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## **Improving the health of adults with autism and their caregivers**

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## Chapter 3

# Psychological, behavioural, and biological factors associated with gastrointestinal symptoms in autistic adults and adults with autistic traits

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## Abstract

### Background

Gastrointestinal (GI) symptoms and their relation to physical and mental aspects in adults with an autism spectrum disorder (ASD) are poorly understood, despite their high prevalence. Therefore, the aim of this study is to examine psychological, behavioural, and biological factors associated with GI-symptoms in adults with ASD(-traits).

### Methods

We included 31,185 adults from the Lifelines Study. Using multivariable logistic regression, we analysed the association between GI-symptoms and psychological, behavioural (questionnaire-assessed), and physically measured biological factors in adults with ASD (n=309), without ASD (n=30,876), and in the quartiles with highest (n=7783) and lowest (n=7783) Autism Spectrum Quotient-10 (AQ-10) sum-scores.

### Results

In the ASD-group, GI-symptoms were associated with psychiatric comorbidity (odds ratio (OR) 2.71, 95% confidence interval (CI) 1.51-4.85), more stress (OR 1.15, 95% CI 1.06-1.26), and worse perceived health (OR 2.32, 95% CI 1.62-3.34). In the quartile with highest AQ-10 sum-scores, GI-symptoms were also associated with these psychological factors, and with less physical activity (OR 0.95, 95% CI 0.92-0.98).

### Conclusion

Our study demonstrates that not only adults with ASD but also adults with ASD-traits are at increased risk for GI-symptoms, which is associated with psychological and behavioural factors. This suggests that an integrated psychosomatic approach of GI-symptoms in adults with ASD(-traits) is needed.

## Lay abstract

### Background

Little is known about factors related to the increased risk for gastrointestinal (GI) symptoms in adults with an autism spectrum disorder (ASD), while the negative impact of GI-symptoms is evident. Especially, the relationship between GI-symptoms and psychological, behavioural, and biological risk factors in adults with ASD(-traits) is unclear. Autistic peer support workers and autism-advocates also emphasized the importance of identifying risk factors, because of the high prevalence of GI-problems in people with ASD. Therefore, our study investigated which psychological, behavioural, and biological factors are associated with GI-symptoms in adults with ASD or with ASD-traits.

### Methods

We analysed data from 31,185 adults in the Dutch Lifelines Study. Questionnaires were used to evaluate the presence of an ASD-diagnosis, autistic traits, GI-symptoms, psychological, and behavioural factors. Biological factors were examined with body measurements.

### Results

We found that not only adults with ASD, but also adults with higher levels of autistic traits were at increased risk for GI-symptoms. Adults with ASD who experienced psychological problems (psychiatric problems, worse perceived health, chronic stress) had a higher risk for GI-symptoms than adults with ASD without these psychological problems. Moreover, adults with higher levels of autistic traits were less physically active, which was also associated with having GI-symptoms.

### Conclusion

In conclusion, our study highlights the relevance of identifying psychological problems and evaluating physical activity when trying to help adults with ASD or autistic traits and GI-symptoms. This suggests that healthcare professionals should be more aware of behavioural and psychological risk factors when evaluating GI-symptoms in adults with ASD(-traits).

## Introduction

Gastrointestinal (GI) problems are more prevalent in people with an autism spectrum disorder (ASD) than in the general population (McElhanon et al., 2014; Croen et al., 2015; Hand et al., 2020). However, previous studies concerning GI-symptoms mainly included children with ASD (Holingue et al., 2018; Lefter et al., 2019; McElhanon et al., 2014). Consequently, in-depth knowledge about GI-symptoms in autistic adults is lacking. A case-control study reported a prevalence of 35% GI-disorders in adults with ASD (mean age 29 years old) compared to 28% in adults without ASD (Croen et al., 2015). In older adults with ASD (65+ years old), GI-disorders seem to be more common than in non-autistic adults (49% versus 25%), as are other GI-conditions such as gastroenteritis or constipation (Hand et al., 2020). It is also known that the risk for GI-disorders is increased in adults with both ASD and intellectual disability (Bishop-Fitzpatrick and Rubenstein, 2019; Gilmore et al., 2021). However, to our knowledge, there are no previous studies investigating other factors (e.g., psychological, behavioural and biological) associated with GI-symptoms in adults with ASD. Thus, comprehensive knowledge on GI-symptoms in autistic adults is limited, while a better understanding of their increased risk for GI-problems is needed (Leader et al., 2021). In this study, we investigated the presence of GI-symptoms, rather than diagnosed GI-disorders, because of the high prevalence of functional GI-problems in a broader sense in adults with ASD (Bishop-Fitzpatrick and Rubenstein, 2019; Croen et al., 2015).

When investigating factors associated with GI-symptoms, psychological factors such as stress, anxiety, and depression, should be taken into account. Stress is a mediator in GI-symptoms, as stress influences both gut function and microbiota composition through the hypothalamic-pituitary-adrenal (HPA) axis, and reduces gut motility via the noradrenergic system (Cacioppo et al., 2007; De Palma et al., 2014). The individual's immune status is also closely intertwined with HPA axis activity, personal stress levels, the gastrointestinal condition and microbiota (Hollins and Hodgson, 2019). Relevant immunological markers that are altered by stress are for example C-reactive protein (CRP) and leukocyte counts (Del Giudice et al., 2018; Dijkstra-de Neijis et al., 2020). In addition, in children with ASD, a positive association between the concentration of the stress hormone cortisol and lower GI-tract symptoms was found (Ferguson et al., 2016). Moreover, stress is related to psychiatric symptoms, such as depression and anxiety (Tafet and Nemeroff, 2015). People with ASD often experience high levels of stress (Baron et al., 2006; Bishop-Fitzpatrick et al., 2015; Hirvikoski et al., 2015) and psychiatric comorbidities are very common (Lai et al., 2014; Hand et al., 2020). Also, anxiety has been associated with GI-problems in children with ASD (Ferguson et al., 2017).

Furthermore, behavioural factors, such as physical (in)activity and alcohol abuse, can have an impact on GI-symptoms through the gut-brain axis (Bilski et al., 2018; Chen and Haber, 2021). It is also known that children with ASD are more susceptible for food selectivity and insufficient oral intake, which can affect the intestinal microbiome (Bandini et al., 2010; Murtaza et al., 2017).

Next to the above-mentioned immunological markers that can be altered in relation to stress and GI-symptoms, other biological factors that are of concern when investigating GI-symptoms are body mass index (BMI) and waist circumference (to measure abdominal obesity). Increased BMI and abdominal obesity are associated with GI-problems, such as irritable bowel syndrome (IBS) and gastroesophageal reflux (Emerenziani et al., 2019; Foxx-Orenstein, 2010; Lee et al., 2015).

In summary, psychological, behavioural, and biological factors affecting the gut-brain axis should be taken into account when researching GI-symptoms in autistic adults.

While adults with ASD tend to have more GI-symptoms than the general population, an integrated approach analysing psychological, behavioural, and biological factors associated with GI-symptoms in autistic adults is missing. A better understanding of these factors and their putative associations in adults with ASD is relevant to optimise prevention and treatment of these GI-symptoms. The underlying theory entails that autistic traits and related factors, such as stress, depression, anxiety, sensory dysregulation, and food selectivity contribute to GI-symptoms (Amos et al., 2019; Harris et al., 2021; Mazurek et al., 2013; Williams and Gotham, 2021). Therefore, the main goal of this study is to use an integrated approach to explore which psychological, behavioural, and biological factors are associated with GI-symptoms in adults with ASD and in adults with autistic traits. In addition, our study leads to more insights regarding psychological, behavioural, and biological characteristics in both adults with ASD and in the general adult population with autistic traits.

## Methods

### Study population

All data were extracted from the Lifelines database. Lifelines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviours of 167,729 persons living in the North of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, biological and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity

and complex genetics (Scholtens et al., 2015). Baseline assessment took place between 2007 and 2013, and the second assessment was performed from 2014 until 2017. Subsequently, in 2019 an autism questionnaire (AUTQ) was sent to 109,352 Lifelines participants. The AUTQ consisted of the short version of the Autism Spectrum Quotient (AQ-10) and a self-report question regarding the presence of an ASD-diagnosis. Thus, to be eligible for this study, participants had to be able to submit self-report surveys.

We included 31,185 participants (Figure 1), aged 18 years or older at the start of the second Lifelines assessment, who reported the presence or absence of an ASD-diagnosis, and provided any answer to one or more questions regarding GI-symptoms in the respective questionnaire (see below). These 31,185 included participants were first divided into two groups: ASD (n=309) and non-ASD (n=30,876). Next, for formation of groups based on AQ-10 sum-scores, cases with an incomplete AQ-10 were excluded from the whole included population (Figure 1). The remaining participants were categorized in quartiles, based on their AQ-10 sum-scores. The highest quartile (HQ-traits-group: n=7783), consisting of 25% of participants with the highest AQ-10 sum-scores, and the lowest quartile (LQ-traits-group: n=7783), consisting of 25% of participants with the lowest AQ-10 sum-scores, were used for analyses.

## Measures

### *Autistic traits*

The AQ-10 is an instrument to quantify the degree of autistic traits in adults with average intelligence (Allison et al., 2012). It should not be used to predict an ASD-diagnosis, since studies found varying sensitivity- and specificity-values of 62-80% and 28-87%, respectively (Sizoo et al., 2015; Booth et al., 2013; Ashwood et al., 2016). However, the AQ-10 is indeed a valid measure in epidemiological studies to grade the level of autistic symptoms in adult participants (Lundin et al., 2019; Warrier et al., 2020).

### *Gastrointestinal symptoms*

The Rome III IBS Diagnostic Questionnaire addresses different variables concerning GI-symptoms (Thompson et al., 2006). In our study, we defined GI-symptoms as the self-report of one or more of the following complaints: abdominal discomfort/pain, diarrhoea, constipation, and/or heartburn. The presence of each symptom was rated on a 5- or 7-point Likert-scale. Cut-off points (described in Figure 2) were based on clinical relevance and definitions of functional bowel disorders (Longstreth et al., 2006; Palsson et al., 2016). As supplementary analysis, the prevalence of GI-diseases (Crohn's disease, ulcerative colitis, coeliac disease, gastric ulcer, and/or gallstones) was compared between adults with and without ASD.



### *Psychological factors*

Psychiatric comorbidity was defined as the presence of one or more of the following diagnoses: any current depressive disorder (major depressive disorder, dysthymia) and/or any current anxiety disorder (panic disorder, agoraphobia, social phobia, generalized anxiety disorder), as determined in a face-to-face Mini International Neuropsychiatric Interview (MINI; based on the DSM-IV-TR). Another psychological measure included self-reported stress, using the Long-term Difficulties Inventory (LDI). LDI sum-scores were calculated to compare stress levels between groups. We assessed perceived health with a RAND-question about general health as a continuous variable, asking participants to answer on a 5-point Likert scale: ‘How would you rate your health generally speaking?’

### *Behavioural factors*

Physical activity was measured using the following question from the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH): ‘Adding everything up, on how many days per week on average are you involved in cycling, doing odd jobs, gardening, sport, or other strenuous activities for at least 30 minutes?’. Alcohol use was assessed with this question from the Flower Food Frequency questionnaire (FFQ): ‘During the past month, how many glasses of alcoholic drinks did you drink per day on average?’. For this variable, the cut-off for binary analyses was set at an average alcohol intake of more than two glasses per day (Wouters et al., 2020; Rausch et al., 2022).

### *Biological factors*

Waist circumference and height and weight (for calculation of BMI) were measured by trained Lifelines’ personnel during a physical visit of the second assessment. In addition to the level of psychological stress as determined by the LDI, we evaluated counts of different types of leukocytes and CRP levels as measures of inflammation and therefore indirect physical components of perceived stress (Del Giudice et al., 2018; Dijkstra-de Neijs et al., 2020). Blood samples were also drawn during a physical visit of the second assessment. CRP levels were processed using a nephelometric assay in a Roche Modular analyser (Roche, Basel, Switzerland).

### *Covariates*

Socioeconomic status was determined with self-reported employment status and educational attainment. Employment status was a dichotomous question on whether the participant, at that moment, did paid work for one or more hours per week. Educational attainment was categorized as low (no education, primary, lower or preparatory vocational education, or lower general secondary education), middle (intermediate vocational education or apprenticeship, higher

general secondary education, or pre-university secondary education), high (higher vocational education or university), or other.

### *Autistic community involvement*

The research team was advised by members of the Dutch Academic Workplace for Autism' (including autistic adults and clinicians) about the research question, selection of relevant variables, and interpretation of the results.

### **Statistical analyses**

We used IBM SPSS Statistics version 25 for all data analyses. Before we performed our main analyses, we executed non-response analyses. The goal of the first non-response analysis was to map characteristics of the 71,428 Lifelines' participants that did receive the AUTQ, but did not submit this questionnaire (and could therefore not be included in our study). The second non-response analysis was performed to compare the total included study population (N=31,185) with the participants that were excluded because of an incomplete GI-questionnaire (n=6726).

Then, we first analysed basic characteristics with univariable analyses comparing the ASD- versus non-ASD-group, and the HQ-traits- versus LQ-traits-group. After that, psychological, behavioural, and biological factors were also compared for the ASD- versus non-ASD-group and the HQ-traits- versus LQ-traits-group, with univariable and multivariable analyses. In univariable analyses, categorical variables were analysed with Chi-square tests or Fisher's exact tests, and continuous variables with Student *t* tests or Mann-Whitney U tests. P-values below 0.05 were considered statistically significant. Correction for age, sex, and socioeconomic status was carried out with multivariable logistic regression for categorical variables and multivariable linear regression for continuous variables.

Next, multivariable logistic regression with the presence of (one or more) GI-symptoms as outcome measure was performed. These logistic regression models were executed for each of the included psychological behavioural, and biological factors in the ASD-, non-ASD-, HQ-traits-, and LQ-traits-group. Age, sex, and socioeconomic status were included as confounders in multiple logistic regression (Bytzer et al., 2001; Knutsson et al., 2010). Transformation of skewed data was not indicated, because the assumptions of logistic regression were met based on the nature of the distributions. In these logistic regression models, exclusion of participants with ASD from the HQ-traits-group did not result in different outcomes. Therefore, these participants were not excluded from the HQ-traits-group.

Primary interaction term analysis was executed in the total included study population (N=31,185), to identify the significance of the interaction effect of an ASD-diagnosis for each investigated psychosocial, behavioural, and biological

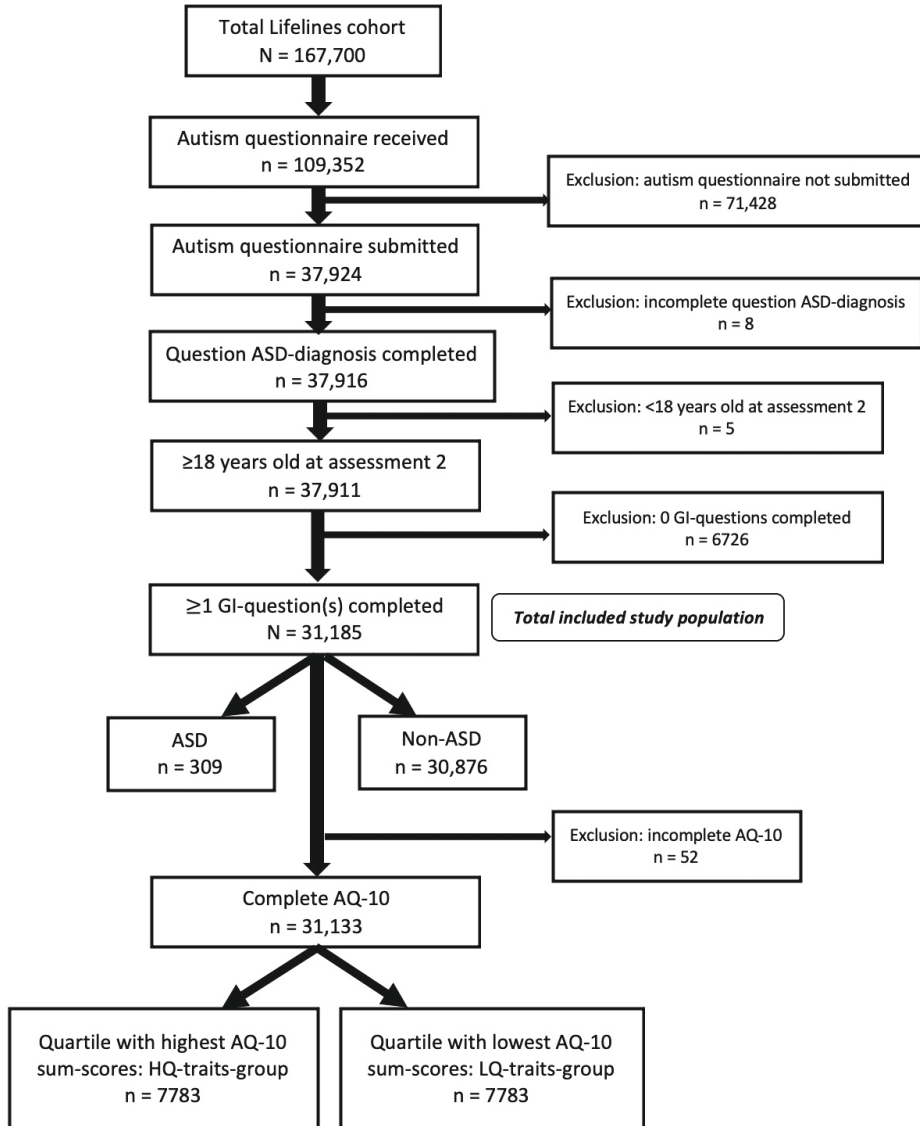
factor with GI-symptoms as dependent variable. Secondary interaction analysis was performed in the combined HQ-traits- and LQ-traits-group (n=15,566), to identify the significance of the interaction effect of reporting the highest levels of autistic traits (HQ-traits-group).

Imputation of missing data was not needed, because prevalence of missing data did not differ between ASD and the control group, and the origin of these missing data was regarded as random. CRP levels were not analysed in the ASD-group, since these data were only available for 3 participants in this group. This was due to the fact that from the total Lifelines cohort (N=167,700), only in approximately 500 participants CRP levels were determined during the second assessment.

### **Ethical approval**

Ethical approval for the Lifelines study (reference number NL17981.042.07) was obtained from the medical ethical committee of the University Medical Center Groningen, The Netherlands.

Figure 1. Flow diagram of study population



**Figure 2.** Definition of gastrointestinal (GI) symptoms

GI-symptoms were defined as the presence of 1 or more of these 4 symptoms:			
Abdominal discomfort:	Diarrhea	Constipation	Heartburn
<ul style="list-style-type: none"> <li>• <math>\geq 1</math> day per week abdominal pain or unpleasant feeling in abdomen in past 3 months.</li> <li>• for women, exclusion if: this unpleasant feeling in belly was only during menstruation and not at other times</li> </ul>	<ul style="list-style-type: none"> <li>• often thin, loose, or watery stools in past 3 months</li> </ul>	<ul style="list-style-type: none"> <li>• <math>\geq 2</math> of the following in past 3 months:               <ul style="list-style-type: none"> <li>• straining during <math>\geq 25\%</math> of defecations</li> <li>• lumpy/hard stools in <math>\geq 25\%</math> of defecations</li> <li>• sensation of incomplete evacuation in <math>\geq 25\%</math> of defecations</li> <li>• sensation of anorectal obstruction/blockage in <math>\geq 25\%</math> of defecations</li> <li>• manual maneuvers to facilitate <math>\geq 25\%</math> of defecations</li> </ul> </li> <li>• <math>\leq 2</math> defecations per week</li> </ul>	<ul style="list-style-type: none"> <li>• <math>\geq 1</math> day per week pain or burning feeling in center of abdomen (above belly button, but not in the chest) in past 3 months</li> </ul>

## Results

### Basic characteristics

In the total study population of 31,185 adults, 1% (n=309) reported an ASD-diagnosis (Table 1). Of these 309 adults with ASD, 86.4% (n=267) was categorized in the HQ-traits-group (i.e., group with highest AQ-10 sum-scores). Moreover, in adults with ASD, the median AQ-10 sum-score of 6.0 (interquartile range (IQR) 4.5-7.5) was 4.0 points higher than in adults without reported ASD ( $p<0.01$ ). Socioeconomic status was lower in adults with ASD compared with adults without ASD, and in the HQ-traits-group compared with the LQ-traits-group.

The first non-response analysis revealed that the 71,428 participants who were not included because they did not submit the AUTQ, were younger in age and consisted of more men compared to the 37,924 participants who submitted the AUTQ. The second non-response analysis showed that the 6726 participants who were excluded because they did not answer any of the questions in the GI-questionnaire, more frequently had an ASD-diagnosis (n=128; 1.9%) compared to the total included study population of 31,185 participants (n=309; 1.0%). Nonetheless, the median AQ-10 sum-score in the excluded 6726 participants was not different from the median AQ-10 sum-score in the included study population.

### Gastrointestinal symptoms

The prevalence of GI-symptoms (abdominal discomfort/pain, diarrhoea, constipation, and/or heartburn) was significantly higher in adults with ASD compared with adults without ASD (36% versus 21%,  $p<0.01$ ) (Table 1). This tendency was also observed in the quartiles: 25% in the HQ-traits-group compared with 19% in the LQ-traits-group ( $p<0.01$ ). In adults with ASD, three out of four investigated GI-symptoms were more frequently reported ( $p$ -values  $< 0.01$ ). Subsequently, in the HQ-traits-group all four investigated GI-symptoms were more frequent, compared with the LQ-traits-group ( $p$ -values  $< 0.01$ ). In addition, GI-diseases (Crohn's disease, ulcerative colitis, coeliac disease, gastric ulcer, and/or gallstones) were not more prevalent in adults with ASD compared with adults without ASD.

### Psychological, behavioural and biological factors

In Table 2A (ASD- versus non-ASD-group) and in Table 2B (HQ-traits- versus LQ-traits-group) the outcomes of differences within groups (based on univariable and multivariable analyses) regarding psychological, behavioural, and biological factors are presented.

Psychiatric comorbidity (anxiety and/or depression) was more common in the ASD-group (OR 5.29, 95% CI 3.94-7.10). Adults with ASD reported higher stress

levels (OR 1.22, 95% CI 1.18-1.27) and worse perceived health (OR 1.71, 95% CI 1.47-2.00; as a higher score resembles worse perceived health), compared with adults without ASD. When comparing the HQ-traits and LQ-traits-group, the same differences were observed: psychiatric comorbidity was more prevalent in the HQ-traits-group (OR 3.27, 95% CI 2.83-3.79), stress levels were higher (OR 1.15, 95% CI 1.13-1.17), and perceived health was worse (OR 1.48, 95% CI 1.41-1.55).

Regarding behavioural factors, the level of physical activity and prevalence of alcohol use of more than two glasses per day in adults with ASD did not differ from adults without ASD. Multivariable analyses, correcting for differences in age, sex and socioeconomic status, also revealed no difference in prevalence of alcohol use between the HQ-traits- and LQ-traits-group. These analyses did demonstrate that adults in the HQ-traits-group were less physically active (OR 0.95, 95% CI 0.93-0.97) than adults in the LQ-traits-group.

Multivariable analyses of biological measurements showed that mean BMI and waist circumference were not different between both the ASD- and non-ASD-group and the HQ-traits- and LQ-traits-group. Similar approaches of immune parameters revealed no differences between the ASD- and non-ASD-group and the HQ-traits- and LQ-traits-group regarding the total leukocyte and leukocyte-subset counts as well as CRP levels.

### Factors associated with gastrointestinal symptoms

Assessing GI-symptoms in the distinct groups and associated factors (Table 3), we observed that GI-symptoms were associated with all investigated psychological variables (psychiatric comorbidity, stress, and perceived health) in the ASD-, non-ASD-, HQ-traits-, and LQ-traits-group. In the ASD-group, GI-symptoms were not associated with behavioural variables (alcohol use and physical activity). In the HQ-traits-group, being less physically active was associated with GI-symptoms (OR 0.95, 95% CI 0.92-0.98). No association was found between alcohol use and GI-symptoms in the HQ-traits-group. Most biological variables (BMI, waist circumference, eosinophils, monocytes, and basophils) were not associated with GI-symptoms in any of the investigated groups. However, in the HQ-traits-group as well as in the non-ASD-group, GI-symptoms were positively associated with total leukocyte, lymphocyte, and neutrophil counts.

Interaction term analyses demonstrated that there were no interactions between the variable 'ASD or non-ASD' and the included psychological, behavioural, and biological factors. The only significant interaction was between the variable 'HQ-traits- or LQ-traits-group' and lymphocyte counts (OR 1.16, 95% CI 1.01-1.33).

**Table 1.** Basic characteristics: ASD- versus non-ASD-group and HQ-traits- versus LQ-traits-group

	ASD n = 309		non-ASD n = 30,876		p-value <sup>a</sup>	HQ-traits- group <sup>b</sup> n = 7783		LQ-traits- group <sup>c</sup> n = 7783		p-value <sup>a</sup>
Age, years (mean, SD)	41.2	(12.0)	50.7	(12.1)	<0.01	50.9	(12.7)	49.8	(11.4)	<0.01
Female sex (N, %)	124	(40.1)	18,089	(58.6)	<0.01	3534	(45.4)	5385	(69.2)	<0.01
ASD diagnosis (N, %)	309	(100)	0			267	(3.4)	2	(0.0)	<0.01
AQ-10 sum score <sup>d</sup> (median, IQR)	6.0	(4.5-7.5)	2.0	(0.5-3.5)	<0.01	5.0	(4.0-6.0)	1.0	(0.0-1.0)	<0.01
Ethnicity (N, %)										
Eastern or Western European	275	(89.0)	29,047	(94.1)	N.S.	7254	(93.2)	7392	(95.0)	0.03
Mediterranean, Arabic, Black or Asian	0		175	(0.6)		33	(0.4)	38	(0.5)	
Other	<10	(<3.2)	174	(0.6)		61	(0.8)	29	(0.4)	
Educational attainment (N, %)										
Low	47	(15.2)	6242	(20.2)	0.03	2025	(26.0)	1009	(13.0)	<0.01
Middle	120	(38.8)	9775	(31.7)		2578	(33.1)	2334	(30.0)	
High	90	(29.1)	10,012	(32.4)		1978	(25.4)	3202	(41.1)	
Employment (N, %)	191	(61.8)	21,502	(69.6)	0.01	5085	(65.3)	5853	(75.2)	<0.01
Gastrointestinal symptoms <sup>e</sup> (N, %)	112	(36.2)	6446	(20.9)	<0.01	1919	(24.7)	1467	(18.8)	<0.01
Constipation	56	(18.1)	2491	(8.1)	<0.01	760	(9.8)	560	(7.2)	<0.01
Diarrhoea	33	(10.7)	2067	(6.7)	<0.01	629	(8.1)	484	(6.2)	<0.01
Heartburn	15	(4.9)	963	(3.1)	N.S.	292	(3.8)	211	(2.7)	<0.01
Abdominal pain/ discomfort	53	(17.2)	3151	(10.2)	<0.01	936	(12.0)	687	(8.8)	<0.01

a. Univariable analysis with Chi-square test, independent sample t-test or Mann-Whitney U test. b. HQ-traits-group = 25% of participants with the highest Autism Quotient (AQ-10) sum-scores. c. LQ-traits-group = 25% of participants with the lowest AQ-10 sum-scores. d. AQ-10 = short version of the Autism Spectrum Quotient. e. Gastrointestinal symptoms = presence of 1 or more of these symptoms: constipation, diarrhoea, heartburn, abdominal pain/discomfort.



**Table 2A.** Psychological, behavioural and biological factors in ASD- versus non-ASD-group

	ASD n = 309		non-ASD n = 30,876		p-value <sup>a</sup>	Adjusted OR (95% CI) <sup>b</sup>
<b>Psychological</b>						
Psychiatric comorbidity <sup>c</sup> (N, %)	88	(28.5)	2042	(6.6)	<0.01	5.29 (3.94-7.10)
Anxiety	75	(24.3)	1735	(5.6)	<0.01	4.98 (3.67-6.76)
Depression	49	(15.9)	812	(2.6)	<0.01	5.50 (3.81-7.92)
Stress (median, IQR) <sup>d</sup>	3	(2-6)	1	(0-3)	<0.01	1.22 (1.18-1.27)
Perceived health (mean, SD) <sup>e</sup>	2.9	(0.9)	2.6	(0.8)	<0.01	1.71 (1.47-2.00)
<b>Behavioural</b>						
Alcohol use, >2 glasses/day (N, %)	67	(21.7)	7638	(24.7)	N.S.	0.57 (0.41-0.80)
Physical activity, days/week (mean, SD)	4.3	(2.0)	4.4	(1.9)	N.S.	N.S.
<b>Biological</b>						
Body mass index (mean, SD)	25.8	(4.9)	25.9	(4.2)	N.S.	N.S.
Waist circumference (cm) (mean, SD)	90.4	(14.2)	90.0	(12.4)	N.S.	N.S.
Leukocytes (10 <sup>9</sup> /L) (median, IQR)	6.00	(5.10-6.90)	5.80	(4.90-6.80)	<0.05	N.S.
Neutrophils (10 <sup>9</sup> /L) (median, IQR)	3.09	(2.60-3.84)	3.06	(2.27-3.80)	N.S.	N.S.
Lymphocytes (10 <sup>9</sup> /L) (median, IQR)	1.97	(1.59-2.36)	1.90	(1.57-2.31)	N.S.	N.S.
Monocytes (10 <sup>9</sup> /L) (median, IQR)	0.49	(0.42-0.60)	0.48	(0.39-0.58)	<0.05	N.S.
Eosinophils (10 <sup>9</sup> /L) (median, IQR)	0.16	(0.09-0.24)	0.16	(0.11-0.24)	N.S.	N.S.
Basophils (10 <sup>9</sup> /L) (median, IQR)	0.04	(0.03-0.06)	0.04	(0.03-0.06)	N.S.	N.S.

a. Univariable analysis with Chi-square test, Fisher's exact test, independent sample t-test or Mann-Whitney U test. b. Multivariable logistic regression, adjusted for age, sex, socioeconomic status (SES: employment and educational attainment); odds ratio = OR; 95% confidence interval = 95% CI. c. Psychiatric comorbidity = any current depressive disorder and/or any current anxiety disorder. d. Long-term Difficulties Inventory sum-score. A higher score equals more perceived stress. e. A higher score equals worse perceived general health.

**Table 2B.** Psychological, behavioural and biological factors in HQ-traits- versus LQ-traits-group

	HQ-traits-group <sup>a</sup> n = 7783		LQ-traits-group <sup>b</sup> n = 7783		p-value <sup>c</sup>	Adjusted OR (95% CI) <sup>d</sup>
<b>Psychological</b>						
Psychiatric comorbidity <sup>e</sup> (N, %)	855	(11.0)	316	(4.1)	<0.01	3.27 (2.83-3.79)
Anxiety (N, %)	714	(9.2)	269	(3.5)	<0.01	3.20 (2.74-3.75)
Depression (N, %)	396	(5.1)	107	(1.4)	<0.01	3.92 (3.10-4.95)
Stress (median, IQR) <sup>f</sup>	2.0	(0.0-3.0)	1.0	(0.0-3.0)	<0.01	1.15 (1.13-1.17)
Perceived health (mean, SD) <sup>g</sup>	2.8	(0.8)	2.5	(0.8)	<0.01	1.48 (1.41-1.55)
<b>Behavioural</b>						
Alcohol use, >2 glasses / day (N, %)	2088	(26.8)	1752	(22.5)	<0.01	N.S.
Physical activity, days / week (mean, SD)	4.2	(1.9)	4.5	(1.9)	<0.01	0.95 (0.93-0.97)
<b>Biological</b>						
Body mass index (mean, SD)	26.1	(4.3)	25.6	(4.2)	<0.01	N.S.
Waist circumference (cm) (mean, SD)	91.5	(12.6)	88.4	(12.1)	<0.01	N.S.
Leukocytes (10 <sup>E9</sup> /L) (median, IQR)	5.80	(5.00-6.90)	5.70	(4.90-6.80)	<0.01	N.S.
Neutrophils (10 <sup>E9</sup> /L) (median, IQR)	3.09	(2.50-3.83)	3.04	(2.43-3.78)	<0.01	N.S.
Lymphocytes (10 <sup>E9</sup> /L) (median, IQR)	1.90	(1.56-2.31)	1.90	(1.57-2.29)	N.S.	N.S.
Monocytes (10 <sup>E9</sup> /L) (median, IQR)	0.49	(0.40-0.59)	0.47	(0.39-0.56)	<0.01	N.S.
Eosinophils (10 <sup>E9</sup> /L) (median, IQR)	0.16	(0.11-0.24)	0.16	(0.11-0.24)	<0.01	N.S.
Basophils (10 <sup>E9</sup> /L) (median, IQR)	0.04	(0.03-0.06)	0.04	(0.03-0.06)	N.S.	N.S.
CRP (median, IQR)	1.1	(0.6-3.0)	1.0	(0.5-2.2)	N.S.	N.S.

a. HQ-traits-group = 25% of participants with the highest Autism Quotient (AQ-10) sum-scores. b. LQ-traits-group = 25% of participants with the lowest AQ-10 sum-scores. c. Univariable analysis with Chi-square test, Fisher's exact test, independent sample t-test or Mann-Whitney U test. d. Multivariable logistic regression, adjusted for age, sex, socioeconomic status (SES: employment and educational attainment); odds ratio = OR; 95% confidence interval = 95% CI. e. Psychiatric comorbidity = any current depressive disorder and/or any current anxiety disorder. f. Long-term Difficulties Inventory sum-score. A higher score equals more perceived stress. g. A higher score equals worse perceived general health.

**Table 3.** Multivariable logistic regression with GI-symptoms as outcome, adjusted for confounders <sup>a</sup>

	ASD n = 309	non-ASD n = 30,876	HQ-traits-group <sup>b</sup> n = 7783	LQ-traits-group <sup>c</sup> n = 7783
	GI-symptoms <sup>d</sup> (OR, 95% CI) <sup>e</sup>	GI-symptoms <sup>d</sup> (OR, 95% CI) <sup>e</sup>	GI-symptoms <sup>d</sup> (OR, 95% CI) <sup>e</sup>	GI-symptoms <sup>d</sup> (OR, 95% CI) <sup>e</sup>
<b>Psychological</b>				
Psychiatric comorbidity <sup>f</sup>	2.78 (1.51-5.01) *	2.72 (2.46-3.01) *	2.68 (2.27-3.15) *	2.45 (1.91-3.13) *
Stress <sup>g</sup>	1.15 (1.06-1.26) *	1.20 (1.18-1.21) *	1.18 (1.15-1.21) *	1.20 (1.16-1.23) *
Perceived health <sup>h</sup>	2.32 (1.62-3.34) *	2.07 (1.99-2.16) *	2.16 (1.98-2.35) *	2.06 (1.89-2.24) *
<b>Behavioural</b>				
Alcohol use, >2 glasses/ day	0.77 (0.38-1.59)	1.07 (0.99-1.16)	1.04 (0.90-1.21)	1.18 (1.00-1.39)
Physical activity	0.92 (0.80-1.05)	0.96 (0.95-0.98) *	0.95 (0.92-0.98) *	0.96 (0.93-1.00)
<b>Biological</b>				
Body mass index	1.01 (0.96-1.07)	1.01 (1.00-1.02)	1.01 (1.00-1.02)	1.02 (1.00-1.03)
Waist circumference	1.00 (0.98-1.02)	1.01 (1.00-1.01)	1.01 (1.00-1.01)	1.01 (1.00-1.01)
Leukocytes	0.99 (0.85-1.14)	1.04 (1.02-1.06) *	1.05 (1.01-1.08) *	1.04 (1.00-1.08)
Neutrophils	1.06 (0.84-1.34)	1.07 (1.04-1.09) *	1.07 (1.02-1.12) *	1.07 (1.01-1.12) *
Lymphocytes	0.76 (0.49-1.19)	1.08 (1.03-1.13) *	1.11 (1.01-1.22) *	0.98 (0.89-1.09)
Monocytes	0.36 (0.05-2.85)	1.23 (0.99-1.52)	0.94 (0.63-1.40)	1.25 (0.80-1.96)
Eosinophils	2.87 (0.48-17.22)	0.96 (0.76-1.21)	1.01 (0.65-1.57)	1.43 (0.92-2.20)
Basophils	0.80 (0.00-597051.25)	2.09 (0.52-8.44)	6.86 (0.51-92.77)	1.34 (0.08-23.88)
CRP	-	1.16 (0.57-2.36)	1.24 (0.31-5.04)	(0.13-3.66)

a. In each of the 4 groups (ASD-, non-ASD-, HQ-traits-, and LQ-traits-group) separate multivariable logistic regression models were performed with the presence of 1 or more GI-symptom(s) as dependent outcome measure. For each of the independent psychological, behavioural, and biological variables a separate multivariable logistic regression model was performed, including adjustment for confounders (age, sex, employment and educational attainment). Thus, the groups were not compared in these analyses. b. HQ-traits-group = 25% of participants with the highest Autism Quotient (AQ-10) sum-scores. c. LQ-traits-group = 25% of participants with the lowest AQ-10 sum-scores. d. Gastrointestinal (GI) symptoms = presence of 1 or more of these symptoms: constipation, diarrhoea, heartburn, abdominal pain/discomfort. e. Odds ratio = OR. 95% confidence interval = 95% CI. Statistically significant odds ratios are marked: \*. f. Psychiatric comorbidity = any current depressive disorder and/or any current anxiety disorder. g. Long-term Difficulties Inventory sum-score. A higher score equals more perceived stress. h. A higher score equals worse perceived general health.

## Discussion

Our results demonstrated an elevated risk for the GI-symptoms abdominal discomfort/pain, diarrhoea, constipation, and/or heartburn in adults with ASD compared to adults without ASD. This increased risk was also found in adults with higher levels of autistic traits compared to adults with lower levels. In both adults with ASD and adults with higher levels of autistic traits, GI-symptoms were strongly associated with psychiatric comorbidity (anxiety and/or depressive disorder), increased perceived psychological stress levels, and worse perceived health. In adults with higher levels of autistic traits, there was a moderate association between GI-symptoms and less physical activity and increased leukocyte, lymphocyte, and neutrophil counts.

With regard to psychological factors, we found no previous studies specifically investigating the association between GI-symptoms in adults with ASD or autistic traits and stress or psychiatric comorbidities. Nonetheless, interactions between autistic traits and several psychological parameters have already been acknowledged (Green and Ben-Sasson et al., 2010; Amos et al., 2019), for example sensory over-responsivity, which could lead to stress and anxiety. Through the gut-brain axis, psychological factors such as stress and anxiety can cause alterations in the GI-tract (e.g., bowel movement and gut microbiota), and vice versa (Chidambaram et al., 2020). Such GI-alterations are related to GI-symptoms. On a biological basis, this bilateral interaction between the physique and the psyche in the gut-brain axis supports the associations we observed between GI-symptoms and psychiatric comorbidity and stress in autistic adults. The current study also showed that in adults with ASD and in adults with higher levels of autistic traits, worse perceived health was associated with GI-symptoms. This result could be explained by the relation between worse perceived health and stress, as found in autistic adults (Moseley et al. 2021).

Regarding investigated behavioural factors, alcohol use of more than two glasses per day was not associated with GI-symptoms in our adults with ASD or autistic traits. Previous studies demonstrated that alcohol (ab)use is less prevalent in adults with ASD (Croen et al., 2015; Vohra et al., 2017; Tolchard et al., 2018; Weir et al., 2020), as also observed in our multivariable analysis. When it comes to physical activity, previous literature has pointed out that children and adults with ASD on average perform less physical exercise (McCoy et al., 2016; Memari et al., 2015; Hillier et al, 2020). Moreover, the current study displayed that being less physically active was associated with GI-symptoms in adults with higher levels of autistic traits. Therefore, it is advisable to take physical activity into account when assessing GI-problems in autistic adults.

With respect to biological factors, BMI is associated with GI-symptoms in the general population (Eslick GD, 2012) and weight problems are an

increasing epidemic in people with ASD (Li et al., 2020; Croen et al., 2015; Hand et al., 2020). In our study, mean BMI and waist circumference did not differ between adults with and without ASD, and both were not associated with GI-symptoms. Regarding immunological factors, we investigated leukocyte and subtype counts, because stress is a mediator in GI-symptoms and stress hormones (such as cortisol) are known to regulate these leukocyte counts (De Palma et al., 2014; Dhabhar et al., 2012). Therefore, we hypothesised that altered immunological blood markers might help to understand the correlation between chronic exposure to stress in individuals with ASD and the occurrence of GI-symptoms (Hirvikoski et al., 2015; Bishop-Fitzpatrick et al., 2015; Baron et al., 2006). Our study demonstrated a moderate association between immunological factors, in particular lymphocyte and neutrophil counts, and GI-symptoms in adults with higher levels of autistic traits. In adults with lower levels of autistic traits neutrophil but not lymphocyte counts were found to be associated with GI-symptoms. Thus, we cannot conclude that these total leukocyte and subtype counts clearly highlight the relationship between stress and GI-symptoms in our target population.

In summary, our findings suggest that stress, psychiatric comorbidities, perceived health, and physical activity are important factors to integrate in the assessment of GI-symptoms in adults with ASD or autistic traits. It should be noted that our study population included adults that were able to provide self-reported AQ-10 answers. Therefore, it is estimated that this study population mostly represents adults with lower support needs. It is hypothesised that future inclusion of adults across a wider range of intellectually abilities, would result in similar or magnified findings compared to our study, since intellectual disability increases the risk for GI-disorders in adults with ASD (Bishop-Fitzpatrick and Rubenstein, 2019; Gilmore et al., 2021). It is also expected that investigating a study population including more adults with ASD, would result in larger significant findings regarding factors associated with GI-symptoms in these adults with ASD, because a larger sample size leads to more precision.

### Strengths

The main strength of this study is the large sample size of a prospective general cohort, offering a wide range of basic, psychological, behavioural, and biological variables in more than 31,000 adults. The approximately 1% (n=309) of adults reporting an ASD-diagnosis in our study sample matches the worldwide prevalence of 1-2% (Dietz et al., 2020; Baio et al., 2018). This makes our study population a representative sample in terms of autism prevalence. Another asset to this study is the assessment of stress levels by perceived psychological stress using questionnaires and a laboratory approach by immunological blood markers. Furthermore, the combination of performing the same analyses

in both adults with ASD and adults with autistic traits, is relevant to include in research because of the prevalence of undiagnosed ASD-cases (Baron-Cohen et al., 2009) and the correlations between autistic traits and GI-symptoms (Amos et al., 2019; Williams and Gotham, 2021). A final strength of this study is the involvement of the autistic community in defining the research question, including relevant variables, and interpreting the results (through advice of autistic team-members).

### Limitations

Since this study had a cross-sectional design, it was not possible to examine temporality. Another limitation of our study is the fact that data from the GI-questionnaire and the autism questionnaire were not available at the same moment in time. This resulted in a mean gap of 4 years between data about the ASD-diagnosis and AQ-10 scores, and psychological, behavioural, biological, and GI-data. Nonetheless, since ASD is considered as a life-long diagnosis, this time gap was not likely to interfere with our results in the ASD- and non-ASD-group. Broadbent et al. (2013) concluded that the AQ-10 test-retest reliability, with a time interval of 6 to 12 months, was satisfactory. As it is known that food selectivity and insufficient oral intake can affect the intestinal microbiome in children with ASD (Bandini et al., 2010; Murtaza et al., 2017), it is a limitation of our study that dietary variables were not included. Our study was limited to self-report regarding the presence of an ASD-diagnosis. However, this uncertainty was partly countered by a median AQ-10 sum-score of 6.0 in adults with ASD, compared to 2.0 in the adults without ASD. It should also be taken into account that GI-symptoms were based on self-report (ROME-III questions). However, Lefter et al. (2019) advised to use ROME-questionnaires to assess GI-problems in ASD. Since people were not eligible for inclusion in the Lifelines Cohort if they did not have the ability to fill in self-report surveys, our study sample does not represent those autistic adults with cognitive disabilities or other types of disabilities impacting self-report. Therefore, the results from our study cannot be generalised to all types of autistic adults, but are limited to autistic adults with the ability to fill in self-report surveys.

### Implications

Both healthcare providers and autistic adults should be aware that psychological and behavioural factors are associated with GI-symptoms in autistic adults. It would be beneficial to people with ASD if this knowledge results in more integrated psychological, behavioural, and somatic care. Since we found that adults with autistic traits exhibit more psychological and behavioural risk factors, it could be useful to consider autistic traits in patients with poorly understood GI-complaints. After exclusion of a somatic cause of GI-problems, stress, perceived health, psychiatric comorbidity, and physical activity should

be evaluated by clinicians. Moreover, health education focusing on psychosomatic interactions could lead to better adherence of treatment or preventive measures. Future research should address the optimisation of prevention and treatment of GI-symptoms in autistic adults. Finally, associations between GI-symptoms and increased leukocyte (subtype) counts in autistic adults should be investigated in more depth to clarify their value for clinical practice.

## Conclusion

Our study demonstrates that not only adults with ASD, but also adults with higher levels of autistic traits are at increased risk for GI-symptoms. In addition, in both adults with ASD and adults with higher levels of autistic traits, GI-symptoms are strongly associated with higher stress levels, psychiatric comorbidity, and worse perceived health. In adults with the highest levels of autistic traits, GI-symptoms are also moderately associated with less physical activity. Together, these results suggest that assessment of GI-symptoms in adults with ASD or autistic traits might benefit from an integrated psychological and behavioural approach, next to the somatic assessment. Future research could focus on optimising clinical practice regarding prevention and treatment of GI-symptoms in autistic adults.

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