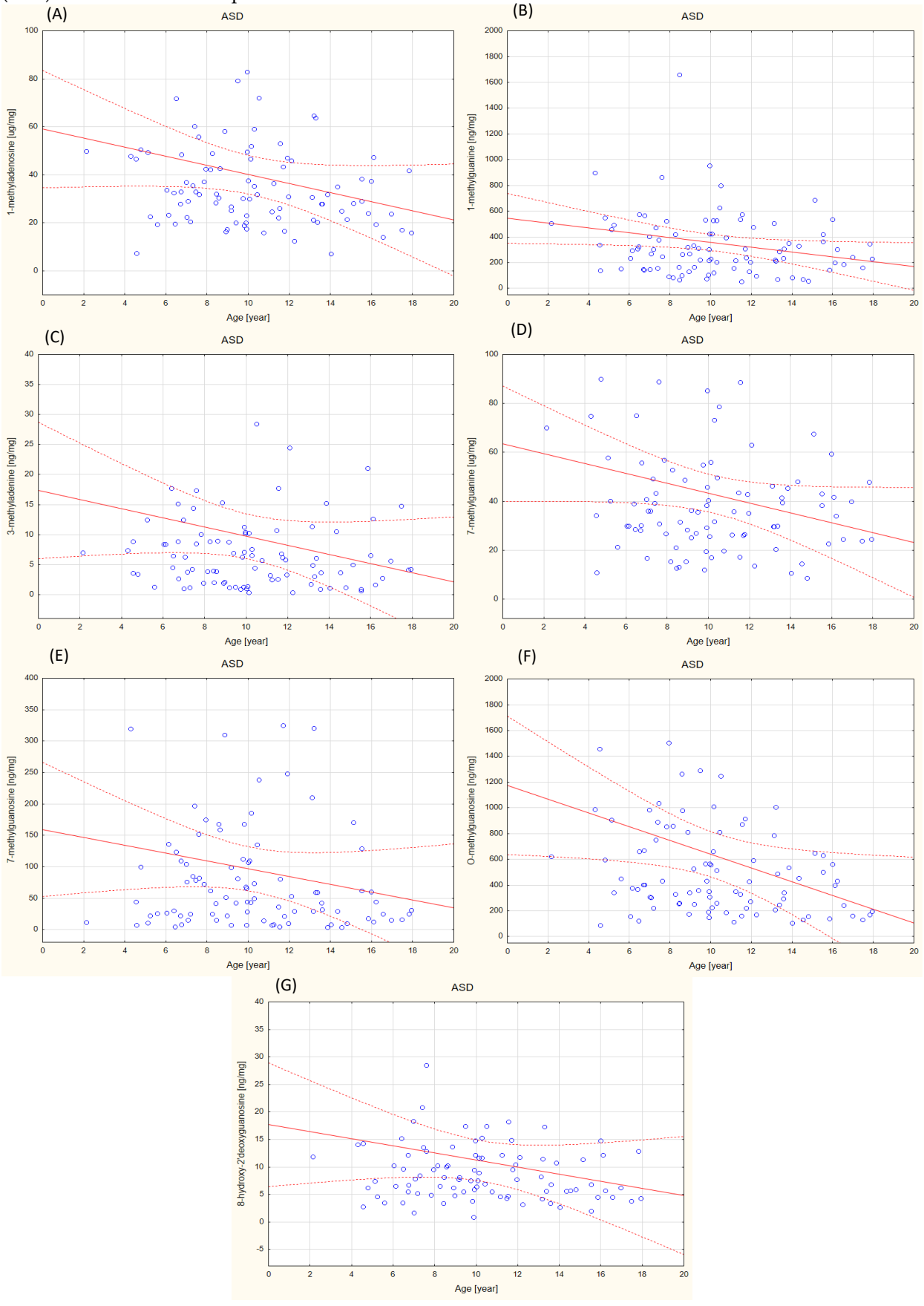


Figure S5. Scatterplots illustrating the relationship between the age and the concentration of metabolites (A-G) in the urine of control group.

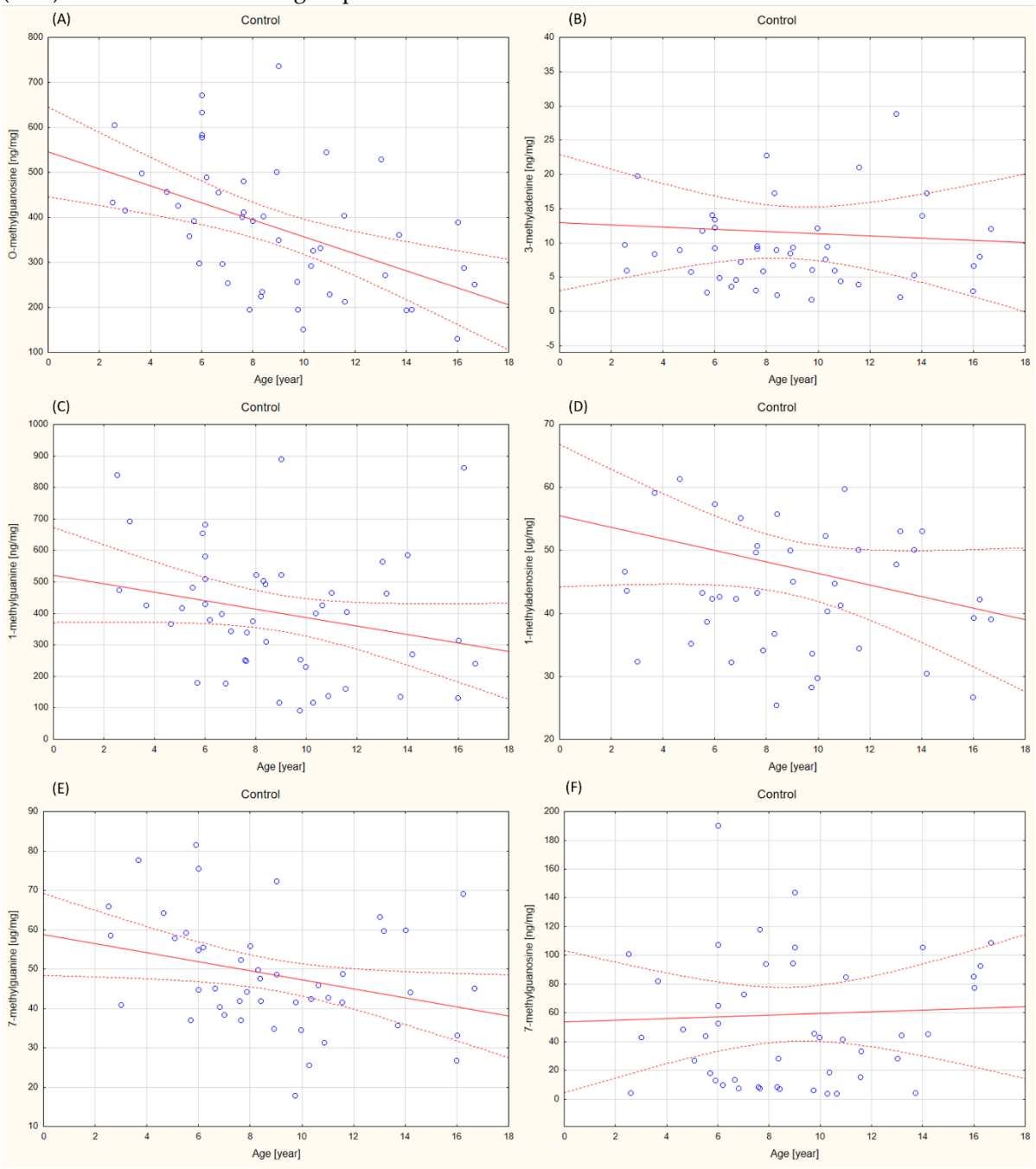


Figure S6. Box and Whisker plots for all determined compounds in group- and gender-categorized. In these box plots, medians inside the 25–75% interquartile range (IQR) are presented.

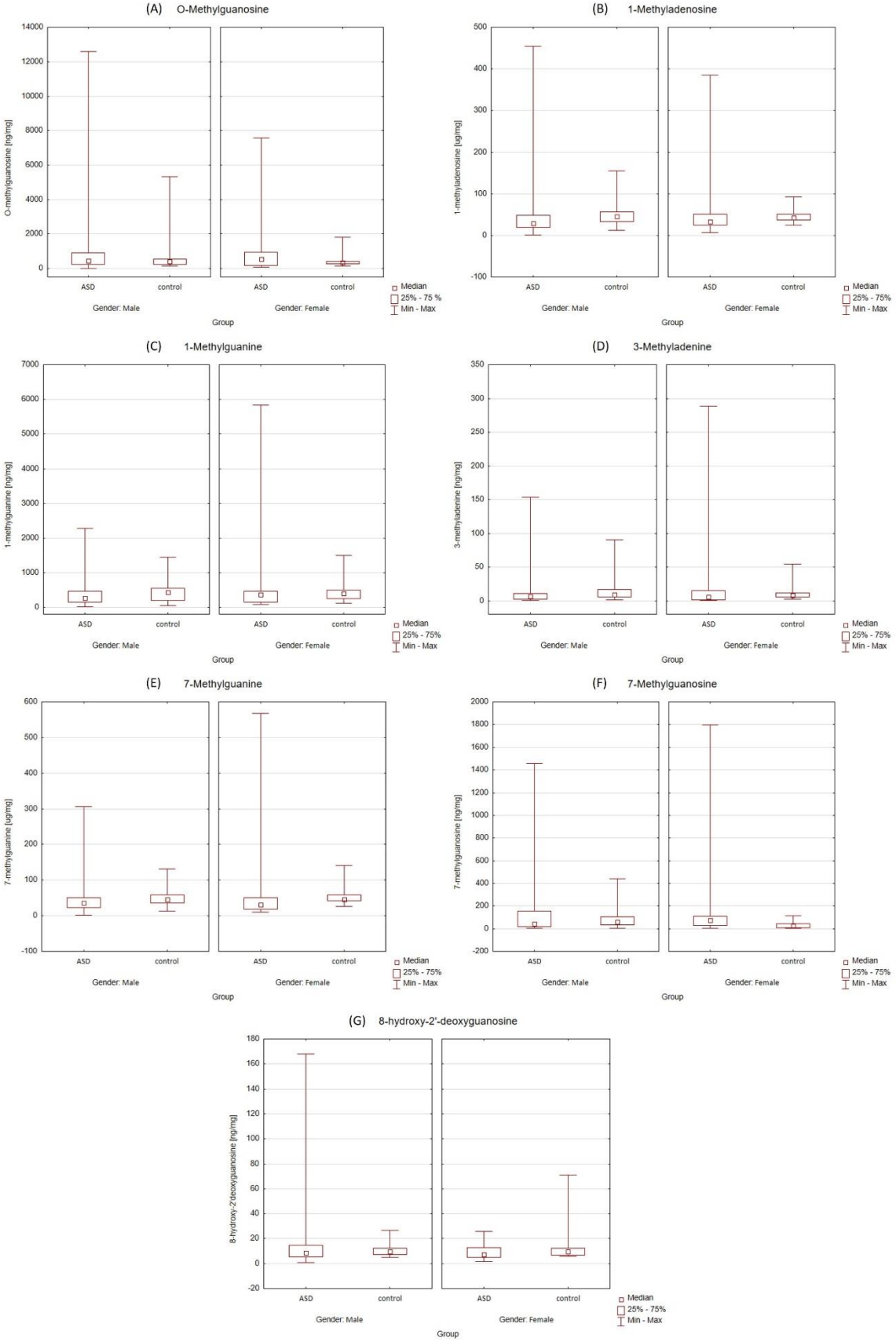
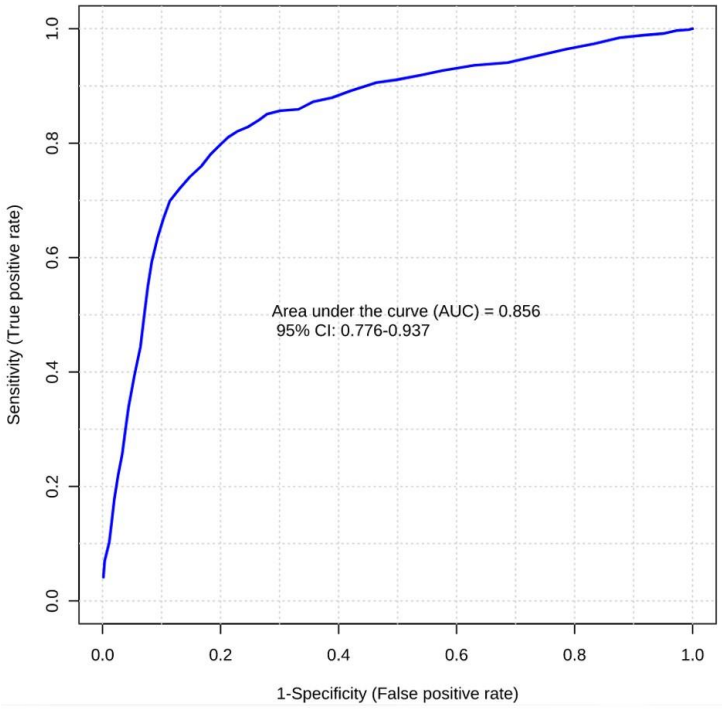
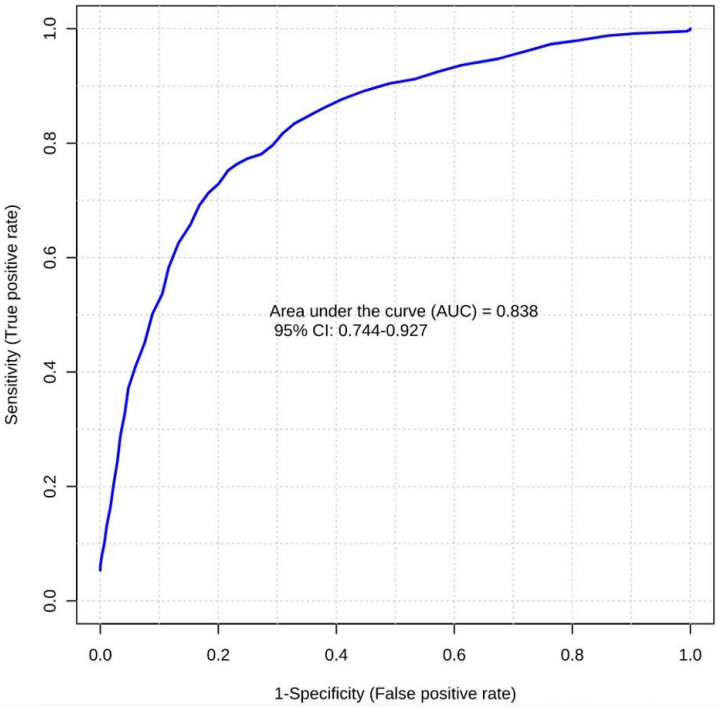


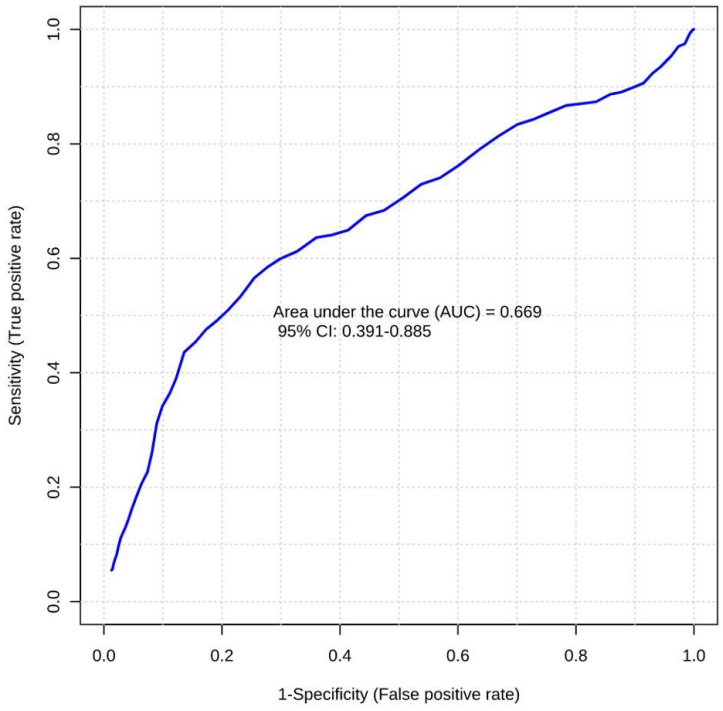
Figure S7. The ROC curves generated in Biomarker Module of Metaboanalyst. (A-C) for mild, moderate and severe severity of the disorder; AUC-area under the ROC curve.



(A)



(B)



(C)